Fate of 2-Chloro-*s*-triazine Herbicides in Soil

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The degradation in soils of 2-chloro-4,6-bis-(ethylamino)-*s*-triazine (simazine), 2-chloro-4-ethylamino - 6 - isopropylamino - *s* - triazine (atrazine), and 2-chloro-4,6-bis(isopropylamino)-*s*-triazine (propazine), all randomly ring-labeled with C[14], was studied. Periodic methanol extractions of treated soils stored at 30° C. recovered decreasing amounts of radioactivity. Within a few weeks after treatment, hydroxy derivatives of the chloro-*s*-triazines were recovered and at eight weeks accounted for as much as 50% of the radioactivity in the methanol extracts. Additional amounts of the hydroxy derivatives were recovered by extraction of the same soil samples with 0.5*N* NaOH. Accumulation of the hydroxy derivatives indicated that the initial hydrolysis at the 2-position of the triazine ring occurred more readily than subsequent degradation steps. The hydroxy derivatives are nonphytotoxic, and their formation represents detoxication of these herbicides.

The 2-chloro-*s*-triazines are widely used as soil-applied herbicides, yet their fate in soil has not been clearly established. Soil microorganisms are capable of degrading the *s*-triazines (*10*), but the importance of microbial degradation in soil is not known. The microbial alteration of simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine] reported by Kaufman, Kearney, and Sheets (*10*) yielded a dealkylated product with the chloro-substituent intact. However, Harris (*8*) found that the 2-hydroxy derivative was the major degradation product occurring in soils. Couch *et al.* (*5*) reported a slow conversion of atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) to hydroxy-atrazine (2-hydroxy-4-ethylamino-6-isopropyl amino-*s*-triazine) by a soil fungus, *Fusarium roseum* (LK.) Snyder and Hansen. Armstrong and Harris (*1*) also reported that atrazine was degraded in soil to hydroxy-atrazine.

The purpose of this study was to follow the disappearance of simazine, atrazine, and propazine (2-chloro-4,6-isopropylamino-*s*-triazine) from soil under controlled conditions, to look at the effects of soil properties and temperature on disappearance, and to isolate and identify the degradation products.

Material and Methods

Soils. The soils used in this study were thoroughly mixed field samples taken from the top 6-inch layers. Chemical and physical properties of the soils are listed in Table I. All soils were passed through a 1/4-inch screen, and soils used in laboratory studies were passed through a 2-mm. screen.

Herbicides. Carbon-14 ring-labeled preparations of simazine, atrazine, and propazine were used in all laboratory experiments, with the exception of the use of chlorine-36–labeled simazine in one experiment. All greenhouse and field experiments were carried out with either technical grade or formulated wettable powder materials.

Tracer Studies

Experiment 1. Triplicate 20-gram samples of Sharkey clay, Lakeland sandy loam, Hagerstown silty clay loam, and Wehadkee silt loam were treated with 1 p.p.m. by weight of ring-labeled simazine C[14] (specific activity = 5 μc. per mg.) in the following manner: One half of each soil sample was spread about 1/4 inch deep on a filter paper and 40 μg. of simazine in 1 ml. of chloroform was pipetted one drop at a time over the soil surface. The treated soil was stirred and allowed to dry. After all traces of chloroform had disappeared, the treated soil from each sample was thoroughly mixed with the remainder of the sample and placed in a 30-ml. beaker. All soils were then supplied with enough water to bring them to field capacity and placed in an incubator maintained at 30° ± 2° C. A damp towel was kept over the beakers—not in contact with the soil—and changed daily. All samples were rewatered to field capacity by weight twice each week.

Sets of samples were removed periodically and extracted with 100 ml. of methanol on a Soxhlet extractor for 2 hours. Recovered radioactivity was determined by plating aliquots of the methanol extracts on aluminum planchets and counting them on a gas flow proportional counter. Per cent recovery was calculated by comparing the counts per minute of radioactivity in the sample extracts with counts per minute for the original treatment solutions determined in the same counting system. All counts were corrected for background. In some cases the soil samples that had been extracted with methanol were extracted a second time with 25 ml. of 0.5*N* NaOH for 2 hours on a reciprocating shaker. Radioactivity in the NaOH extracts was determined in the same manner as for the methanol extracts.

The NaOH extracts required considerable cleanup before they could be successfully chromatographed. The extracts were acidified to pH 4.0, and the precipitated material was removed by centrifugation. The pH was then adjusted to 7.0 and the additional precipitate

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Table I. Chemical and Physical Properties of Soils[a]

Soil Type	pH	Organic Matter, %	Clay, 0.002 Mm., %	Cation Exchange Capacity, Meq./100 G.	Simazine Adsorption, %
Chillum silt loam	4.6	4.4	22.1	7.6	34
Hagerstown silty clay loam	5.5	4.3	30.0	12.5	25
Lakeland sandy loam	6.2	3.3	10.5	2.9	12
Sharkey clay	6.2	3.9	67.1	40.2	54
Wehadkee silt loam	5.6	1.9	25.2	10.2	20

[a] See (9) for description of methods used.

removed by centrifugation. The radioactive triazines were adsorbed on activated charcoal from the neutral aqueous solutions, and the charcoal was collected on filter paper in a suction filter assembly. The radioactive materials were then eluted from the charcoal with hot methanol and concentrated for chromatography.

Experiment 2. This experiment was set up similar to experiment 1 with ring-labeled simazine, atrazine, and propazine with specific activities of 4.0, 4.0, and 2.0 μc. per mg., respectively. Duplicate samples of Lakeland sandy loam, Hagerstown silty clay loam, and Wehadkee silt loam soils were used.

Chromatography. Alterations in the chloro-s-triazine herbicides were determined by paper and thin-layer chromatography. The soil extracts were concentrated and the radioactive components resolved in the chromatographic systems listed in Table II. Quantitative distribution of the radioactivity on paper chromatograms was determined by counting the different compounds either in a 4π strip scanner and comparing areas under the various peaks or with a gas flow hand counter and comparing corrected counts per minute. Radioactive compounds on the thin-layer plates were detected by preparing radioautographs on no-screen x-ray film. Carbon-14 hydroxy analogs for standards were prepared by hydrolysis of the carbon-14 chloro-s-triazines

in equal volumes of 6N HCl and 95% ethanol at 50° C. for 8 hours.

Bioassay Studies. Soils were treated with herbicides in 3-inch plastic pots and inactivation of the herbicides was determined by oat bioassays. After the desired treatment, 13 oat seeds (var. Markton) were planted per pot and fresh weights of the tops determined after two to three weeks.

Results of Tracer Studies

Experiment 1. The recoveries of radioactivity from simazine C^{14}-treated soils by methanol extraction are shown in Figure 1. The recoveries decreased rapidly until 8 to 12 weeks after treatment and showed a gradual decline from 12 to 32 weeks.

The 32-week samples were re-extracted with 0.5N NaOH, and both the methanol and NaOH extracts were chromatographed in systems A and B (see Table II). Comparison of R_f values of authentic and extracted samples suggested that most of the labeled s-triazine in soils was present as either simazine or hydroxysimazine. The approximate distribution in the methanol extracts was as follows: Hagerstown silty clay loam, 90% hydroxysimazine; Lakeland sandy loam, 20%

Table II. R_f Values for Chloro- and Hydroxy-s-triazines

Compound	Chromatographic Systems[a] A	B	C	D
Simazine	0.98	0.98	0.55	0.92
Hydroxysimazine	0.41	0.08	0.86	0.68
Atrazine	0.98	0.98	0.40	...
Hydroxyatrazine	0.45	0.13	0.88	...
Propazine	0.99	0.98	0.24	...
Hydroxypropazine	0.51	0.20	0.90	...

[a] The chromatographic systems were as follows:
A Benzene–acetic acid–water, 10:10:1, v./v./v. on silica gel G TLC.
B Ethyl acetate–chloroform–acetic acid, 3:4:3, v./v./v. on silica gel G TLC.
C 30% acetic acid on kerosine-treated Whatman No. 1 paper (descending).
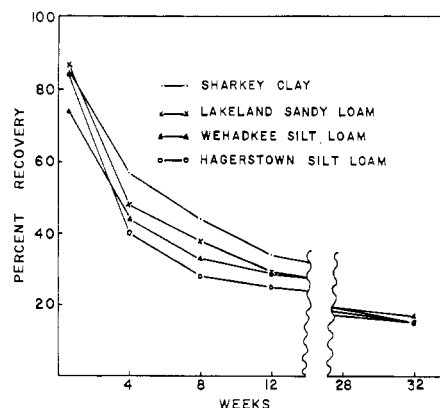D Isoamyl alcohol saturated with 0.3N HCl on Whatman No. 1 paper (descending).

Figure 1. Recoveries of radioactivity by methanol extraction from soils incubated at 30° C. after treatment with simazine C^{14}

Average of three replications

simazine and 70% hydroxysimazine; Sharkey clay, 70% simazine and 15% hydroxysimazine; Wehadkee silt loam, 49% simazine and 46% hydroxysimazine. All of the radioactivity in the NaOH extracts chromatographed with hydroxysimazine and accounted for 10 to 15% of the initial application of simazine. Recoveries of simazine were less than 10% of the initial application for all soils and less than 2% for the Hagerstown silty clay loam. The most important degradation product observed appeared to be hydroxysimazine.

Soils were selected for their range in adsorption of simazine (Table I) to determine if adsorption might decrease the rate of simazine degradation. Sharkey clay adsorbed the most simazine and gave the highest recoveries of total radioactivity up to 12 weeks. The 32-week extraction of Sharkey showed no more total radioactivity than the other soils but the amount of simazine recovered was greater. These results suggest that the adsorption in the Sharkey clay might have protected the simazine against degradation. However, degradation was most rapid in the second most adsorptive soil, Hagerstown silty clay loam. This might be explained by the higher montmorillonite content in the Sharkey clay and the higher organic matter content in the Hagerstown silty clay loam. Simazine adsorbed on clay may be protected more against degradation than simazine adsorbed on organic matter. Also, the degradation process may be closely associated with the organic matter and proceed faster in soils with a high organic matter content.

The rate of conversion of simazine to hydroxysimazine was enhanced by increasing the temperature. Faster conversion made some short-term studies possible. Further evidence for the identification of the degradation product as hydroxysimazine was obtained in a comparison of recoveries of simazine labeled with Cl^{36} and C^{14} (Table III). The soils were treated in an identical manner and aliquots of the methanol extracts were dried on aluminum planchets and counted on a gas flow proportional counter. Since the samples were the same except for the radioactive label, the amount of simazine recovered should have been the same. The much greater recovery of Cl^{36} than C^{14} was assumed to reflect Cl^{36} that had been displaced from the simazine molecule.

Results in Table IV indicate that organic matter may be an important factor in the degradation of simazine. Organic matter was removed from the soil by making repeated additions of hydrogen peroxide. Although the methanol extracts were not chromatographed and further extractions of the soils were not attempted, the author assumed that the greater recovery of radioactivity from the soil without organic matter represented less inactivation.

Experiment 2. The recoveries of radioactivity from the triazine C^{14}-treated soils are shown in Figure 2. Results for the different soils did not vary greatly, and the data reported are averages for all soils. Recovery was greatest from the propazine- and least from the simazine-treated soils. Recoveries diminished with time in all cases. The 8-week samples were re-extracted with 0.5N NaOH and both the methanol and NaOH

Table III. Methanol Extractable Radioactivity from Soils 5 Days after Treatment with Labeled Simazine and Incubation at 60° C.

Soil Type	Radioactivity Recovered, % of Total Added	
	C^{14}	Cl^{36}
Lakeland sandy loam	31	79
Chillum silt loam	21	71
Wehadkee silt loam	19	69

Table IV. Methanol-Extractable Radioactivity from Simazine C^{14}-Treated Soil after 1 Week of Incubation at 25° and 60° ± 2° C.

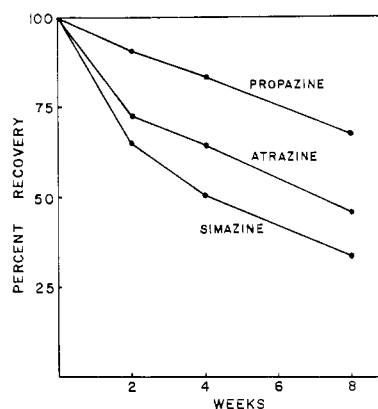| Treated Media	Radioactivity Recovered, % of Total Added	
	25° C.	60° C.
Ottawa sand	97	90
Wehadkee silt loam minus organic matter	79	54
Wehadkee silt loam	71	19

Figure 2. Recoveries of radioactivity by methanol extraction from soils incubated at 30° C. after treatment with C^{14} propazine, atrazine, and simazine

Average of duplicate samples of four soils

extracts chromatographed on systems A, C, and D. More than 40% of the atrazine and propazine appeared to have been converted to the hydroxy forms in Wehadkee silt loam, and more than 25% conversion occurred for all triazines and soils studied (Table V).

In a follow-up study, a microbial inhibitor, sodium azide, in soil at 200 p.p.m. by weight did not appreciably affect the accumulation of the hydroxy derivatives of simazine, atrazine, and propazine during an 8-week incubation at 30° ± 2° C. as compared with similar samples without azide.

Table V. Recovery of 2-Hydroxy-s-triazines from Soil after 8 Weeks at 30° C.

| Sample	Amount Recovered, % of Initial Total Triazine		
	Methanol extract	NaOH extract	Total
ATRAZINE			
Hagerstown silty clay loam	26	14	40
Lakeland sandy loam	23	15	38
Wehadkee silt loam	22	25	47
PROPAZINE			
Hagerstown silty clay loam	26	13	39
Lakeland sandy loam	20	14	34
Wehadkee silt loam	28	17	45
SIMAZINE			
Hagerstown silty clay loam	10	19	29
Lakeland sandy loam	11	24	35
Wehadkee silt loam	Trace	28	28
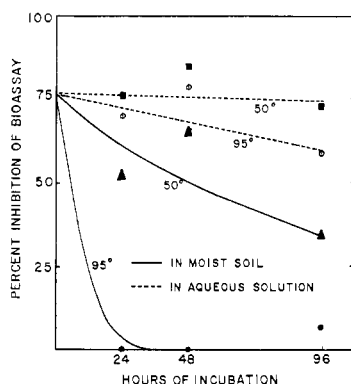
Figure 3. Effect of time, temperature, and contact with soil on the alteration of atrazine as indicated by inhibition of growth of oat seedlings

Average of three replications

Bioassay Studies. Higher temperatures and contact with soil greatly accelerated the detoxication of atrazine (Figure 3). The atrazine treatments in all cases consisted of 0.15 p.p.m. by weight of soil added in enough water to bring the soil to field capacity in plastic pots. The pots were covered with plastic to prevent vapor losses during incubation at high temperatures. Aqueous atrazine solutions in covered flasks were also incubated at high temperatures and then added to pots of soil for bioassay. Atrazine detoxication in soil occurred to such an extent that oats were not injured following 24 hours at 95° C., and injury was reduced by more than 50% following 96 hours at 50° C. Only slight detoxication of atrazine in aqueous solution occurred in the absence of soil at either 50° or 95° C. Additional treatments at 5° and 25° C. showed no change in atrazine effectiveness after 96 hours incubation as compared with 24 hours.

A number of s-triazines and monuron [3-(p-chlorophenyl)-1,1-dimethylurea] were rapidly detoxified in soil incubated at 60° C. (Table VI). Monuron and prometone [2-methoxy-4,6-bis(isopropylamino)-s-triazine] were not detoxified as readily as prometryne [2,4 - bis(isopropylamino) - 6 - methylmercapto - s-triazine] and the chloro-s-triazines by the 60° C. treatment. The treated soils were incubated in plastic pots covered with plastic to prevent vapor losses.

Field Studies. Soil samples from atrazine-treated field plots of Wehadkee silt loam at Beltsville, Md., were analyzed for hydroxyatrazine (see Table I for soil properties). The samples were collected 5 months after treatment with atrazine at the rate of 30 pounds per acre. The samples had been stored at −5° C. until extraction. Methanol extracts were cleaned up on filter paper strips with chloroform and then with isoamyl alcohol saturated with 0.1N HCl. The material from the paper strips where hydroxyatrazine would be expected was eluted, concentrated, spotted on a silica gel G thin-layer plate, and developed in benzene–acetic acid–water, 10:10:1, v./v./v. Silica gel from the hydroxyatrazine spot on the thin-layer plate was washed with 0.01N HCl in methanol. The solution was made up to volume with methanol and scanned on an ultraviolet spectrophotometer (Figure 4). Comparison of the spectrum from the soil extract with the spectrum from authentic hydroxyatrazine shows the presence of

Table VI. Fresh Weights of Oats (Grams) Planted in Treated Soil after a 5-Day Incubation (Average of three replications)

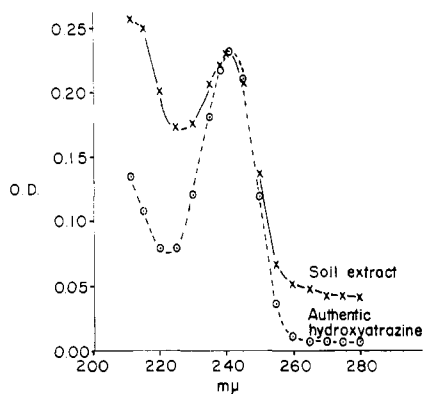| Soil	Incubation Temp., °C.	Herbicide					
		Atrazine	Propazine	Simazine	Prometone	Prometryne	Monuron
Lakeland	29	0.7	0.7	0.3	0.3	1.7	0.6
	60	3.3	3.2	3.3	0.4	2.4	2.1
Chillum	29	0.4	0.7	0.6	0.6	0.7	0.6
	60	2.9	3.0	2.5	1.3	3.5	2.0
Wehadkee	29	0.5	0.7	0.6	0.9	1.0	1.0
	60	3.7	3.6	3.3	1.6	3.5	2.3

Figure 4. Ultraviolet spectra comparison for authentic hydroxyatrazine and a purified soil extract

hydroxyatrazine in the soil. The material absorbing at 240 mμ was equivalent to approximately 10% of the original application of atrazine.

Discussion

The s-triazine recovery data in this study when compared with CO_2 evolution data of Kaufman, Kearney, and Sheets (10) and MacRae and Alexander (12) suggest that the chloro-s-triazines are either complexing with some soil constituent or are undergoing alteration to compounds that are more difficult to extract. Kaufman, Kearney, and Sheets (10) reported only 3 to 4% degradation of simazine C^{14} in soil in 30 days based on $C^{14}O_2$ evolution. MacRae and Alexander (12) found very little simazine, atrazine, or propazine C^{14} went to $C^{14}O_2$ in 16 weeks. The rapid reduction in methanol-extractable simazine C^{14} in the present study suggested that some alteration or complexing of the chloro-s-triazines must be occurring that does not involve conversion to CO_2. The greater recovery of Cl^{36} than of C^{14} from soil is evidence that the chlorine in the 2-position is being displaced. This would occur with either simple hydrolysis to the 2-hydroxy form or in the case of complex formation at the 2-position. The 2-hydroxy forms were evident in the methanol extracts in most cases, but the additional amounts that were recovered by 0.5N NaOH suggested that a complex was involved. However, samples of Wehadkee silt loam were treated with radioactive hydroxysimazine and after 3 days only 23% of the added radioactivity was recovered in a 2-hour Soxhlet extraction with methanol. Therefore, the additional recovery of hydroxytriazine from the test samples with NaOH does not necessarily indicate that complex formation is involved, because hydroxysimazine is not easily recovered by methanol extraction.

Kaufman, Kearney, and Sheets (10) reported rapid degradation in pure cultures of *Aspergillus fumigatus* Fres. to a product that was later identified (11) as 2-chloro-4-amino-6-ethylamino-s-triazine. They noted

that the chlorine in the 2-position remained intact. This product was not observed in soil in the present study and the 2-hydroxy derivatives appeared to be major products. Couch *et al.* (5) reported microbial conversion of atrazine to hydroxyatrazine. However, in the present study formation of the 2-hydroxy derivatives in soil was not inhibited by 200 p.p.m. sodium azide, and atrazine was rapidly detoxified in soil at 95° C. but was only slowly altered in aqueous solution at the same temperature. Both of these findings suggest that some soil constituent is catalyzing a nonbiological hydrolysis at the 2-position. Ercegovich and Frear (6) found a similar effect of temperature on the loss of amitrole (3-amino-1,2,4-triazole) in soil. Monuron also may be subject to chemical inactivation. That the rapid detoxification observed at 60° C. could be attributed to soil microorganisms is doubtful. Other workers have observed increased rates of inactivation of the s-triazines at higher temperatures (3, 4, 13). However, these studies were conducted mostly within the normal range of active microbial populations, and they concluded that the increased rate of inactivation was related to greater microbial activity.

These results implicate a number of factors as affecting the rate of chloro-s-triazine inactivation. High content of adsorptive clays, which has been shown to reduce the initial effectiveness of simazine (9), appeared also to protect the simazine from degradation and prolonged its persistence. Gast (7) observed a similar phenomenon with activated carbon. Further evidence was obtained for the more rapid inactivation at higher temperatures. Soil organic matter appears to be important in triazine degradation. This has been cited as evidence for microbial causes of inactivation, since microbial activity generally increases with increasing organic matter content (4). However, the organic matter itself may be a catalytic agent in the hydrolysis of the 2-chloro-s-triazines. The effect of moisture on s-triazine inactivation was not studied, but neither chemical nor biological inactivation would be likely to occur readily in dry soil. Birk and Roadhouse (2) recovered 96% as much atrazine from air-dry soil samples 55 days after treatment as they did from similar samples right after treatment.

The 2-hydroxy-s-triazines appear to be important degradation products of the 2-chloro-s-triazines. Since they are nonphytotoxic, their formation represents a detoxication mechanism. The importance of dealkylation of the s-triazines in soil was not ascertained, but the lability of the hydroxy derivatives to dealkylation should be examined.

Acknowledgment

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