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9. ABSTRACT

Soil-applied pesticides transported by erosion from the site of application to waters and sediments are subject to conditions sufficiently different from those of aerated soils to alter pesticide persistence. Microbial degradation of ^{14}C ring-labeled 2-choro-4-ethylamino-6-isopropylamino-s-triazine (atrazine) and its 2-hydroxy analog (hydroxyatrazine) in submerged soils was studied by measurement of $^{14}CO_2$ evolution and chromatographic separation of metabolites in soil extracts. In 90 days, 1.67% of hydroxyatrazine-^{14}C was evolved as $^{14}CO_2$, whereas only 0.005% of atrazine-^{14}C was recovered as $^{14}CO_2$. With an energy source added to the soil, 3.8% of hydroxyatrazine-^{14}C was recovered as $^{14}CO_2$ in 60 days. Chemical hydrolysis of atrazine to hydroxyatrazine is the principal pathway of detoxication in soil and is conducive to ring cleavage and total breakdown by microbes. Biological dealkylation without dehalogination occurs simultaneously; 2-chlor-4-amino-6-isopropylamino-s-traizine was identified by TLC in the extracts from atrazine-treated soil. Ring-labeled atrazine or hydroxyatrazine in soil did not evolve $^{14}CO_2$ or $^{14}CH_4$ gas when the system was subjected to an anaerobic condition. Total breakdown of atrazine is expected to be slower in submerged sediments than in aerated soils because of the higher pH and restricted aeration of sediments.

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Microbial Degradation of the Herbicide Atrazine and Its 2-Hydroxy Analog in Submerged Soils

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■ Soil-applied pesticides transported by erosion from the site of application to waters and sediments are subject to conditions sufficiently different from those of aerated soils to alter pesticide persistence. Microbial degradation of ^{14}C ring-labeled 2-choro-4-ethylamino-6-isopropylamino-*s*-triazine (atrazine) and its 2-hydroxy analog (hydroxyatrazine) in submerged soils was studied by measurement of $^{14}CO_2$ evolution and chromatographic separation of metabolites in soil extracts. In 90 days, 1.67% of hydroxyatrazine-^{14}C was evolved as $^{14}CO_2$, whereas only 0.005% of atrazine-^{14}C was recovered as $^{14}CO_2$. With an energy source added to the soil, 3.8% of hydroxyatrazine-^{14}C was recovered as $^{14}CO_2$ in 60 days. Chemical hydrolysis of atrazine to hydroxyatrazine is the principal pathway of detoxication in soil and is conducive to ring cleavage and total breakdown by microbes. Biological dealkylation without dehalogination occurs simultaneously; 2-chloro-4-amino-6-isopropylamino-*s*-triazine was identified by TLC in the extracts from atrazine-treated soil. Ring-labeled atrazine or hydroxyatrazine in soil did not evolve $^{14}CO_2$ or $^{14}CH_4$ gas when the system was subjected to an anaerobic condition. Total breakdown of atrazine is expected to be slower in submerged sediments than in aerated soils because of the higher pH and restricted aeration of sediments.

Pesticides are used increasingly in both agricultural and urban areas. The ultimate fate of these materials is important to the maintenance of an environment which is largely free of toxic chemicals, especially those displaced from the site of their original application. Transport of soil-applied pesticides to streams, ponds, lakes, or shoreline ocean waters can be accomplished by vertical movement through the soil profile and subsurface strata to groundwaters; however, surface transport by runoff water and eroded soil is more likely in most cases. Eroded soils containing pesticides become sediments in wet, low-lying areas or in bodies of water so that any further transformation of the transported pesticide must occur under conditions of limited aeration. Although chemical reactions should not be affected much by the degree of aeration, pesticide transformations by soil microflora may be quite different under aerated and submerged conditions. Guenzi and Beard (1967) found that normally persistent DDT was converted to DDD relatively fast in an anaerobic environment. Raghu and MacRae (1966) and MacRae et al. (1967) showed that benzene hexachloride, which was relatively resistant to microbial decomposition in aerated soils, was readily degraded under flooded conditions. These studies emphasize the need for assessing the importance of microbial degradation of pesticides with limited aeration—i.e., under conditions corresponding to the environment of submerged sediments.

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) is the most widely used chemical for pre-emergence weed control in corn. In Hawaii, it is important to the culture of sugarcane, pineapple, and macadamia nut. Soil-applied atrazine is subject to displacement from the site of application and subsequent deposition in either fresh- or saltwaters; such transport occurs most likely soon after application when fields are bare, thus more susceptible to runoff and erosion.

Various pathways of chloro-*s*-triazine detoxication in plants, soils, and microbial cultures have been reported in the literature. Nonenzymatic hydrolysis is an important initial degradation step in higher plants (Hamilton and Moreland, 1962; Roth and Knusli, 1961; Castelfranco et al., 1961). The fungus *Aspergillus fumigatus* was found capable of causing dealkylation of 2-chloro-4,6-*bis*(ethylamino)-*s*-triazine (simazine) without intermediate hydroxylation in culture solutions (Kearney et al., 1965). In natural soils, however, detoxication of atrazine occurs principally by chemical hydrolysis at the 2-position (Armstrong et al., 1967; Harris, 1967; Skipper et al., 1967; Obien and Green, 1969). The role of soil microorganisms in degrading atrazine has been clarified considerably by Skipper et al. (1967). They measured $^{14}CO_2$ evolution from chain- and ring-labeled atrazine subjected to various microbial isolates, to determine the extent of dealkylation and ring cleavage. Degradation of the ethylamino chain was accomplished by both bacteria and fungi, but neither produced $^{14}CO_2$ by ring cleavage. Evolution of $^{14}CO_2$ from ring-labeled atrazine and hydroxyatrazine in nonsterile soils was also measured. A threefold greater rate of $^{14}CO_2$ production from ring-labeled hydroxyatrazine indicated that microbial destruction of the chloro-*s*-triazine ring is preceded by chemical hydrolysis. Thus, detoxication of atrazine in aerated soils may result from either chemical hydrolysis or microbial dealkylation, or both; but the rate of total decomposition of the *s*-triazine ring to CO_2 is considerably enhanced by prior chemical hydrolysis. A similar mechanism in plants was indicated by the rapid degradation of hydroxyatrazine to CO_2 by oats which could not degrade the parent atrazine (Montgomery and Freed, 1964).

The purpose of this investigation was to study microbial degradation of atrazine and its hydrolysis product, hydroxyatrazine, under limited aeration conditions similar to those prevailing in the sediments of lakes and bays where transported atrazine may reside. Determinations of hydroxyatrazine degradation under aerobic conditions and both atrazine and hydroxyatrazine degradation under anaerobic conditions (N_2 environment) were also made.

Experimental

Degradation in Submerged Soil. In separate preliminary experiments, the degradation of ring-labeled atrazine was measured in submerged Kapaa and Molokai soils. Soil properties are given in Table I. Atrazine, ring-labeled with ^{14}C was added in aqueous solution to 5.0 g soil to give 10 and 2 μg of atrazine per gram of soil for the Kapaa and Molokai soils, respectively. A 1.0-ml suspension of a bacterial culture inoculum (developed for 10 months on submerged atrazine-treated Molokai soil) was added at the initiation of the experiments. Sufficient water was applied to maintain the soil/water ratio at 1:2 (weight basis). Incubation was performed in triplicate

cate 50-ml centrifuge tubes, each of which was equipped for displacement and collection of $^{14}CO_2$. Control measurements were obtained on both soils.

The tubes were incubated at room temperature (24.5°C ± 1°) and the contents were shaken daily. Evolved $^{14}CO_2$ was periodically displaced and collected in 10-ml portions of hydroxide of hyamine 10-X (Packard Instruments Co.) by passing CO_2-free humidified air through each system separately at 70 cc/min for 30 min. A 1.0-ml aliquot of the hyamine solution was mixed with 15 ml of 7:3 toluene/ethanol scintillation solution, and ^{14}C activity was determined by liquid scintillation spectroscopy. The systems were kept closed between displacements. Separate tests revealed no ^{14}C activity in ethanol traps when dry and humidified air were passed at the above-mentioned rate for 4 hr over ^{14}C-labeled atrazine and hydroxyatrazine solutions or over soils treated with these solutions. Thus, volatilization losses were neligible.

In a second experiment, the degradation rates of ^{14}C ring-labeled atrazine (10 μg/g) and hydroxyatrazine (9.6 μg/g) were measured for 90 days in Molokai soil under the same conditions described above. The effects of additional nitrogen and carbon sources on triazine-ring cleavage by soil microorganisms were determined by additions of 25 mg sucrose at 40 days and 10 mg ammonium sulfate after 67 days of incubation.

Hydroxyatrazine degradation was studied further in a third experiment, in which both a carbon source (50 mg potato starch) and a nitrogen source (40 mg ammonium sulfate) were supplied initially to 10 g of Molokai soil, which was treated with 3 ppmw of hydroxyatrazine. The need for the additional energy source was indicated in the second experiment.

The products of degradation in the latter two experiments were separated and analyzed by thin-layer chromatography (TLC). At the end of incubation, the tubes were centrifuged at 16,000 rpm for 15 min, and the aqueous extracts were decanted. The soils were then extracted with 10 ml 0.05*N* calcium chloride in methanol by shaking for 2 hr. After centrifugation, the aqueous and methanolic extracts were mixed; 2.0-ml aliquots of the extracts were concentrated in the dark at 24.5°C ± 1°. The separation and measurement of the residues and degradation products of atrazine and hydroxyatrazine were done by TLC according to Obien and Green (1969). Nonlabeled standards of probable metabolites of atrazine were subjected to the same TLC evaluation as was used to separate metabolites from soil extracts. R_f values for nonlabeled chemicals were determined by iodine vapor staining. Solutions of ^{14}C-labeled atrazine and hydroxyatrazine were also analyzed by TLC to test the purity of these compounds as supplied by the manufacturer. The specific activities of atrazine and hydroxyatrazine utilized in these experiments were 6.1 μc/mg and 7.1 μc/mg, respectively. Both atrazine and hydroxyatrazine were uniformly labeled with ^{14}C in the triazine ring.

Degradation in Aerobic Soil. Hydroxyatrazine applied at 3 μg/g was incubated with Molokai soil maintained at a water content corresponding to 85% of "saturation." Evolution of $^{14}CO_2$ was measured in triplicate as described previously.

Degradation in Anaerobic Soil. Molokai soil samples (30 g) in 250-ml conical flasks were treated at 10 μg/g of either atrazine or hydroxyatrazine (^{14}C-ring labeled) and mixed with 80 ml of water. A culture inoculum was prepared from the sediment of a stagnant water pool in a marshy area. Twenty milliliters of the water suspension were mixed with the contents of the flask. Half of the flasks was flushed with N_2 gas for 30 min to displace air and create anaerobic conditions. Gases produced during the 90 days of incubation were collected by the displacement of saturated sodium chloride solution while maintaining a slight negative pressure (−18 cm water head) in the system. Collected gases were transferred into gas sampling tubes containing concentrated sulfuric acid and kept under slight pressure for 24 hr for removal of traces of water vapor. The gas samples were then analyzed by mass spectrography under high resolution for ^{14}C-methane and ^{14}C-carbon dioxide gas fractions.

Results and Discussion

Atrazine Degradation in Submerged Soils. Evolution of $^{14}CO_2$ from ring-labeled atrazine in Kapaa and Molokai soils is shown in Figure 1. In 30 days, Molokai soil evolved only 0.02% of the added atrazine, whereas $^{14}CO_2$ evolved from Kapaa soil, represented 0.59% of the input chemical. If the ring cleavage represented here by $^{14}CO_2$ evolution resulted from microbial attack on hydrolyzed atrazine, as indicated by Skipper et al. (1967), the results in Figure 1 may reflect faster hydrolysis of atrazine in Kapaa than in Molokai soil. Obien and Green (1969) found that 48% of atrazine was hydrolyzed in Molokai soil in 30 days, compared with 85% in Kapaa soil. The high organic matter content and high microbial population of the Kapaa soil (Table I) probably enhanced degradation of the hydrolyzed atrazine to CO_2 relative to that in Molokai soil. Although the soils were submerged, the CO_2 evolution shown in Figure 1 indicates the viability of organisms capable of metabolizing the triazine ring in the submerged environment of sediments.

Table I. Properties of Kapaa and Molokai Soils

Soil	Depth sampled, cm	pH	Organic matter, %	Total N, %	Total bacterial counts[a] (×10⁶/g soil)
Kapaa	0–10	4.7	10.5	0.38	31.83
Molokai	0–5	6.4	3.4	0.13	1.36

[a] Viable on sodium caseinate agar medium.

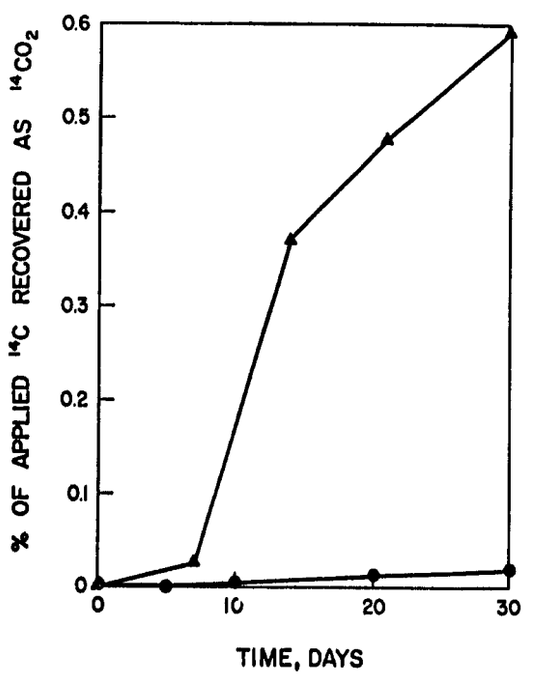


Figure 1. Cumulative ^{14}C recovered as $^{14}CO_2$ from atrazine in submerged soils

▲ Kapaa soil ● Molokai soil

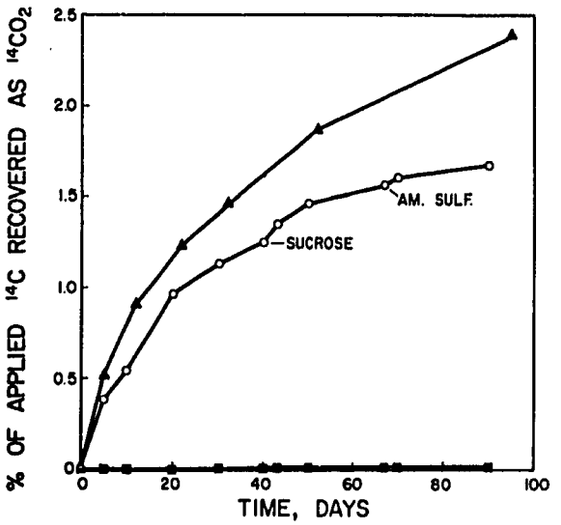


Figure 2. Cumulative ^{14}C recovered as $^{14}CO_2$ from atrazine and hydroxyatrazine in a submerged system and hydroxyatrazine in an aerobic system with Molokai soil

▲ Hydroxyatrazine (aerobic) ○ Hydroxyatrazine (submerged) ■ Atrazine (submerged)

Hydroxyatrazine vs. Atrazine Degradation. A comparison of degradation rates for atrazine and its 2-hydroxy analog under submerged conditions is indicated by relative $^{14}CO_2$ recovery curves (Figure 2). The upper curve in Figure 2 shows $^{14}CO_2$ evolution from hydroxyatrazine with increased aeration. Total destruction of the atrazine ring in Molokai soil was minimal—only 0.005% of the input ^{14}C being recovered as $^{14}CO_2$. Hydroxyatrazine, on the other hand, was degraded to $^{14}CO_2$ to a much greater extent—1.67%. Faster degradation of hydroxyatrazine resulted from more adequate aeration; a total of 2.38% of the applied ^{14}C was evolved as $^{14}CO_2$ in 95 days. Applications of sucrose (at 40 days) and ammonium sulfate (at 67 days) after initiation of the experiment enhanced hydroxyatrazine degradation (Figure 2, middle curve). When starch and ammonium sulfate were supplied at initiation of incubation in the third experiment, $^{14}CO_2$ evolution was greater than in any of the previous experiments; 3.8% of the applied ^{14}C was recovered as $^{14}CO_2$ within 60 days (Figure 3). This increased $^{14}CO_2$ evolution resulting from additional energy sources indicates a nonpreferential utilization of hydroxyatrazine by the soil microbes.

Percentages of ^{14}C recovery given here do not represent only detoxication of atrazine, but rather the extent of ring cleavage and subsequent breakdown. Three atoms of ^{14}C on the hydroxyatrazine ring constitute about 20% of the molecule that produced $^{14}CO_2$. Microbial degradation of the side chains of atrazine has been shown to be much more rapid than ring cleavage. McCormick and Hiltbold (1966) calculated a factor of 74 to 104 molecules of atrazine inactivated for each molecule of CO_2 derived from its ring.

The results in Figures 2 and 3 show that (*a*) under submerged conditions, microorganisms were capable of degrading the ring of hydroxyatrazine to a much greater extent than the atrazine ring; and (*b*) ring cleavage was accomplished principally by facultative aerobic and aerobic organisms.

The measurement of evolved gases from a system maintained in an anaerobic condition gave further evidence for aerobic metabolism. Mass spectrographic analyses revealed no $^{14}CH_4$ in gases produced during the incubation of hydroxyatrazine and atrazine under anaerobic conditions in Molokai soil. A small peak at 46 (mol. wt.) for $^{14}CO_2$ was recorded for gases produced in the hydroxyatrazine-treated system which was partially anaerobic. Nonlabeled methane and carbon dioxide were found in all gas samples collected from incubated soils, with or without triazine application. It is concluded, therefore, that atrazine and hydroxyatrazine were not metabolized by the anaerobic microflora.

Products of Microbial Degradation of Atrazine and Hydroxyatrazine. Figure 4 shows radiochromatographs of the extracts obtained from Molokai soil after incubation with atrazine and hydroxyatrazine for 90 days. The upper curve shows (*a*) a large residue of applied atrazine (R_f = 0.93); (*b*) a lesser amount of the 2-hydroxy analog produced by chemical hydrolysis (R_f = 0.13); and (*c*) a small quantity of product-B at R_f = 0.64. Table II contains R_f values obtained for the standard compounds and the extracts of soils incubated with atrazine and hydroxyatrazine. The metabolite designated product-B appears to be 2-chloro-4-amino-6-isopropylamino-*s*-triazine. This is probably the same metabolite which Obien and Green (1969) reported, with R_f = 0.70. Kearney et al. (1965) also reported production of a similar metabolite from simazine in culture solutions supporting the growth of *Aspergillus fumigatus*. This monodealkylated chloro-*s*-triazine has not been positively identified in soil previously.

The radiochromatograph for hydroxyatrazine-treated soil (Figure 4, lower graph) shows evidence of an unknown metabolite at R_f = 0.92. This peak appeared repeatedly in extracts of soils treated with hydroxyatrazine and did not appear in chromatographs of the added standard compound. The anticipated low mobility of dealkylated 2-hydroxy-*s*-triazines in the chloroform-acetone solvent would eliminate these compounds as possible metabolites responsible for the measured peak. Conjugation of some labeled breakdown product with lipoidal molecules might be responsible for the peak at 0.92. This peak would normally be masked by atrazine when atrazine breakdown is being studied.

It is now clear that alternative first steps in the degradation of atrazine are chemical hydroxylation at the 2-position and microbial dealkylation with chlorine intact in the 2-position. The former product appears to be the most susceptible to ring

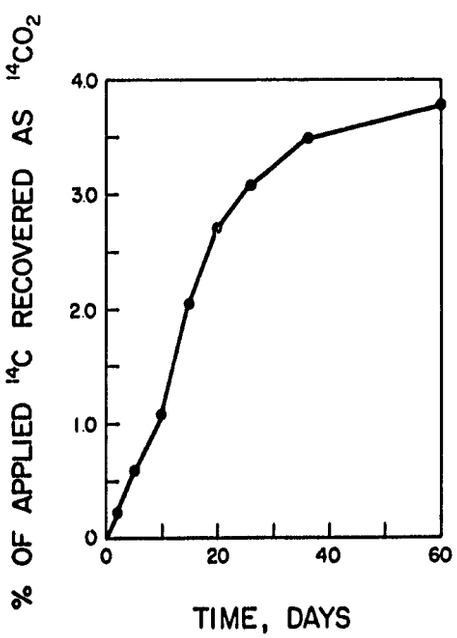


Figure 3. Cumulative ^{14}C recovered as $^{14}CO_2$ from hydroxyatrazine when starch and ammonium sulfate were added in submerged Molokai soil

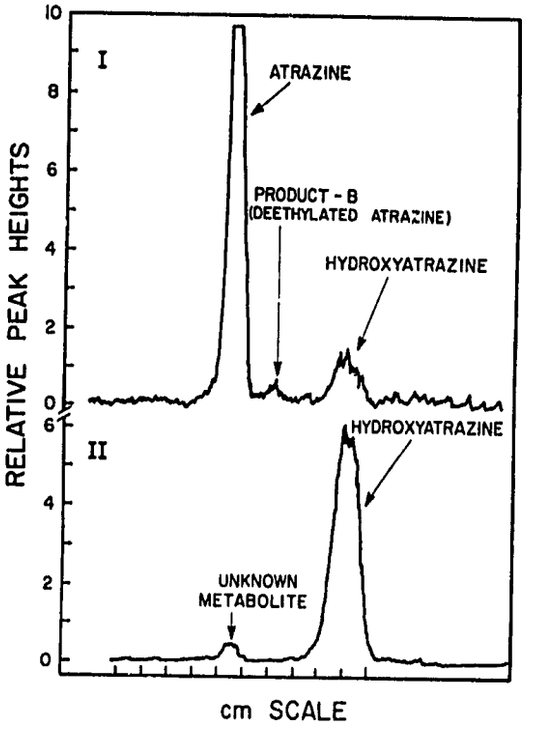
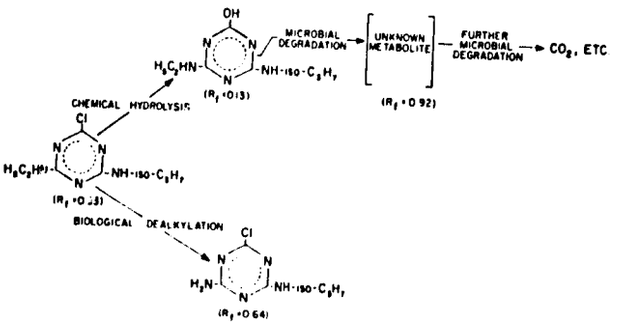


Figure 4. Radiochromatograph of TLC on water–methanol extract from Molokai soil 90 days after treatment with atrazine (I) and hydroxyatrazine (II)

cleavage by microbial attack. The two pathways, proposed in the following diagram, could operate simultaneously in soil.



The results obtained in submerged soil were consistent with those obtained previously by Skipper et al. (1967) with aerated soil. Recently completed research by Skipper (1970) confirmed his earlier work and, in addition, showed that the isopropyl side chain of atrazine was degraded more slowly than the ethyl group.

While chemical hydrolysis might occur as rapidly under anaerobic conditions as in an aerobic system at the same pH, anaerobic soil systems tend to have higher pH values. Patrick and Wyatt (1964) measured pH 6.7 on submerged samples of a silt loam soil from Louisiana used for rice production, whereas the pH of the same soil fell to 5.3 when the soil was held at a water content which was optimum for nitrification. We have measured pH values of 6.9 to 7.6 (average, 7.3) in several submerged marine sediments in Hawaii; terrestrial soils from which these sediments were eroded had pH values of 4.0 to 6.8 (average, 5.1). These observations indicate the likelihood of low rates of hydrolysis for atrazine transported to either fresh or marine waters. The pathway or detoxication most likely in sediments is that of dealkylation by facultative aerobic microorganisms. In sediments having limited aeration the rates of dealkylation of atrazine and ring cleavage of hydroxyatrazine are expected to be relatively slow. While the submerged environment enhances microbial degradation of DDT and BHC, the degradation of chloro-*s*-triazines will be retarded. The anticipated decrease in the rate of degradation of triazines in sediments does not suggest that these materials are detrimental to environmental quality. Simazine has been used successfully for control of algae and aquatic plants in fish ponds with no damage to fish (Walker, 1964). Thus, the chloro-*s*-triazine herbicides are not expected to be hazardous pollutants of shallow waters, but they are expected to persist longer in submerged sediments than in terrestrial soils.

Table II. R_f Values of Standard Compounds and Metabolites in Soil Extracts Measured by TLC in a 9:1 (v/v) Chloroform–Acetone Solvent Mixture

Compounds	R_f values
Standard compounds	
2-Chloro-4-amino-6-isopropylamino-*s*-triazine	0.66
Atrazine	0.93
Hydroxyatrazine	0.13
Soil extracts	
Product–B	0.64
Unknown metabolite	0.92
Atrazine	0.93
Hydroxyatrazine	0.13

Acknowledgment

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Literature Cited

Armstrong, D. E., Chesters, G., Harris, R. F., *Soil Sci. Soc. Amer. Proc.* **31**(1), 61–6 (1967).
Castelfranco, P., Foy, C. L., Deutsch, D. B., *Weeds* **9**(4), 580–91 (1961).
Guenzi, W. D., Beard, W. E., *Science* **156**(3778), 1116 and 17 (1967).
Hamilton, R. H., Moreland, D. E., *Science* **135**, 373 (1962).
Harris, C. I., *J. Agr. Food Chem.* **15**(1), 157–62 (1967).
Kearney, P. C., Kaufman, D. D., Sheets, T. J., *J. Agr. Food Chem.* **13**(4), 369–72 (1965).
MacRae, I. C., Raghu, K., Castro, T. F., *J. Agr. Food Chem.* **15**(5), 911–4 (1967).
McCormick, L. L., Hiltbold, A. E., *Weeds* **14**(1), 77–82 (1966).
Montgomery, M. L., Freed, V. H., *J. Agr. Food Chem.* **12**(1), 11–4 (1964).
Obien, S. R., Green, R. E., *Weed Sci.* **17**(4), 509–14 (1969).
Patrick, W. H., Wyatt, R., *Soil Sci. Soc. Amer. Proc.* **28**(5), 647–53 (1964).
Raghu, K., MacRae, I. C., *Science* **154**(3746), 263 and 4 (1966).
Roth, W., Knusli, E., *Experientia* **17**, 312 (1961).
Skipper, H. D., Gilmour, C. M., Furtick, W. R., *Soil Sci. Soc. Amer. Proc.* **31**(5), 653–6 (1967).
Skipper, H. D., Ph.D. thesis, Oregon State University, June 1970.
Walker, C. R., *Weeds* **12**(2), 134–9 (1964).

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