Bull. Environ. Contam. Toxicol. (1987) 38:232–239

Environmental Contamination and Toxicology

Persistence and Degradation of the Herbicide Hexazinone in Soils of Lowbush Blueberry Fields in Nova Scotia, Canada

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Hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione; tradename Velpar] is a broad spectrum herbicide used primarily in forestry, industrial and right-of-way weed control. Hexazinone is very water soluble (330 g/L at 25°C). It readily leaches in soils (Rhodes 1980) and, depending on rainfall and slope, can be transported laterally following surface applications. (Harrington et al 1982, Neary et al 1982). Rhodes (1980) reported the half-lives of hexazinone in silt loam soils in Delaware, Illinois and Mississippi to be one, two and six months, respectively. Eight metabolites were extracted from ^{14}C-hexazinone treated soils and metabolite C [Rhodes' terminology; 3-(4-hydroxycyclohexyl)-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione] was the major metabolite at each location (Rhodes 1980). In Alabama (Sung et al 1981), the half-life of hexazinone in a clay soil was four to six weeks, and in a loamy sand less than four weeks. In this case, metabolite B [3-cyclohexyl-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)dione] was the major degradation product and metabolite A [3-(4 hydroxy-cyclohexyl)-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione] was the secondary one. Hexazinone is degraded primarily by microorganisms in the soil with little degradation occurring under sterile or anaerobic conditions (Rhodes 1980).

The native lowbush blueberry (Vaccinium angustifolium) is tolerant to hexazinone at rates that give selective control of many weedy species associated with this crop. This blueberry is an important fruit crop of Maine and the Eastern Canadian provinces where commercial fields have been developed by management of wild stands originating from forests or abandoned farmland. Hexazinone is now widely used in all blueberry producing areas (Jensen, 1986; Jensen and Kimball 1985) with thousands of hectares treated annually. The following study examines the fate of this herbicide in several typical soil types in the field and under laboratory conditions.

MATERIALS AND METHODS

Four soil types were used in these studies (Table 1). The three

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loam sites were typical commercial fields that had had a vegetation and litter cover for many years hence the higher organic matter content and cation exchange capacity (C.E.C.) in the upper 15 cm. The Somerset sand site was a cultivated field used for select clone blueberry plantings with a largely bare surface. The plot areas were burn-pruned prior to treatment as is the recommended practice. Plots measured 2x10 m and treatments were replicated 4X at each site.

Table 1. Characteristics of the four soils used in these studies.

Soil	Depth (cm)	Composition (%) Organic Matter	Sand	Silt	Clay	C.E.C. (meq/100 g)
Tormentine sandy loam	0-5	10.2	54	13	33	18.91
	5-15	5.1	55	14	31	10.82
	15-30	2.8	56	16	28	8.86
Pugwash sandy loam	0-5	8.7	55	10	35	15.30
	5-15	4.9	60	11	29	10.73
	15-30	2.2	63	12	25	7.31
Somerset sand	0-5	2.0	78	7	15	5.62
	5-15	1.8	82	7	11	5.25
	15-30	1.8	84	8	8	5.33
Shulie silt loam*	0-5	21.2	40	50	10	30.36

*Only 0-5cm soil was sampled

In one test (Table 2) repeated at three locations, 2 and 4 kg/ha (active) hexazinone (Velpar 90% SP) was applied on either Nov 23, 1980 or May 2, 1981 and soils were sampled on May 2 (fall treatments only), July 6, Dec 3, 1981 and April 28, 1982. Because of roots and stones on the Pugwash and Tormentine sandy loam sites, plots were sampled by excavating three random 25x25 cm areas on each plot at each date to 0-5, 5-15, 15-25, 25-35 and 35-45 cm. Because of surface litter, plots at these sites were not sampled on day of application. Instead, levels on that date were calculated from residues extracted from 10 filter paper disks that had been placed on the plots during treatment and the weight of the screened (2.0 mm mesh) soil in the 0-5 cm zone. This would overestimate levels on day of application (day 0). At the Somerset site, 15 2.5 cm cores were taken of the same depths on each plot except on day 0 which was only sampled to 5 cm. Roots, stones and litter were discarded and only soil passing a 2.0 mm screen was saved for analysis. These samples were air-dried and stored at -15°C until analysis.

In this trial, hexazinone was extracted by shaking 30 g soil with 60 mL [80:20 (v/v)] methanol:water for 1 h. This extract (40 ml) was diluted with 100 mL water and partitioned 3X with 50 mL chloroform.

The combined chloroform fractions were flash evaporated and the residues dissolved in ethyl acetate:hexane which could be injected directly without further clean-up. Recoveries of 84% were obtained from soils fortified with 0.25 ppm hexazinone. Residue analysis employed a Tracor Model 220 gas-chromatograph equipped with a N/P detector. The column (60 cm x 2.0 mm id) was packed with 15% OV17 on 80/100 Gas Chrom Q and operated at 260°C with the inlet at 270°C. The carrier gas was helium 20 cm^3/min, hydrogen 1.5 cm^3/min, and air 120 cm^3/min. Under these parameters, the retention time for hexazinone was 108 sec, and at attenuation 10x1, 2 µg hexazinone gave 25% FSD. Only data from plots treated with 4.0 kg/ha hexazinone is presented here. Holt's (1981) method of determining hexazinone metabolites involving derivatization with trifluoroacetic anhydride was also attempted on these samples but because of low levels, only metabolite B could be consistently detected in all samples (metabolite data not presented).

A second series of plots, established in three newly burned commercial fields, was treated May 10, 1984 with 2.0 kg/ha hexazinone and sampled on day of application, July 19 and Nov 20 by taking 24 2.5 cm cores 10 cm deep on each plot. In this case surface litter was removed during seiving of samples resulting in a lower level of hexazinone extracted on day 0 (Table 3). Holt's (1981) method was used for extraction of metabolites A, B, C and D. After flash evaporation and liquid paritioning, the samples were concentrated to dryness, made up to volume with hexane and injected into the GC without derivatization (C. McIntosh, pers. comm). This method proved superior to Holt's earlier one for detecting metabolites in these soils. Recoveries of 76, 83 and 80% were obtained from soils fortified with 0.5 ppm metabolite A, B and C and 72% for D fortified at 1.0 ppm.

Residue analysis employed a Tracor Model 220 gas-chromatograph equipped with a N/P detector. The column (60 cm x 2.0 mm id) was packed with 10% SP2250 on 80/100 Gas Chrom Q and operated at 263°C with the inlet at 270°C. The carrier gas was helium at 50 cm^3/min, hydrogen 1.5 cm^3/min, and air 120 cm^3/min. Under these parameters, retention times were 1.7, 2.0 and 3.8 min respectively for hexazinone, metabolite B, and metabolite A. With a column temperature of 225°C the retention time for metabolite D was 1.4 min. Metabolite E came off at 1.6 min at 230°C. At an attenuation of 10x1, 2 ng hexazinone, metabolite A and B gave 80, 55 and 45% FSD. Metabolite D and E were less sensitive requiring 10 ng and 20 ng to give 30 and 40% FSD, respectively.

Metabolite C was determined using a (60 x 2.0 mm id) column packed with 1.5% OV17/1.95% OV210 on 80/100 Gas Chrom Q at 245°C with the inlet at 255°C and carrier gas of 50 cm^3/min. Retention time of metabolite C was 1.7 min with 2 ng giving 55% FSD.

To study the degradation of hexazinone under laboratory conditions, 50 g samples of the Tormentine sandy loam and the Somerset sand soils containing 5.0 ppm hexazinone were amended with 0.5 µCi ring-labelled hexazinone (98% pure, 1 mg = 7.11 µCi), then placed in

flasks and brought to 75% moisture. One series of soils (non-sterile) was incubated at 24°C for 0, 2, 4, 8 and 16 weeks. Another series (sterile) was autoclaved for 30 min prior to incubation for 0, 8 and 16 weeks. After incubation, ^{14}C-labelled compounds in 5.0 g air-dried soil samples were extraced by shaking for 1 h with 80 mL methanol. The extract was centrifuged, filtered and concentrated to 0.2 mL by flash evaporation. Total recoverable ^{14}C-activity exceeded 99%. Concentrated extracts were banded and co-chromatographed with known unlabelled standards on silica gel TLC plates with fluorescence indicator. The plates were developed first to 7.5 cm in chloroform:methanol (9:1), dried, and then to 15.0 cm in ethyl acetate:methanol (9:1). Autoradiographs of developed plates were used to locate and identify ^{14}C-compounds after 80 d exposure. Unlabelled standards were visualized by spraying plates with 0.1 N silver nitrate in water:acetone (50:50 v/v). Under UV light, hexazinone and metabolite A appear as pink spots; B, D and E appear whitish immediately after spraying, while metabolite C appears as a dark spot. R_f values for metabolites C, A, B, hexazinone, E and D in this sytem were 0.26, 0.35, 0.49, 0.56, 0.66 and 0.78, respectively. Areas of the plates corresponding to ^{14}C-activity were removed and assayed by liquid scintillation counting techniques.

RESULTS AND DISCUSSION

Hexazinone rapidly dissipates from blueberry field soils. On May 2, only 8.6 to 20.7% of the calculated hexazinone was recovered from samples of the upper 45 cm of soils treated the previous November (Table 2). Only 6.2 to 8.5% of the May application was recovered (0-45 cm) from samples taken July 6. Loss of hexazinone was greater from the essentially bare Somerset sand than from the sandy loams in native stands. In the U. S., half lives of one to several months have been reported (Sung et al 1981, Rhodes 1980), and it appears that persistence in Nova Scotia is similar. Additional residues not accounted for by this sampling technique would be those found below 45 cm, those in the surface litter and trash, and those absorbed by vegetation.

The major pathways of degradation of hexazinone in field soils involves demethylation of the N-alkyl sidechain and hydroxylation of the cyclohexyl group (Rhodes 1980). Rhodes (1980) has reported that metabolite C, a product of both demethylation and hydroxylation, was the major metabolite in soils from Delaware, Mississippi and Illinois. However, others (Sung et al 1981; Neary et al 1983) report that metabolite A, the 4-hydroxycyclohexyl derivative, and metabolite B, the N-demethylated derivative, were the major soil metabolites. In the present study, metabolite B was detected in all depths in which hexazinone was detected (data not shown). In the second field test (Table 3), metabolite B was also the major metabolite 10 and 28 wk after application, and levels of metabolite C exceeded those of metabolite A. Metabolite D was detected at low levels in two of the three soils studied. Metabolite E was not detected in any sample. That metabolite B is the major metabolite is significant since this metabolite retains considerable herbicidal

Table 2. Levels of hexazinone in three soils following fall and spring applications of 4.0 kg/ha hexazinone.

Soil depth (cm)	Fall application (ppm)* Nov. 23 1980	May 2 1981	July 6 1981	Dec. 3 1981	Apr. 28 1982	Spring application (ppm)* May 2 1981	July 6 1981	Dec. 3 1981	Apr. 28 1982
					Tormentine Loam				
0-5	11.24‡	0.595	0.193	0.069	0.064	12.16‡	0.625	0.312	0.178
5-15		0.440	0.023	0.123	0.036		0.131	0.081	0.029
15-25		0.172	0.015	0.077	0.020		0.044	0.063	0.007
25-35		0.129	0.007	0.077	0.018		0.017	0.012	0.007
35-45		0.125	0.007	0.023	0.018		0.013	0.006	0.007
Total (% of applied)		(20.7)	(2.6)	(5.6)*	(2.2)		(8.5)	(5.2)	(2.2)
					Pugwash sandy loam				
0-5	12.26‡	0.705	0.082	0.067	0.042	10.96‡	0.514	0.422	0.310
5-15		0.400	0.041	0.020	0.028		0.051	0.049	0.061
15-25		0.181	0.030	0.015	0.013		0.018	0.027	0.026
25-35		0.100	0.017	0.013	0.017		0.012	0.010	0.017
35-45		0.072	0.017	<0.010	<0.010		0.005	0.004	0.015
Total (% of applied)		(18.0)	(2.4)	(1.5)	(1.4)		(6.2)	(5.5)	(5.0)
					Somerset sand				
0-5	8.62	0.060	<0.010	Tr	ND§	9.02	0.125	0.041	0.031
5-15		0.070	0.010	Tr	ND		0.097	0.028	0.016
15-25		0.080	0.020	<0.01	ND		0.081	0.019	<0.010
25-35		0.110	0.020	<0.01	ND		0.031	0.013	<0.010
35-45		0.090	0.020	<0.01	ND		0.019	<0.01	<0.010
Total (% of applied)		(8.6)	(1.8)	(Tr)			(6.5)	(1.8)	(1.3)

*Means of 4 replications; ‡Calculated; §ND = not detected

activity; metabolites A, C and D do not (Sung et al 1981).

Table 3. Levels of hexazinone and some of its metabolites in two soils (0-10 cm) following a May 9 application of 2.0 kg/ha hexazinone.

Sampling date	Soil type	Hexazinone* (ppm)‡	Metabolites (ppm)‡ A	B	C	D
July 19	Tormentine sandy loam	0.59	0.07	0.25	0.017	<0.01
	Pugwash sandy loam	0.70	0.07	0.23	0.016	ND
	Shulie sandy loam	0.69	0.09	0.28	0.017	<0.01
Nov. 20	Tormentine sandy loam	0.34	0.04	0.18	0.016	0.014
	Pugwash sandy loam	0.47	0.04	0.16	0.014	ND
	Shulie sandy loam	0.35	0.04	0.20	0.010	<0.01

*Levels of hexazinone on date of application was: Tormentine s.l. (1.43), Pugwash s.l. (1.47) and Shulie s.l. (1.76)
‡Means of 4 replications.

Incubation of the Tormentine sandy loam and the Somerset sand amended with ^{14}C-hexazinone indicated that under these conditions the trione, metabolite D, is a major metabolite in both non-sterile and autoclaved soils (Table 4). Significant amounts of metabolite D formed during autoclaving (Table 4) and this was confirmed by its formation in soils heated in a boiling water bath (data not shown). Rhodes (1980) reported only minor amounts of metabolite D (< 5%) extracted from field treated soils. He also concluded from lack of $^{14}CO_2$ evolution from soil incubation studies that little degradation occurs under sterile conditions. Our data suggests that the trione forms by chemical means and may be a significant metabolite under moist, warm conditions. Again in these studies, metabolite B exceeded both metabolites A and C. In addition to these metabolites, several unknowns, X and Y, were detected (Table 4). Low levels of most ^{14}C-products were detected after extracting the prepared sample (week 0) and low, unchanging levels may be considered artifacts of extraction or impurities in the ^{14}C-hexazinone sample.

Previously air-dried soils were used in these studies which may have affected the microflora of the soils, which in turn may have influenced the pathways of degradation. There was a loss of total extractable ^{14}C-activity from the non-sterile soil over the 16 wk incubation period reflecting cleavage of the s-triazine ring with significant differences between the two soils. A 10% loss of total ^{14}C-activity was detected in the sterilized Somerset sand. Plating out sterilized soils after incubation indicated all samples had remained sterile throughout the test.

Table 4. Distribution of hexazinone and its metabolites extracted from sterile and non-sterile soils incubated with ^{14}C-hexazinone over 16 weeks.

Compound	Percent of extracted ^{14}C-activity*							
	Non-sterile (weeks)					Sterile (weeks)		
	0	2	4	8	16	0§	8	16
				- Tormentine sandy loam -				
Hexazinone	96.4	83.9	80.0	60.8	61.7	81.4	52.5	50.4
A	0.6	1.6	1.1	1.0	2.7	1.0	0.9	1.1
X‡	0.6	6.9	7.3	5.3	5.5	0.8	0.5	0.5
B	1.0	4.0	3.8	6.2	5.8	1.2	2.7	2.6
C	0.3	0.7	1.8	2.8	1.7	0.2	0.7	0.7
D	0.6	3.0	5.2	12.8	19.3	12.8	40.7	42.5
E	1.0	0.9	1.4	0.6	0.8	7.6	1.6	1.3
Y	-	-	0.9	1.5	2.5	1.0	0.6	0.9
^{14}C-activity (% of applied)	(100.0)	(95.7)	(90.9)	(84.2)	(83.9)	(100.0)	(102.4)	(101.4)
				- Somerset sand -				
Hexazinone	95.1	88.0	79.1	59.3	52.5	81.2	51.6	49.0
A	0.8	0.5	1.3	2.5	2.7	0.8	0.8	0.8
X	0.3	2.6	8.0	16.6	15.4	0.5	0.5	0.5
B	1.1	3.6	4.9	8.3	10.3	1.4	2.2	2.3
C	0.5	0.5	0.6	2.8	4.5	0.4	0.7	0.8
D	0.6	2.5	4.0	7.4	10.2	13.7	42.4	44.7
E	1.2	1.6	1.4	1.2	1.2	1.4	1.2	1.1
Y	0.5	0.7	1.0	1.8	3.4	0.5	0.7	0.7
^{14}C-activity (% of applied)	(100.0)	(90.8)	(82.2)	(67.0)	(49.9)	(100.0)	(91.9)	(89.2)

*Means of 3 replicates; ‡Compounds X and Y where unknowns with R_f values of 0.43 and 0.71, respectively; §samples extracted after autoclaving.

Although residue levels were generally highest in the 0-5 cm zone, hexazinone readily leached to lower depths (Table 2). Because of its high water solubility, leaching is an important means of dissipation from the soil (Rahman 1981, Rhodes 1980). In the field we have observed that there is little or no herbicidal activity with hexazinone in ruts or depression were water accumulates. Patterns of weed control in the plot areas also suggests some lateral movement of hexazinone. Loss of hexazinone from forest watersheds has been monitored (Neary et al 1983). Blueberry fields burned prior to application would favor greater surface losses. Although weed control from hexazinone treatments often lasts several years, it appears this is a result of good initial kill of weedy vegetation, and not herbicide carry-over. Carry-over from year-to-year was <5%, and

accumulation of hexazinone from such residues from biennial applications would not be expected according to Smith's (1982) calculations.

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Received April 2, 1986; accepted July 29, 1986.