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Impact of Hexazinone on Invertebrates after Application to Forested Watersheds

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Abstract. The impact of the herbicide, hexazinone, was assessed on aquatic macrophytes, aquatic and terrestrial invertebrate communities within forested watersheds in the Piedmont region of Georgia. Four replicate watersheds received hexazinone on April 23, 1979, and were subsequently monitored for eight months. Residue levels in terrestrial invertebrates were a maximum of two orders of magnitude greater than comparable levels (0.01 to 0.18 ppm) found in forest floor material. Aquatic organisms in a second order perennial stream were exposed to intermittent concentrations of hexazinone (6 to 44 ppb). Hexazinone and its metabolites were generally not detected (<0.1 ppm) in aquatic invertebrates and macrophytes. No major alterations in species composition or diversity were detected in the aquatic macroinvertebrate community. Terrestrial microarthropod samples collected near the end of the study period revealed no major community changes.

In the southeastern U.S., herbaceous weeds and hardwood trees are a major problem for pine silviculture. Reestablishment of harvested stands requires some form of site preparation. Current reforestation practices favor intensive mechanical systems which produce a loss of ground cover and often reduce site productivity (Brender 1973). Soil erosion is often a serious problem for years following mechanical site preparation, especially in the Piedmont, where sediment losses of 5 to 11 metric tons $\cdot ha^{-1} \cdot yr^{-1}$ can occur (Nutter and Douglass 1978). Herbicides provide an alternative site preparation technique which does not contribute to erosion and have been used in the past to control hardwood competition in pine stands (Fitzgerald 1980). However, some of these chemicals have been banned because of their potential to produce adverse impacts on nontarget organisms. New compounds are being developed and tested by chemical manufacturers to meet a growing demand for herbicides that are effective, inexpensive, easy to apply, and environmentally safe. One new chemical which shows potential for forestry use in the South is hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4-(*1H,3H*)-dione][2]. It is an effective herbicide on a broad spectrum of annual and perennial species. Velpar®, with hexazinone as the active ingredient, was registered by the U.S. Environmental Protection Agency in 1975 for noncropland and right-of-way use. The pelleted formulation was granted registration for conifer release and site preparation in all states, except Alaska, California, Hawaii, Idaho, Nevada, Oregon, Utah, and Washington, in March, 1981.[3,4]

This study is part of an investigation on the loss of hexazinone in storm runoff from small forest watersheds (Neary *et al.* 1980). The objectives were: (1) to monitor accumulations of hexazinone and its metabolites in aquatic macrophytes, aquatic invertebrates, and terrestrial macroinvertebrates and (2) to determine if changes occurred in the species composition or diversity of terrestrial and aquatic invertebrate communities.

Materials and Methods

Site

The study area is in a headwater drainage of the Broad River on the Chattahoochee National Forest, Habersham County, GA,

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[2] Manufacturer E. I. duPont de Nemours, Inc., Wilmington, DE, under Trade Name Velpar® Gridball® Brushkiller. The use of trade and corporation names does not constitute endorsement by the U.S. Department of Agriculture, but is provided as a reference.

[3] This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

[4] CAUTION: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or wildlife—if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.

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and consists of a series of well-defined, ephemeral and first-order perennial drainages at the 400-500 m elevations. The ridgetops are broad and have been extensively eroded by past agriculture. The drainages are typically broad, bowl-shaped in their upper reaches, incised at their mid points, and broad-bottomed in their lower reaches. The valley bottoms are overlaid with extensive alluvial deposits which occasionally reach 2 m in depth. The soils are mainly Cecil sandy loam, a typic hapludult derived from gneiss and mica schist bedrock.

The study area consists of five small watersheds approximately one ha each (Figure 1). These watersheds contain similar mixed hardwood-pine stands of chestnut oak (*Quercus prinus*), white oak (*Q. alba*), black oak (*Q. velutina*), blackjack oak (*Q. marilandica*), red maple (*Acer rubrum*), sourwood (*Oxydendrum arboreum*), hickory (*Carya* spp.), and shortleaf pine (*Pinus echinata*). Four of the five watersheds, (WS-1 through WS-4) were treated with herbicide. The treatment watersheds form ephermeral drainages that merge to form a first-order perennial stream, (Stream "b"), which enters Moonshine Creek, a second-order perennial stream. Moonshine Creek watershed encompasses 104.4 ha, with most of the area forested; however the upper 10 to 15% is primarily agricultural or residential. Baseflow measured at gauge Station 88 on Moonshine Creek ranged from 14-42 $l \cdot sec^{-1}$ and maximum discharge measured was 776 $l \cdot sec^{-1}$. The fifth watershed (WS-5) served as a control and was not treated with herbicide.

Hexazinone pellets (10% a.i.; pellet size—2 cm^3) were applied by hand to WS-1 through WS-4 on April 23, 1979 over the entire watershed, including ephemeral drainage channels, on a grid spacing of 1.2 × 1.8 m at a rate of 16.8 $kg \cdot ha^{-1}$.

Rainfall was measured with a standard storage gauge and Belfort recording rain gauge located 40 m north of Watershed 5. Stream stage was measured at gauging Station No. 88 with a Belfort FW-1 water level recorder. Flow was determined by the 0.2 and 0.8 depth method using a pygmy current meter.

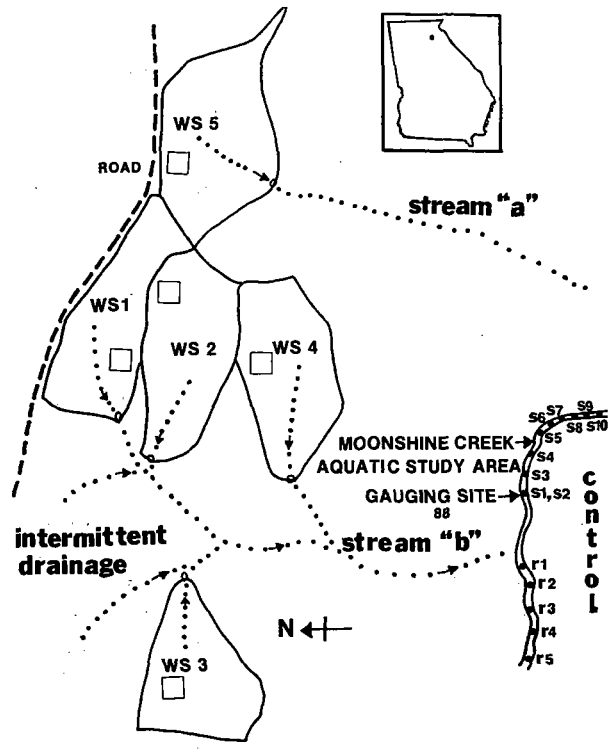

Fig. 1. Map of study areas showing treatment (WS-1 to WS-4) and control (WS-5) study sites on Moonshine Creek, Clarkesville, GA. Terrestrial invertebrate sampling plots are indicated by square (□). "R" indicates sampling locations for aquatic invertebrates and macrophytes in the stream control section and "S" indicates sample sites in the section receiving hexazinone residues

Water Sample Collection

Water samples were collected at Gauging Site 88 (Figure 1) throughout the study. The weekly bulked samples were collected by a Brailsford[4] sampler during most of the sampling period. A Manning S-4040T[5] operated from late May through June and again from late October through the end of the year. This automatic sampler collected water every two hr compositing four of these into one bottle. Grab sampling was used intermittently to supplement the automatic samplers. Water samples collected for herbicide residue analysis were placed in 1000-ml washed and methanol-rinsed jars, briefly stored at the Coweeta Hydrologic Laboratory at 4°C, transported to the University of Georgia in insulated, ice-packed containers, and stored at 4°C prior to extraction and analysis.

Forest Floor Sample Collection

Leaf litter samples were collected 3, 14, 30, 60, and 90 days after the first rain following herbicide application from areas adjacent to invertebrate collection plots. During herbicide application, individual hexazinone pellets were marked to locate individual sampling spots. Samples were collected at three equidistant points on radii of 0.6 m from each sampled pellet. Individual values for each watershed were determined from litter samples composited from samples collected from upper, mid, and lower slope areas of each of the four treated watersheds. A control sample was collected from the control watershed. Litter material was placed in labelled bags, and frozen until used for residue analysis. Studies conducted by E. I. duPont chemists indicate no loss of residues with freezer storage for up to 3 yr (Holt, personal communication).

Invertebrate and Macrophyte Collection

Macrophytes: Samples of aquatic macrophytes from rock substrate in the spray zone of the stream were collected on each date (April 1, May 12, June 16, August 17, December 1, 1979) for residue analysis. Plant material was scraped from the substrate and processed as described for stream invertebrates. Only one site per stream section (R and S) and date was sampled. Macrophytes were collected from a number of areas within each site and combined to form a representative sample for chemical analysis. Additional material was preserved in 70% ethanol for identification.

Aquatic Invertebrates: Only fifteen riffle areas similar in stream flow, depth, and substrate were available for invertebrate sampling (Figure 1). Samples were collected from a large area be-

[5] The use of trade and corporation names does not constitute endorsement by the U.S. Department of Agriculture but is provided as a reference.

cause of the extreme heterogeneity of substrate in the riffles and its consequent effect on species distribution. A sample of at least 1 m^2 was necessary in order to assume the samples represent all species in the community and are from a homogeneous "parent population" (Pielou 1966a). On April 1, 1979, prior to herbicide application, samples were collected from replicate sites S-2, S-3, S-10, and R-1 to determine the adequacy of a 1 m^2 sample area. On May 12, 1979, samples were collected at replicate treatment sites S-4 and S-6 and at control site R-2. Samples were collected at respective treatment and control sites S-5 and R-3 on June 16, 1979, S-7 and R-4 on August 17, 1979, and S-8 and R-5 on December 15, 1979.

A "kick" sampling technique was used to collect samples. Substrate was turned by hand and invertebrates and debris allowed to float into a net with 1.6 mm mesh opening. The mesh size tended to select only the larger members of the invertebrate community. Organisms were sorted and counted in the field and stored in glass vials on ice. Upon return to the laboratory, the samples were frozen and stored at 0°C for analyses. The invertebrate samples were composited to form a single representative sample for chemical analysis per site and date. A number of representative individual specimens of each taxonomic group were preserved in 70% ethanol to determine a precise taxonomic description.

Terrestrial Invertebrates: In each watershed, a 100 m^2 study was established that had similar general slope, soil type, and aspect. Within each plot, individual herbicide pellets were marked with flags. On each sampling date (April 1, May 12, June 16, August 17, December 1, 1979) circular areas of 2 m^2 around two flags were sampled by removing the leaf litter and humus with a rake and shovel, composited for each study plot, and stored. Invertebrates were extracted from subsamples of composited leaf litter using standard Berlese funnel methods (Edwards and Fletcher 1971). Litter was stored at room temperature for about one month during subsampling and extraction.

After extraction, the macroinvertebrates were sorted, counted, and stored at 0°C prior to chemical analysis. A single representative macroinvertebrate sample was obtained by combining the entire extraction of litter subsamples for chemical analysis. No representative individuals were preserved for the determination of a precise taxonomic description of terrestrial invertebrates. In addition to macroinvertebrates, microarthropods were isolated from samples collected on August 17 and December 1, 1979, and preserved in 70% ethanol.

Community Evaluations: A diversity value for each aquatic invertebrate sample was calculated by an index based on information theory derived from the Shannon formula (Shannon and Weaver 1963). Wilhm and Dorris (1968); Wilhm (1970a), and Bradt (1978) have used this index to quantify the diversity of aquatic macroinvertebrate communities. A coefficient of community value was calculated for each sample using an index presented by McIntosh (1967) and Whittaker (1975). This index has been used in determining similarity among communities in various ecosystems including aquatic systems containing benthic macroinvertebrates (Burlington 1962). Values may range from 0, indicating perfect dissimilarity, to 1, indicating perfect similarity.

Extraction and Analysis of Hexazinone

Analysis for hexazinone and its metabolites (A, B, D, and E) utilized a modification of a method by Holt (1981). A 25-g sample of the total invertebrate or macrophyte sample was weighed into a Waring® blender and 120 ml of chloroform added together with 50 g of sodium sulfate (Na_2SO_4). Each sample was blended at high speed for about 5 min, and vacuum filtered through Whatman glass filter paper in a Büchner funnel. The chloroform extraction was repeated twice using additional 100 ml portions of solvent. Finally, the sample was blended for 5 min with 150 ml of ethyl acetate and the homogenate was filtered. The combined extracts were filtered through double glass filter papers with 50 g Na_2SO_4 on the filter. The combined filtrates were quantitatively transferred to a 1000-ml round-bottom flask. Water (75ml) was added to the combined extracts and the organic solvents were removed with a rotary evaporator at 60°C. The remaining water was transferred through a glass wool filter to a 250 ml separatory funnel with small volumes of water as rinse. The flask was rinsed with chloroform and methanol until clean and then rinsed with water. Hexane (50 ml) was added to the separatory funnel. The funnel was shaken gently for about one min, and the phases allowed to separate. The water layer was run back into the 1000-ml round-bottom flask, discarding the hexane. The hexane wash was repeated twice with 50 ml portions of solvent and the sample was rinsed back into the separatory funnel each time with water. After the last hexane wash, the 1000-ml round-bottom flask was rinsed with methanol several times and finally with chloroform. Chloroform (75 ml) was added to the aqueous phase in the separatory funnel and shaken for 2 min. The phases were allowed to separate, and the chloroform was filtered through glass wool and Na_2SO_4 into the 100 ml round-bottom flask. The chloroform extraction was repeated twice with additional 75 ml portions of solvent. The volume was reduced to 4 ml on a rotary evaporator and transferred to a 10-ml culture tube with several chloroform/ethyl acetate rinses. The samples were stored in a freezer for future analysis.

At the time of analysis, extracts were concentrated to dryness in a water bath at 60°C under a stream of nitrogen. The residue was dissolved in one ml of chloroform and the sample reacted with trifluoroacetic anhydride. The derivatives were analyzed by gas-liquid chromatography on the same day as derivitization with a Tracor Model 222 Gas Chromatograph equipped with a Tracor Model 702 N/P detector. The chromatographic column was 15% OV-17 on 100/120 Chromosorb W HP (Supelco, Inc., Bellefonte, PA); 914 mm glass, 6.35 mm O.D., 1.59 mm I.D. The gas chromatograph operating conditions were: Inlet temperature, 230°C; detector temperature, 280°C; helium carrier gas flow, 35 ml/min. The column was temperature-programmed from 230° to 280° C at a rate of 10°/min. The column was held at the final temperature for about 8 min. Hexazinone and metabolite levels were determined by comparison of peak height in sample chromatograms to those of an analytical standard. All reference standards of hexazinone and metabolites A, B, D, and E were obtained from the Biochemicals Department, Agrichemicals Marketing Division, E. I. duPont de Nemours & Co., Inc., Wilmington, DE. A reagent blank and a spiked sample were included with each set of analyses, and values were corrected for percent recovery.

Results

Water Sample Residues

The residues of hexazinone and metabolite (hexazinone plus metabolites A and B) detected at Gauging Site 88 and monthly rainfall are presented in Figure 2. Metabolite B was never detected in streamflow at the 88 site. For much of the experi-

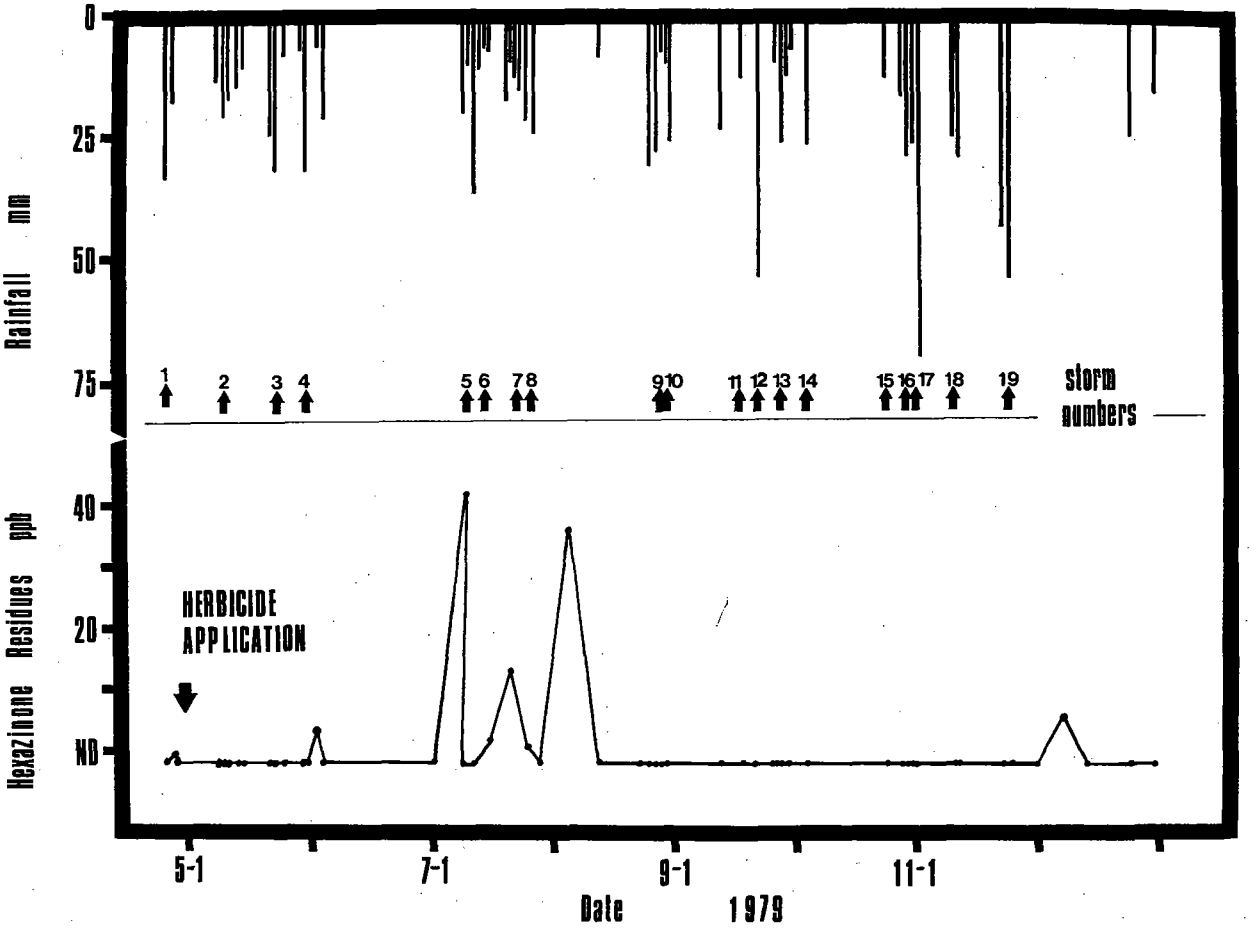

Fig. 2. Hexazinone residue (hexazinone plus metabolite A and B) in solution at Moonshine Creek Gauging Site No. 88

mental period, levels were below the 1 ppb analytical detection limit. Detectable concentrations (6 to 44 ppb) occurred during July and early August. Residues of metabolite A first appeared in the peak of July 9, 1979, contributing nearly one-half of the total residue concentration. The August 8th peak, which was not associated with a storm was composed of 62% hexazinone and 38% metabolite A (not detected again at Gauging Site 88). Peak residue levels did not show any direct relationship to the magnitude of storm events nor did periods of elevated concentrations coincide with all storm events. Hexazinone residues were detected only during storms 1, 4, 5, 7, and 8. The residue peaks were short in duration, and low in concentration.

Forest Floor Residues

Samples of the forest floor, litter and decomposed humus material lying above the mineral soil, contained residues of hexazinone for up to 90 days following the first rain after herbicide application (Table 1). Residues of metabolite A were present three days after the first rainfall but were not detected thereafter. Metabolite B was not detected in the forest floor material. Since samples were collected at a maximum distance from application points, these concentrations are minimum levels of exposure for terrestrial invertebrates in the forest floor.

Hexazinone and Metabolite Residue in Organisms

Residue levels of hexazinone and its metabolites above the limit of analytical detection (0.1 ppm) were observed in aquatic macrophytes (composed primarily of aquatic mosses *Ecrhynchium rusciforme* and *Leptodictyum riparium*) and macroinvertebrates on only a few occasions. Macroinvertebrates collected on May 12, 1979, from S-4 and S-6 exhibited respectively a trace of metabolite B and 0.24 ppm (wet weight) metabolite D. A trace of metabolite B was again encountered in a macroinvertebrate sample collected on December 15,

Table 1. Residues of hexazinone and metabolites A and B in forest floor material, Moonshine Creek study area, 1979

Sample date 1979	Days from first rainfall	Herbicide residue[a]		
			Metabolites	
		Hexazinone	A (ppm, oven dry weight)	B
4/30/79	5	0.18 ± 0.19	0.10 ± 0.17	ND[b] —
5/10/79	15	0.01 ± 0.01	N.D. —	ND —
5/24/79	29	N.D. —	0.16 ± 0.22	ND —
6/25/79	61	0.07 ± 0.10	N.D. —	ND —
7/25/79	91	0.06 ± 0.06	N.D. —	ND —

[a] Values are means determined for replicate watersheds (WS-1 to WS-4, inclusive) ± 1 standard deviation

[b] Limit of analytical detection of 0.01 ppm; for purposes of mean concentrations of hexazinone, a value of 0.01 was used for nondetectable (ND) residues. Due to the presence of interfering substances and small sample size, metabolites D and E were not reported

1979, from S-8. Also, trace amounts of compounds similar to metabolites E and D were observed respectively on March 31 and June 16, 1979, in macrophytes from the control section suggesting that interfering substances, chemicals similar to hexazinone's metabolites, are present in the aquatic environment.

Most soil macroinvertebrate samples showed detectable concentrations of hexazinone and/or its metabolites. However, the values from replicate watersheds on any one sampling date proved to be highly variable (Table 2). This variability probably resulted from the small, heterogenous samples of invertebrates and the variation in microhabitats between replicate watersheds. As in aquatic samples, unidentified interfering substances are present as evidenced by the detection of these substances in samples from the control watershed.

Community Composition

I. Aquatic Macroinvertebrates

Samples from 1 m^2 proved to be adequate for diversity estimates. The diversity values for 1 m^2 samples collected on April 1, 1979, were within 10% of the asymptotic diversity as estimated by the pooling of samples (Pielou 1966b). Similar variations in diversity with time were noted for hexazinone receiving and control sections of stream (Figure 3). With the assumption of no interaction between time and treatment effects supported by the similar variations in diversity with time for each stream section, an analysis of variance without replication was used with collection dates as random effects and hexazinone receiving and control sections as fixed treatment effects (Sokal and Rohlf 1969). Dates May 12 through December 15, 1979, were included in the analysis. No significant differences were noted between hexazinone receiving and control sections at the 0.10 level, $F = 1.30 < 4.54$. The coefficient of community values were assembled into a similarity matrix for each sampling date (Table 3). Values equal to or greater than 0.65 were considered to indicate ecological similarity by Beckett (1978), Hurd (1961), and Hanson (1955). All values in this study were equal to or greater than 0.65 indicating marked similarity between the control section and the section of the stream receiving hexazinone residues. Apparently, the residues had little effect on the composition of the aquatic macroinvertebrate community.

As a measure of the abundance of a taxon in the control and treated sections, the numbers of individuals of a specific taxon per sample were expressed as a percentage of the total number of aquatic invertebrates collected per sample. Only taxa that composed 10 percent or more of a minimum of one sample were compared graphically (Figures 4 and 5). Taxa present in samples in percentages of less than 10% were found in samples only sporadically and consequently did not provide meaningful information on the effects of hexazinone on their appearance in the community. The taxa compared in samples collected from both stream sections showed similar temporal variations in abundance throughout the sampling period. There is no evidence that hexazinone altered taxa abundance.

II. Terrestrial Invertebrates

Terrestrial macroinvertebrate samples were too small to derive meaningful information regarding the effects of hexazinone on community composition. The diversity values of pooled replicate samples indicated that sample size was not adequate for diversity and similarity studies (Pielou 1966b; Wilhm 1970b). The only observation that may be of significance occurred in samples collected on August 17, 1979, when at least twice the number of

Table 2. Concentration means and ranges of hexazinone and metabolites A and B in terrestrial invertebrate samples from treated and untreated watersheds, Moonshine Creek study area, 1979

		ppm[a]					
		Hexazinone[b]		met. A		met. B	
Date		Level	Range	Level	Range	Level	Range
3/31/79	Before application	ND		ND		ND	
5/12/79	Control	ND		ND		0.67	
	Treated	0.35	ND–1.34	ND		0.43	ND–0.95
6/16/79	Control	ND		ND		ND	
	Treated	0.13	ND–0.50	0.97	ND–3.88	0.74	ND–1.72
8/17/79	Control	ND		ND		ND	
	Treated	ND		ND		1.43	ND–4.29
12/1/79	Treated	ND		ND		ND	

[a] Ppm is determined on an "as is" basis. Values for the control watershed WS-5 were determined from a single sample. Values for 12/1/79 were from a single sample from watershed WS-4. Means and range were determined from samples collected from watersheds WS-1 to WS-4. ND is interpreted as zero for calculation of means

[b] Due to the presence of interfering substances and small sample size, Metabolites D and E were not reported. Presence of hexazinone in Control 5/12/79 sample probably indicates interference in the analytical procedure

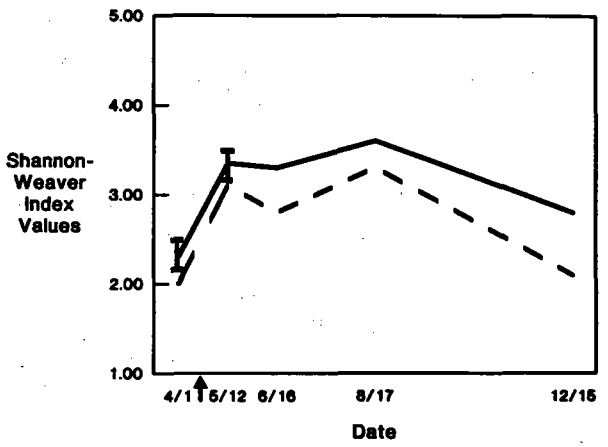

Fig. 3. Shannon-Weaver Index values calculated for aquatic invertebrates in treated and control sections of Moonshine Creek, 1979. Error bars indicate the range of values for a mean of replicate samples. Values without error bars were determined from a single sample. (——treated area, – – – control, ↑ date of hexazinone application)

Table 3. Similarity matrices for the coefficient of community index calculated for sites receiving hexazinone residues and control sites, Moonshine Creek, 1979

April 1	Sites[a]	S-2	S-3	S-10	R-1
	S-2	1	0.92	0.88	0.88
	S-3		1	0.80	0.80
	S-10			1	0.85
	R-1				1
May 12	Sites	S-4	S-6	R-2	
	S-4	1	0.69	0.65	
	S-6		1	0.86	
	R-2			1	
June 16	Sites	S-5	R-3		
	S-5	1	0.79		
	R-3		1		
August 17	Sites	S-7	R-4		
	S-7	1	0.88		
	R-4		1		
December 15	Sites	S-8	R-5		
	S-8	1	0.65		
	R-5		1		

[a] Sites designated with "S" indicate sites below the entry point of hexazinone into the stream; sites designated with "R" indicate sites above the entry point of hexazinone

individuals were collected in the control watershed (WS-5) compared to the number collected in any one herbicide treated watershed (WS-1 to WS-4, inclusive).

The variation in soil microarthropod numbers in samples from treated watersheds was large, suggesting that microhabitat differences exist within watersheds. Since microarthropods were not identified below family, the number of taxa per sample was consistently less than that needed for a meaningful calculation of the Shannon-Weaver Index or Coefficient of Community Index. Therefore, the abundance of a specific taxon was expressed as a percentage of the total number of individuals collected per sample. Only those taxa that composed 5% or more of a minimum of one sample were compared tabularly (Table 4). Acarina composed a large fraction of the microarthropod community in all watersheds, and its abundance did not vary appreciably between control (WS-5) and herbicide treated watersheds (WS-1 to WS-4, inclusive). The remaining taxa also showed no consistent variation between herbicide treated and control watersheds.

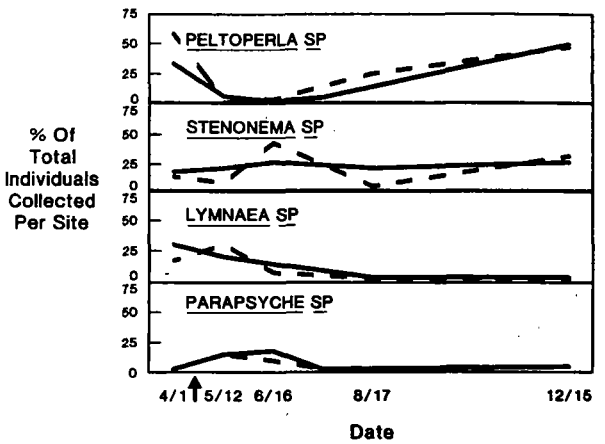

Fig. 4. Percent of the total number of individuals per site for 4 major taxa (*Peltoperla* sp., *Stenonema* sp., *Lymnaea* sp., and *Parapsyche* sp.) collected from Moonshine Creek, 1979. (—— treated area, – – – control, ↑ date of hexazinone application)

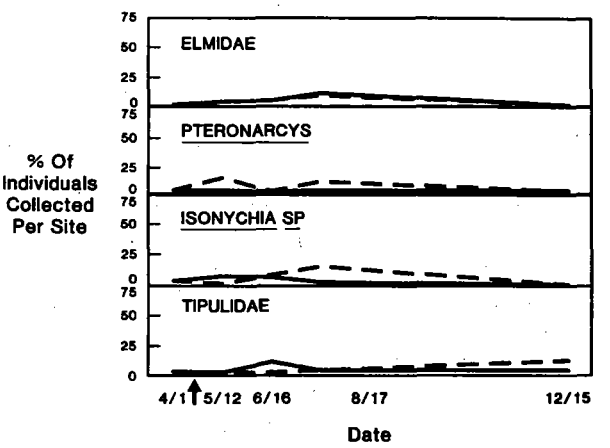

Fig. 5. Percent of the total number of individuals per site for four major taxa (Elmidae, *Pteronarcys dorsata*, *Isonychia* sp., Tipulidae) collected from Moonshine Creek, 1979. (—— treated area, – – – control, ↑ date of hexazinone application)

Table 4. Abundance of taxa as a percentage of the total number of microarthropods in a sample, and the total number of individuals and taxa in samples from treated and untreated watersheds, Moonshine Creek study area, 1979

		August 17 sites				
Order	Organisms[a]	WS-1	WS-2	WS-3	WS-4	WS-5
Hymenoptera	Formicidae	1.6	0.0	0.6	0.2	2.1
Acarina		60.8	83.5	89.5	89.4	88.1
Araneae		5.8	5.0	1.1	1.5	1.3
Collembola		19.0	0.3	0.6	3.6	1.4
Psocoptera		0.0	5.9	0.2	0.0	0.0
Total per sample		189	661	2879	1826	1573
Taxa per sample		14	10	16	12	15
		December 1 sites				
		WS-1	WS-2	WS-3	WS-4	WS-5
Hymenoptera	Formicidae	8.3	17.5	0.0	10.5	6.8
Acarina		58.3	68.2	71.0	62.0	69.9
Araneae		4.2	1.6	1.3	4.4	2.7
Collembola		25.0	4.8	19.7	19.6	15.1
Psocoptera		0.0	0.0	0.8	0.0	1.4
Total per sample		24	63	238	229	73
Taxa per sample		5	6	8	9	8

[a] Organisms could not be identified to a lower taxonomic unit as a result of the lack of late instar specimens. Only those taxa that composed 5% or more of any one sample were tabulated. The remaining taxa composed less than 5% of any one sample on each sampling date

Discussion

Mainstream Residues

Mainstream residue levels for hexazinone plus its metabolites were low (<44 ppb), because of the small loss of hexazinone from the treated watersheds and dilution downstream. The effect of mainstream dilution during storm discharge is considerable; during Storm 1, which produced the highest average residue concentrations of 442 ppb from the small watersheds (Neary *et al.* 1980), only 1.5 ppb hexazinone was detected in a 7-day bulk sample. Losses of herbicide residues were episodic and unpredictable. They occurred as pulses of short duration during most of the study period, and did not coincide with peak storm runoff, but occurred several days later. This suggests that other mechanisms, such as subsurface flow, may be the dominating factors in the loss of residues. As a consequence, aquatic organisms were exposed inter-

mittently to short duration, low-level concentrations of hexazinone residues over a period of 8 months. The concentrations of hexazinone residues to which aquatic organisms were subjected in this study are typical of anticipated uses of hexazinone as a forest herbicide where <5% of a second-order watershed is treated. The degree of streamflow dilution is dependent upon the relative sizes of treated and untreated areas of the watershed. The maximum concentration of herbicide residues to which aquatic organisms would be exposed would occur only where the entire drainage area of a perennial stream, such as Stream "b", was treated with hexazinone. In this study, the maximum measurement was 442 ppb (Neary *et al.* 1980).

Forest Litter Residues

Hexazinone residues in the forest litter were much higher than mainstream residues. Residues were mainly the parent compound (0.01 to 0.18 ppm). Attenuation of hexazinone concentrations is likely to follow normal residue decay curves. Since no forest litter samples were collected from August to December, no comparisons of levels of hexazinone in the forest litter and terrestrial invertebrates can be made.

Accumulation of Hexazinone in Organisms

Hexazinone residues in mainstream flow did not reach levels shown to affect aquatic organisms in laboratory studies nor was there any evidence that hexazinone accumulated in stream organisms. Fowler (1977) has shown in the laboratory that the common algae *Cladophora clomerata, Rhizochonium hieroglyphium* and *Vaucheria dichotoma* are inhibited at 0.5, 0.5, and 1.0 ppm, respectively. *Daphnia* sp., a freshwater invertebrate, has an LC_{50} (48 hr) of 151 ppm and, in a 21-day life cycle study, the effective concentrations for an LC_{50} with 7- and 3-day renewal of hexazinone were 33.1 and 20 to 50 ppm, respectively (Summers, personal communication). In this study stream, organisms were exposed for periods of 1 to 10 days to detectable concentrations of hexazinone that were one to four orders of magnitude less than the effective concentrations in laboratory studies. Although stream organisms were intermittently exposed to detectable herbicide residues throughout the study period, the accumulation of residues, if any, in organisms was less than one order of magnitude above the maximum concentration observed in Moonshine Creek. Notwithstanding, there is limited information regarding the impact of hexazinone on stream organisms.

In contrast to the aquatic biota, hexazinone and its metabolites accumulated in terrestrial macroinvertebrates. Mean levels of hexazinone or at least one of its metabolites were one to two orders of magnitude greater than comparable levels found in forest litter. This suggests a significant uptake of hexazinone by the macroinvertebrate community, either by active biological uptake or passive accumulation. Since invertebrate levels were determined on a wet weight basis and levels in the forest litter were determined on a dry weight basis, the actual differences in invertebrate and forest litter hexazinone levels are somewhat greater than indicated by the data. More information on the mechanism of uptake and the concentrations which impair the function of, or kill, invertebrate organisms would be needed to evaluate the significance of the observed accumulations of terrestrial macroinvertebrates.

Community Composition

The marked similarity in diversity and species composition exhibited between control and herbicide treated sections of stream indicate that no gross changes in the aquatic invertebrate community occurred as a result of the introduction of hexazinone residues into the aquatic environment. No large or consistent variations in the abundance of major taxa of soil microarthropods were observed between control and herbicide treated watersheds. Nevertheless, information on the effects of the herbicide treatment on terrestrial and aquatic invertebrate communities is limited. Reduced sample replication, large variation between replicate samples, incomplete taxonomic description of samples and a sampling schedule of less than one year can mask subtle effects. Diversity values at all stream sites on all sampling dates were somewhat lower than expected. Diversity values equal to or greater than 3.0 are indicative of "clean" water and are expected to range from 3.0 to 4.0 for small mountain streams (Wilhm 1970a). Only samples collected in the summer fell into this category. The low values may be the result of incomplete taxonomic description (*i.e.*, the lack of separation of species present in higher taxonomic units) and/or the lack of complete sampling (*i.e.*, sandy areas in pools), or the result of other perturbations of the stream from farmland, roads, and a railroad right-of-way occurring upstream of the study area.

Bormann and Likens (1979) demonstrated that devegetation of a forested ecosystem disrupts the regulation of hydrologic, energy flow, decomposition, mineralization, erosion, and nutrient cycling processes. The disruption of regulation of these

processes would probably also alter the abundance of invertebrates and the organization of aquatic and terrestrial invertebrate communities within the ecosystem. The removal of vegetation rather than herbicide toxicity is probably responsible for the decrease in the abundance of macroinvertebrates during the later summer months. The increased heat and light reaching the forest litter most likely forced the invertebrates into the mineral soil. The impacts of devegetation on ecosystem processes often occur several years later; therefore, the full impact of hexazinone application on invertebrate communities may not be evident in this study. The extent that devegetation will disrupt steady-state regulation, and consequently invertebrate communities, will depend upon the extent of devegetation and the rate and type of revegetation that consequently restores biotic regulation. Methods for separating indirect and long-term effects of vegetation removal from direct toxic effects of hexazinone are needed to more thoroughly assess the impact of hexazinone on the invertebrate communities of forested ecosystems.

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