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Effect of Soil Moisture and Sample Depth on Pesticide Photolysis

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The effects of soil depth and moisture on pesticide photolysis were studied. Moist soil at depths of 3, 2.5, 2, 1.5, 1, and 0.5 mm were each dosed at 2.5 μg/g with ^{14}C-niclosamide and photolyzed under a xenon lamp at constant temperature. Samples were removed after 20, 40, 110, and 153 h of continuous irradiation. The decrease in percent of niclosamide and the appearance of degradates were followed by analyzing the soil extracts by HPLC. A corresponding set of experiments used air-dried soil. An experiment was also performed using initially moist soil which was permitted to dry during photolysis but returned to moist conditions at each sampling. Qualitative and quantitative differences were found in the rate and route of degradation of niclosamide under these conditions. These differences have resulted from a combination of reduced photochemical activity and microbial population in dry soil. The half-lives of niclosamide in the dry soils were 2 to 5 times longer than those in the moisture-maintained soil. There was also a noticeable difference in the half-lives in soil of different depths. Moisture-maintained soil showed a uniform linear increase in half-life from 95 to 195 h as soil depth increased from 0.5 mm to 3.0 mm. With air-dried soil the half-lives were greatly dependent on soil depth, showing a much broader range of 199 h at 0.5-mm to 1064 h in 3.0-mm soil. An experimental design is described which maintains soil temperature and moisture to preset conditions.

KEYWORDS: Soil photolysis; soil depth; moisture; half-life

INTRODUCTION

Chemicals such as insecticides and herbicides, when applied to soil, are very effective in controlling their intended targets. Once in contact with the environment, these chemicals themselves undergo changes brought on by microbes, moisture, and sunlight, which are integral components of the environment. Although each environmental factor has a unique effect on the chemical, collectively their effects can be more pronounced.

For example, moisture acts as a hydrolytic agent, whereas sunlight imparts photolytic energy upon the chemical. Yet hydrolysis in the absence of sunlight may yield different results, just as photolysis in the absence of moisture may yield very different results. On the other hand, the effect of microbes from the soil on a chemical is very dependent on the moisture content of the soil. Loss of moisture, or a dry soil, results in the decrease of microbial population (unpublished data). This decrease may have a dramatic effect on the transformation of the chemical.

Changes in environmental conditions can manifest different factors to degrade the chemical. Soil exposed to sunlight leads to the formation of various oxidants at the soil surface (*1*). Gohre and Miller (*2*) reported the generation of singlet oxygen on soil surface by trapping the singlet oxygen with 2,3-dimethyl-1-buten-3-ol and 2,5-dimethylfuran. Mabury and Crosby (*3*) reported the generation of hydroxyl radical in rice field water. These publications show that sunlight can generate reactive ion species under different environmental conditions. These reactive ions will react with pesticides to yield degradates.

Because water plays an extremely important role in the degradation of chemicals in aquatic environments, degradation of chemicals due to hydroxyl radical has received considerable attention (*4*−*8*). Soil also contains several other ions, including those from metals and nitrates which aid in the generation of hydroxyl radical (*5, 9*−*11*), which in turn reacts with the chemical. Therefore, if the soil is dry, there is far less possibility of generation of hydroxyl radicals. Also, if irradiation is conducted on soil that is allowed to dry between irradiations, there is less likelihood of generating hydroxyl radical. To understand the fate of a chemical in the environment it is essential for the environmental conditions to be accurately simulated in the laboratory. Hence, it is essential that soil moisture is maintained to understand the fate of the chemical during irradiation.

The purpose of this paper was to investigate the effect of soil depth on pesticide photolysis in moisture controlled, moisture adjusted, and air-dried nonsterile loamy sand soils of varying depths.

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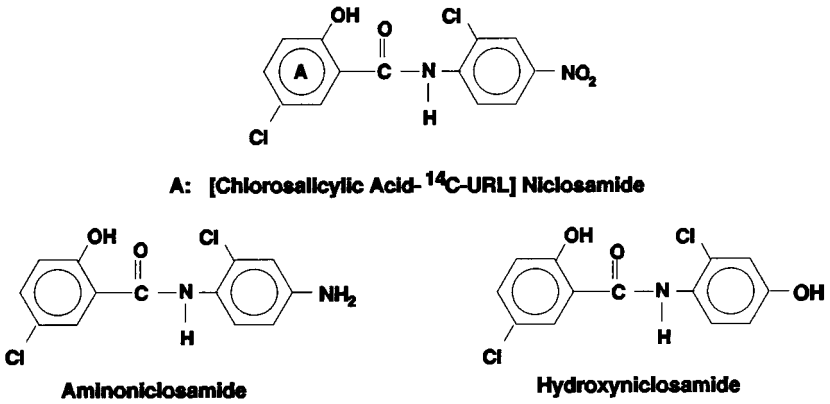

Figure 1. Structures of test and reference substances.

Table 1. Soil Characteristics

organic matter	2.7%
pH (1:1)	5.4
sand	87%
silt	08%
clay	05%
USDA Textural Class	loamy sand
cation exchange capacity	7.2 meq/100 g
bulk density	1.26 g/cm^3

base saturation data

cation	percent	ppm
calcium	34.7	500
magnesium	11.6	100
sodium	2.1	35
potassium	2.9	81
hydrogen	48.8	35

MATERIALS AND METHODS

Test and Reference Substances. ^{14}C-Niclosamide (2′,5-dichloro-4′-nitrosalicylanilide, **Figure 1**) was synthesized by DuPont/New England Nuclear (Boston, MA). The compound was uniformly ring-labeled at the chlorosalicylic acid moiety. Nonlabeled reference substances included niclosamide obtained from Sigma Chemical Company, aminoniclosamide (2′,5-dichloro-4′-aminosalicylanilide) synthesized by Derse & Schroeder Associates of Madison, WI, and hydroxyniclosamide (2′,5-dichloro-4′-hydroxysalicylanilide) produced by Natland International. All reference substances were used without further purification. Stock solutions of reference substances were prepared in methanol and stored refrigerated. Prior to use, a 650 μg/mL solution of ^{14}C-niclosamide in methanol was prepared and analyzed by high-performance liquid chromatography (HPLC) for purity. The stock and dosing solutions were found to contain about 7% of an impurity, eluting at 27.5 min, and 93% niclosamide. The presence of this impurity in the dosing solutions was inconsequential, as only the decline of niclosamide was followed in all studies.

Soil Acclimation. Loamy sand soil was obtained from Sauk County, WI and classified by Agvise Laboratories (Northwood, ND) as loamy sand. The soil classification is detailed in **Table 1**. The moisture content of 75% at $^1/_3$ bar field moisture capacity (FMC) was determined by saturating a 50-g portion with water in a filter-paper-lined Büchner funnel and drawing off the water under vacuum at 253 mmHg. The net weight of soil and water was then obtained, which represented 100% FMC at $^1/_3$ bar. Multiplication by 0.75 resulted in the weight of 50 g of natural soil at 75% FMC at $^1/_3$ bar. Prior to use, the soil was brought to 75% FMC at $^1/_3$ bar and incubated at 25 °C. Oven drying resulted in a loss of 0.15 g of water per g of natural soil. A portion of the natural soil was also air-dried for 3 days, causing a loss of 0.14 g of water per g of natural soil.

Microbial Population. Soil microbial viability was determined at the end of the 1.5 and 2.0 mm moist soil exposure and at the end of the 0.5 and 1.0 mm dry soil exposure. A 1−2 g aliquot of the reference soil was removed and mixed with 10 mL of sterile calcium chloride solution. Serial dilutions of 1- to 5-fold of the soil slurry were prepared aseptically in the calcium chloride solution. Aliquots of the dilutions were then applied and spread aseptically in duplicate onto Standard Methods Agar (BBL, Cockeysville, MD) for the enumeration of microbes. Sterile calcium chloride solution was also plated as a control. After ∼72 h of plate incubation at 25 °C, the colonies were counted, and the colony forming units per gram (CFU/g) of soil/water slurry was calculated.

Light Penetration through Soil. The ability of light to penetrate through soil was determined by measuring the intensity of light through various soil depths. A Suntest photounit (Heraeus DSET Laboratories, Inc., Phoenix, AZ) and xenon lamp (Atlas, Linsengericht, Germany) were used as the light source. Lamp intensity was measured with a radiometer and photo detector assembly (International Light, Inc., Newburyport, MA) using 280, 365, and 440 nm sharp cut (high pass) filters and a wide-eye quartz diffuser. A baseline value of zero light transmittance was obtained for each filter. To obtain the upper limit of 100% transmittance, the lamp intensity was measured at each filter directly beneath a quartz glass plate. The quartz plate served as the surface upon which the soil would be placed.

To obtain the desired soil depths, forms were constructed by attaching together layers of paper to the required dimension as measured with a micrometer. The form was placed on the quartz plate, and the soil was spread across the form to create a soil bed of the correct thickness. The light intensity was measured through 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 mm depths of both dry and moist soil.

Test Systems. The irradiated test vessel consisted of an open-top stainless steel chamber with a water jacket to maintain the soil temperature. A water bath provided temperature control by circulating water through the floor of the photolysis chamber underneath the samples. An airtight system was attained by sealing a quartz glass plate on the open top of the vessel. The photolysis chamber was attached to an air inlet to allow constant purging of the sample headspace. The soil trays were irradiated continuously by the xenon lamp 23 cm above the plates. The apparatus was designed to continuously monitor and maintain soil temperature and moisture at preset values. A soil tray containing undosed reference soil was equipped with probes to monitor temperature and moisture. An automated water spray nozzle next to each soil tray dispensed deionized water to the soil, each calibrated to deliver the same amount of water during the spray cycle as the reference soil nozzle. The water spray cycle was automatically initiated when the recorded soil moisture level fell below the preset value (75% FMC at $^1/_3$ bar). The water spray period per cycle for a soil tray was about one second (40−60 μL of water per second). The various soil depths were obtained in the trays by filling a tared tray with soil and leveling the soil with the top of the 4-mm deep tray. The net weight of the soil per mm of depth was determined for both moist and air-dried soils.

Incubated test soil was weighed into the reference plate, and the reference plate was inserted into the photolysis chamber to obtain the moisture reference value. The initial reading of the instrument at 25

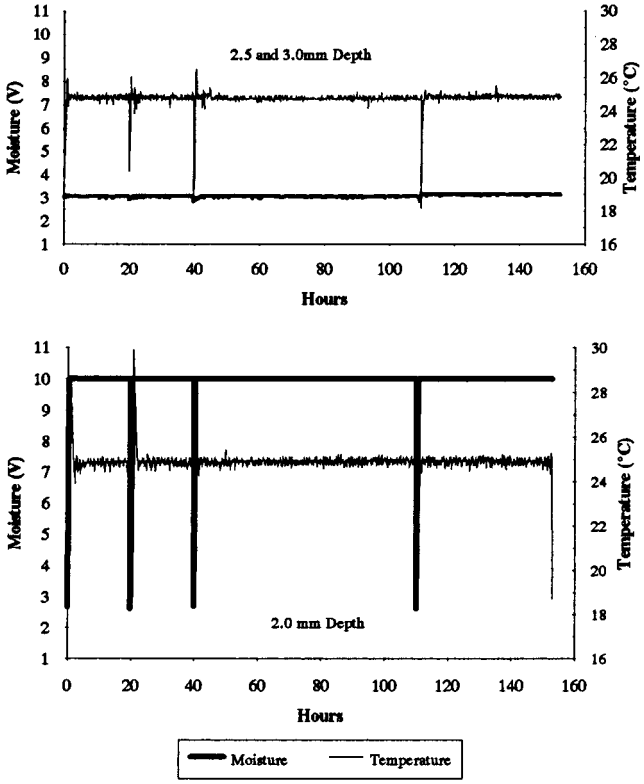

Figure 2. Environmental control of irradiated moisture-controlled soil samples (top panel) and moisture-maintained soil samples (bottom panel). The spikes in the bottom panel represent the rapid drying of initially moist soil.

°C was 3.1 V. Soil temperature and moisture values were recorded every six minutes and were analyzed by spreadsheet application software. The performance of the moisture and temperature control system is represented graphically in **Figure 2** for the moisture-controlled and moisture-adjusted soils. The soil moisture was maintained at the sensor value to which the system equilibrated after each sampling. At each sampling the weight of each soil tray was manually recorded and adjusted with water if necessary to ensure that the soil was being maintained at its initial weight and moisture content. For the moisture-adjusted experiment, soil at 75% FMC at $^1/_3$ bar was dosed but allowed to dry under the xenon lamp during the exposure phase. Moisture was adjusted only at the time of sampling. The temperature control was set at 25 °C and was not lowered prior to lamp ignition. The quartz plate was not sealed to the chamber for this experiment, leaving a gap of about 5 mm, and so no volatiles were collected. Under these experimental conditions, the soil became dry within 25 min if moisture was not maintained, and the soils remained at elevated temperatures for up to 3 h following lamp ignition if the temperature was not initially compensated. No moisture control or adjustment was performed in the air-dried soil study.

The volatile trapping system consisted of a 100-mL Pyrex bottle directly connected to the air outlet port of the test system. The bottle was filled with 80 mL of 1N NaOH. The trapping solution was replenished at each sampling time. A backflow trap prevented liquid from inadvertently being drawn into the soil chamber.

The dark control test vessel consisted of a stainless steel chamber with access ports for air circulation. The air inlet was diffused to maintain an even flow throughout the chamber. The test container was equipped with a stainless steel plate to provide an airtight dark system. The test chamber housed stainless steel soil trays similar to those of the irradiation test system. The test container with soil trays was sealed and maintained in the dark in an incubator (Precision Scientific, Cleveland, OH) at 25 ± 1 °C. A volatile-trapping system was connected to the output of the dark control test vessel.

Liquid Scintillation Counting (LSC). Liquid scintillation analyses were conducted using a TriCarb liquid scintillation analyzer (Packard, Meriden, CT). Aliquots of stock, dosing solution, soil extract (100 or 200 μL), and trapping solution samples (1000 μL) were directly analyzed in ULTIMA GOLD scintillant (Packard). All counts were automatically corrected for instrument background (about 25 cpm) and efficiency (95.8 ± 0.17%; $n = 15$). Results are reported in disintegrations per minute (dpm).

Dosing Procedure and Study Initiation. The dosing solutions were prepared from a 650 μg/mL ^{14}C-niclosamide stock solution by diluting with acetonitrile to a final concentration of 250 μg/mL. The dosing solution concentrations were determined by LSC. The stock solution was stored in the dark below −20 °C.

For the Time 0 samples, air-dried soil or preincubated soil at 75% FMC at $^1/_3$ bar was brought to ambient conditions and dispensed into tared 40-mL vials. The calculated volume of dosing solution was added to the soil with a Hamilton syringe to yield a concentration of 2.5 μg/g. Two soil depth experiments were conducted at a time, resulting in two different application rates. The soils were thoroughly mixed after dosing.

For the remaining samples, soil was weighed into uniquely identified stainless steel trays. The dosing solution was dispensed evenly across the soil surface via syringe, applying approximately 30 drops per plate. The soils were mixed and uniformly distributed across the plate. The plates were then placed inside the photolysis apparatus and kept covered until all soil samples were dosed for irradiation.

Once all samples for irradiation were dosed, the test vessel was covered with a quartz glass plate and sealed. A continuous flow of compressed air at about 10 mL/min was started through the test chamber into the sodium hydroxide trapping solution. The lamp was ignited, and the moisture control and monitoring program was started. The temperature of the soil, initially kept at 13 °C to prevent overheating, equilibrated under the lamp to 25 °C within about 45 min. The time and chronometer hours at lamp ignition were recorded.

Samples were removed after 20, 40, 110, and 153 h of continuous irradiation. At each sampling, the lamp was shut off and the air flow was stopped. The selected samples were removed and weighed. The remaining soils were also weighed, and their moisture was adjusted if necessary. Any water that condensed on the floor of the photolysis chamber was collected and counted by LSC. The soil plates were returned to the photolysis chamber and sealed. Air flow and irradiation were resumed.

The volatile trapping solution was changed at each sampling interval. Trap volumes were determined, and three 1-mL aliquots were assayed by LSC. The volatile radioactivity collected at each sampling was calculated on a per sample basis by prorating according to the application rate of each sample. The total was accumulated throughout the study. The radioactivity in the condensed water was calculated in the same manner.

A dark control experiment was conducted on moist and air-dried soils of 2-mm depth. Dosing was performed in the same manner as for the irradiated soils. The moist and air-dried soils were kept separated in stainless steel chambers. Samples were removed after 20, 40, 112, and 160 h of incubation in the dark at 25 °C. The moist soils were brought back to their initial weight with water at each sampling.

Soil Analyses. After exposure, the samples were transferred into tared 40-mL vials and extracted three times with 7−10 mL portions of acetonitrile/1 N phosphoric acid, 9:1 v/v, by thoroughly vortexing, sonicating for 6 min in an ultrasonic bath, and centrifuging for 10 min. The three portions of extract were pooled and then analyzed by LSC. Aliquots of the extracts were filtered and concentrated by vacuum evaporation to a final volume of 1 mL. The extracted soils were air-dried overnight in a hood.

Aliquots of the extracted, air-dried soil were combusted to determine the soil-bound radioactivity. The combustion analysis was performed using a biological oxidizer (R. J. Harvey Instrument Corporation, Hillsdale, NJ) followed by LSC analysis. The instrument combustion efficiency was determined before and after the combustion of each set of test samples. The efficiency of the oxidizer was calculated based on the recovery of radioactivity from cellulose containing a known quantity of ^{14}C-niclosamide. The average efficiency of the biological oxidizer was 97.0% during the course of the study.

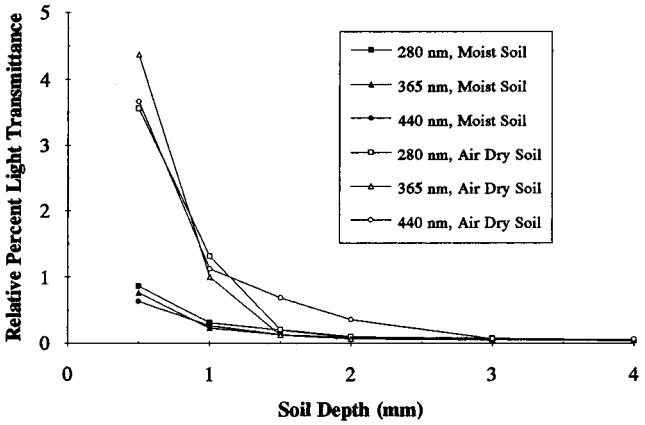

Figure 3. Light penetration in relation to soil depth.

Extract concentrates were analyzed by HPLC. A model 501 HPLC system (Waters, Milford, MA), configured with a Waters 715 WISP autosampler, Waters 484 tunable UV detector, and a Packard FLO-ONE/*βeta* BD radioflow detector using FLO-SCINT II (Packard) scintillant at 3 mL/minute was used for the analysis. The HPLC column was an Ace 5 4.6 × 250 mm C18 (Mac-Mod Analytical, Inc., Chadds Ford, PA) with guard column. The mobile phase was 0.1% TFA and acetonitrile. The gradient began with a 3 min hold at 10% acetonitrile, was ramped to 80% acetonitrile at 15 min, and then held at 80% acetonitrile until 35 min. The flow rate was 1.0 mL/min. Concentrates were diluted with water and spiked with 10−30 μL of a three-component 200 μg/mL standard mix in acetonitrile. The nonradiolabeled reference standards were analyzed with UV detection at 220 nm. Typical retention times were 27.3 min for niclosamide, 22.4 min for aminoniclosamide, and 24.5 min for hydroxyniclosamide. The quantitation of the test substance was carried out from beta detection area response. Radioflow limit of detection was about 1000 dpm (20 ng).

Thin-layer chromatography (TLC) was used as a second method of analysis for confirmation and identification work. Silica gel 60 plates (EM Science, Gibbstown, NJ) containing fluorescent indicators were spotted with soil extract and reference standard solutions. Two solvent systems were used: dichloromethane/ethyl acetate/acetic acid (solvent system 1) and dichloromethane/methanol/10% ammonium hydroxide (solvent system 2). The reference standards were visualized by illumination under a 254-nm lamp and/or iodine stain, and the radioactive areas were imaged using a radioanalytical scanner (Ambis, San Diego, CA). Identification was confirmed when radioactive areas overlapped positions corresponding to reference standards.

RESULTS AND DISCUSSION

Light Transmittance in Relation to Soil Depth. Our studies indicate that even 0.5-mm thin layers of soil are sufficient to block 95% of the incident light, and even less light is able to be transmitted through soil depths of 1.5 mm or greater (**Figure 3**). Light penetration was inhibited in both moist soil and air-dried soil at these depths. At more shallow soil depths (<1.5 mm), the major factor affecting light transmittance was moisture content. Only 20% of the light penetrating air-dried soil was transmitted through moist soil. In dry soil the particles are only loosely packed, leaving room for light to scatter and permeate the soil. In moist soil, the water serves to seal up the crevices between particles, so that light cannot penetrate through the soil. As a result, moist soil at 0.5- and 1.0-mm thickness showed the same low level light transmittance as air-dried soil of 1.5- and 2.0-mm thickness.

Moisture-Maintained Verses Air-Dried Soil. Moisture plays an important role in the distribution of radioactivity throughout the test systems and the degradation of niclosamide (**Table 2**). Moisture greatly increases the amount of radioactivity bound to the soil. The amount extractable from moisture-controlled soils was approximately 40% less than that from air-dried soils. Bound radioactivity was three times greater in moisture-maintained irradiated soil than in air-dried irradiated soil, and seven times greater in moist dark control samples than in the air-dried dark control samples. The nonextractable radioactivity for air-dried samples showed very little increase from 20 h to 153 h of irradiation, but in moisture-controlled soils the percent bound material increased throughout the exposure period. Even when soil is initially moist but is allowed to dry under irradiation, the amount of extractable material is comparable to that of 2.5- and 3.0- mm air-dried soil. In our experiments, the initially moist soil dried and baked under irradiation even with the temperature maintained at 25 °C, so that the soil surface became hard. In the dark control samples the bound material was constant at 2.5% throughout the air-dried soil study, but the amount of extractable radioactivity from the moisture-adjusted samples declined from 97% initially to 68% after 160 h of incubation. Concurrently, soil bound material increased to 18%. Volatile compounds are also more likely to be formed in the presence of moisture. About 2% of the applied radioactivity was volatilized from irradiated air-dried soil, but twice as much radioactivity was observed in the trapping solutions of the irradiated moisture-controlled soil. The pattern of increased volatiles with moisture exhibited by the irradiated samples was upheld in the dark control as well. In the air-dried dark control test systems, very few volatiles were formed. However, almost 3% of the applied radioactivity was evolved from the moist dark control soil.

Figure 4 compares the degradation of niclosamide in the dark control sample and the irradiated air-dried and moist soil samples of varying depths. When moisture was maintained, nearly twice as much niclosamide was degraded after 7 d of irradiation compared to that in air-dried soil of equal depth. When soil moisture was merely adjusted at each sampling but not maintained throughout the study, niclosamide did not degrade as readily as in nonirradiated moist soil. The decline of niclosamide in dark control moist soil was greater than the photolytic decline in air-dried soil for all but the 0.5- and 1.0-mm depths. No degradation of niclosamide was observed in the dark control experiment performed with air-dried soil.

The data in **Table 2** suggest that moisture plays a significant role in the degradation of a pesticide in a soil photolysis study. The degradation pattern from irradiated air-dried soil displayed a vast difference in soil depths of 1 mm and 1.5 mm, whereas the degradation pattern of the moisture-controlled photolyzed soil was similar through all soil depths. Three major degradates observed were aminoniclosamide, 5-chlorosalicylic acid, and hydroxyniclosamide, each identified by HPLC and TLC co-chromatography. In general terms, these compounds reached greater levels in the more shallow soils. Aminoniclosamide, appearing after 40 h of irradiation, attained levels of 8% in the 0.5-mm soil and 5% in the 2.5-mm soil. Aminoniclosamide was first reported as a byproduct of niclosamide degradation in aquatic systems by Muir and Yarechewski (*12*) and confirmed by Graebing et al. (*13*). Previously, aminoniclosamide was shown to be formed only in rat studies (*12*). Hydroxyniclosamide and 5-chlorosalicylic acid ranged from 1 to 5%. The formation of 5-chlorosalicylic acid has only been inferred in previous studies involving environmental systems by the observation of the corresponding degradate 2-chloro-4-nitroaniline (*12*), although it has been observed in direct photolysis on glass slides (*14*). Hydroxyniclosamide has not been previously identified in fate and metabolism studies. Degradate 1, which was formed in the methanol dosing solutions, was fully

Table 2. Distribution of Degradates in Moist Soils as a Percent of Original Compound Applied

hours	degradate #3	amino-niclosamide	5-chloro-salicylic acid	hydroxy-niclosamide	degradate #6	degradate #7	degradate #1	niclosamide	degradate #8
	3.7 min	23.0 min	24.5 min	25.0 min	26.4 min	27.1 min	27.5 min	28.5 min	29.8 min
				dark control; 2.0 mm; moist soil					
0				0.0			5.7	91.2	
20				0.0			0.0	83.4	
40				2.9			0.0	79.0	
112				1.3			0.0	69.7	
160				4.0			0.0	64.4	
				irradiated; 2.0 mm; moisture-adjusted soil					
0		0.0	0.0				6.3	97.2	0.0
20		0.0	2.6				3.4	88.6	0.0
40		0.0	3.3				3.6	86.5	0.0
110		0.0	2.5				4.1	73.0	9.7
153		2.8	5.1				4.8	71.8	3.3
				irradiated; 0.5 mm; moisture-maintained soil					
0	0.0	0.0	0.0	0.0			4.7	87.4	
20	0.0	0.0	0.0	0.0			0.0	72.9	
40	0.0	6.2	4.0	3.0			0.0	47.5	
110	0.0	8.4	4.6	5.1			0.0	36.6	
153	3.1	6.3	5.6	1.1			0.0	26.9	
				irradiated; 1.0 mm; moisture-maintained soil					
0	0.0	0.0	0.0	0.0			6.4	89.7	
20	0.0	0.0	0.0	0.0			0.0	79.2	
40	0.0	1.5	2.9	2.4			0.0	64.3	
110	0.0	2.1	2.2	1.7			0.0	51.6	
153	2.9	5.8	4.0	5.0			0.0	34.7	
				irradiated; 1.5 mm; moisture-maintained soil					
0	0.0	0.0	0.0	0.0			7.1	91.1	
20	4.3	0.0	0.0	0.0			0.0	74.0	
40	0.0	1.3	4.6	3.0			0.0	63.3	
110	0.0	5.9	1.9	3.2			0.0	42.0	
153	0.0	3.8	0.0	5.9			0.0	41.3	
				irradiated; 2.0 mm; moisture-maintained soil					
0		0.0	0.0	0.0			5.7	90.0	
20		1.4	2.7	0.0			0.0	66.6	
40		0.0	0.0	0.0			0.0	73.5	
110		2.1	3.3	3.5			0.0	54.0	
153		5.1	2.2	2.5			0.0	42.4	
				irradiated; 2.5 mm; moisture-maintained soil					
0		0.0	0.0	0.0			6.5	93.7	
20		0.0	2.6	0.0			0.0	82.4	
40		2.8	2.0	2.2			0.0	77.0	
110		5.4	2.6	1.1			0.0	61.3	
153		5.6	1.4	0.0			0.0	50.1	
				irradiated; 3.0 mm; moisture-maintained soil					
0		0.0	0.0	0.0	0.0	0.0	6.6	91.4	
20		0.0	2.2	0.0	0.0	0.0	0.0	79.3	
40		0.0	2.6	2.1	0.0	0.0	0.0	75.6	
110		2.2	2.0	1.0	0.0	0.0	0.0	60.7	
153		3.3	2.4	2.9	0.9	1.3	0.0	50.9	

extractable in the air-dried soils, but was degraded in moisture-maintained samples in both irradiated and dark control systems. This compound did not contribute any components to the HPLC degradation pattern which would erroneously be attributed to niclosamide, based on the fact that the only component observed by HPLC in the 20-h samplings of moist dark control and moisture-maintained irradiated 0.5- and 1-mm soils was parent material. Degradate 1, seen in all 0-h samples, was not observed thereafter when moisture was maintained, leading to the conclusion that it binds to the moist soil, because the amount of volatiles evolved is not sufficient to completely account for this compound. Several other compounds appeared intermittently at singular sampling times.

Two degradates eluting at 26 and 27 min were observed by HPLC in the 3.0-mm soil in the 153 h sampling. No available reference standards matched these compounds by HPLC. A polar peak at 3.7 min was apparent in individual sampling points of soils of 1.5-mm and less depths. This peak is similar to degradates reported in the aqueous photolysis of niclosamide in pH 9 buffer (*15*). In that study a 3-min peak was discovered to be made up of carbon dioxide and several organic acids, indicating complete breakdown of the aromatic ring system. In addition, two compounds, 4-chlorophenol and Degradate 5, were formed only in irradiated air-dried soils. They were not observed in the moisture-controlled samples. 4-Chlorophenol was confirmed by TLC and HPLC co-chromatography. In the dark control studies, the only degradate observed in the air-dried soil was Degradate 1, which was seen in the dosing solution. This degradate exhibited no change in concentration in any of the air-dried dark control samples. Niclosamide concentration held constant in the air-dried dark control study, revealing no degradation. Another effect of moisture in the dark control studies was the degradation of niclosamide and the formation of hydroxyniclosamide which was not observed in the dry soil.

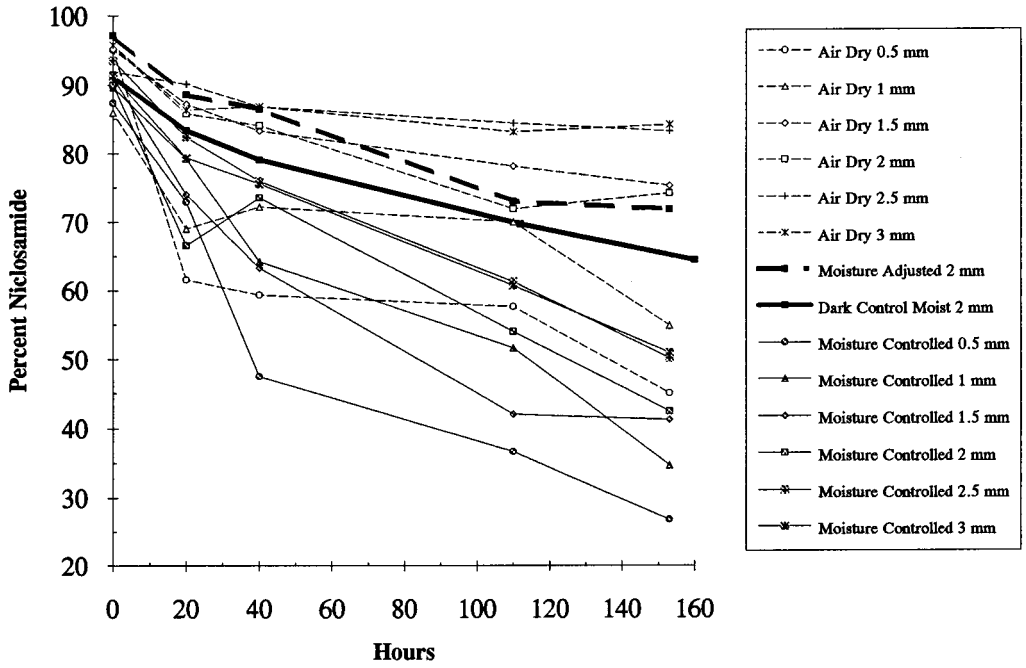

Figure 4. Photolytic decline of niclosamide in moisture-controlled, moisture-adjusted, and air-dried soils.

Table 3. Half-Lives of Niclosamide in Moisture-Maintained (75% FMC at $^1/_3$ bar), Moisture-Adjusted, and Air-Dried Soils of Various Depths

depth (mm)	moisture-controlled soil[a] (hours)	moisture-adjusted soil[b] (hours)	air-dried soil[c] (hours)
		irradiated	
3.0	194.57	N/A[d]	1063.87
2.5	181.26	N/A	1109.04
2.0	161.88	352.01	419.86
1.5	132.74	N/A	511.62
1.0	121.12	N/A	369.62
0.5	95.58	N/A	199.19
		dark control	
2.0	339.51	N/A	not degraded

[a] Moist soil automatically controlled and maintained at 75% FMC at $^1/_3$ bar by photolysis apparatus. [b] Moist soil adjusted manually to 75% FMC at $^1/_3$ bar at each sampling only. [c] Air-dried soil not adjusted for moisture. [d] Not applicable.

The degradation pattern of the moisture-adjusted soils was comparable to that of the moisture-maintained soils, except that hydroxyniclosamide was not observed. Instead, a heretofore unobserved compound which eluted after niclosamide by HPLC was formed at 9% of the applied radioactivity in the 110 hour sample and declined to 3% after 7 days of irradiation. This compound has not been identified in any previous reports. The degradates formed in air-dried soil or in soil where moisture is not maintained can be very different from the degradates formed in moist irradiated soil.

As a result of the dependence on moisture for degradation, the half-life of niclosamide is significantly shorter in cases where the moisture is maintained during irradiation than in cases where the moisture is not maintained (**Table 3**). The microbial population of the moisture-maintained soil was 5.15×10^6 CFU/g, and for the unmaintained soil it was 5.9×10^5 CFU/g. The soil microbial population is an order of magnitude higher in the moisture-maintained soil. The moisture also acts as a source of hydroxyl radicals. However, in the dry soil, the microbial population is low, and the generation of hydroxyl radical is diminished. **Figure 5** is a graphical comparison of the half-lives of niclosamide as determined from the various depth experiments. Clearly, moisture is the more influential factor. When moisture was maintained, only a gradual increase in half-life with soil depth was observed, and the relationship of that increase with depth was strongly linear ($R^2 = 0.989$). If moisture is not maintained, the half-life obtained in a particular soil photolysis experiment is directly related to the depth of the soil. The variance in half-life of niclosamide in moist soil is not as pronounced. Adjustment of soil moisture at each sampling was not as effective as continual automated maintenance. The half-life of the irradiated moisture-adjusted study was comparable to the half-life of the moisture-adjusted dark control study.

Effect of Soil Depth. Soil depth is also an important consideration in a photolysis study. The amount of extractable material increased for both air-dried and moist soil with increasing depth. Apparently that portion of niclosamide in deeper soils is not photolytically transformed and remains easily extracted. Bound material reached 30−35% in irradiated moist soils of 0.5- to 2.5-mm depths, but decreased to 24% in the 3.0-mm soil. In fact, the extractable and soil-bound distribution for the 3.0-mm moist soil is similar to that for the dark control moist samples, another indication of the inability of light to have an appreciable effect at this depth.

With regard to niclosamide decline and degradate formation (**Table 2**), soil depth was an important factor only when moisture was not maintained. The 0.5- and 1.0-mm depth air-dried soils displayed an extensive degradation pattern, but only Degradate 1 was seen in depths greater than 1 mm. This supports the conclusion that light penetration is minimal in soil depths greater than 1.0 mm. Degradate 1 maintained a relatively constant concentration in all levels except the 0.5 mm depth, where it ranged from 5 to 12% of applied. In the moisture-controlled soils, Degradate 1 was seen only in the 0-h samples, and the degradation pattern was similar at all depths. 5-Chlorosalicylic acid was found to have been formed earlier in deeper soils than in the 0.5- to 1.5-mm soils.

In both the air-dried and moisture-maintained soils, there was no difference in the amount of niclosamide remaining in soils of 2.5- and 3.0-mm depths (**Figure 3**). This suggests that photolysis is effective only to a depth of 2.0 mm or less. Certainly, the greater degradation of niclosamide from both moist and air-dried soils of 0.5 and 1.0 mm suggests that

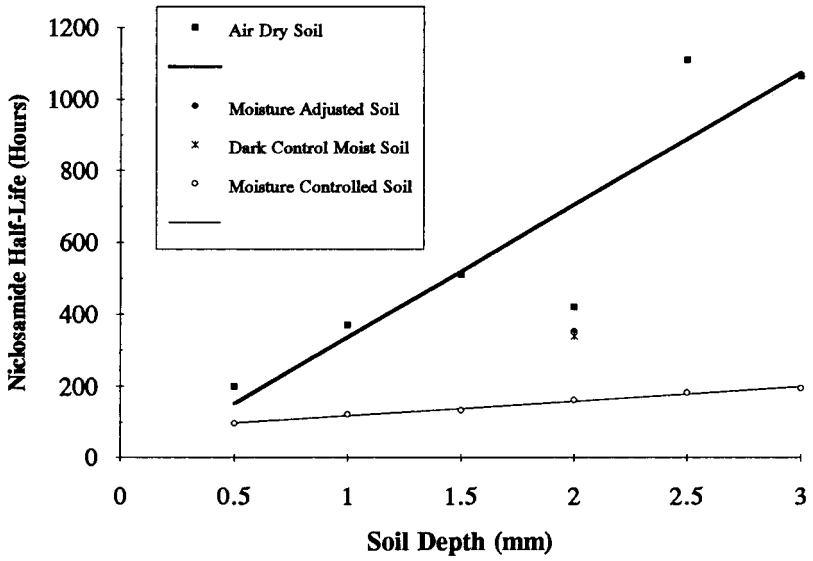

Figure 5. Variation in half-life in relation to soil depth.

photolysis is primarily effective at these depths. Increasing depth of soil sample leads to an increase in half-life, as shown in **Figure 5**. Soil depth has a much greater effect on photolysis in air-dried soil than it does in moist soil. Although our light penetration experiments indicate light cannot permeate soils greater than 1 mm deep (**Figure 3**), moisture in the soil (through evaporation at the surface and condensation) appears to increase the mobility of niclosamide, allowing concentrations deeper in the soil to migrate to the surface where they can be photolyzed. The solubility of niclosamide in water is 5−8 mg/L.

These data have important implications for scientists conducting regulatory soil photolysis studies in support of pesticide registration. Our study showed no difference in light penetration through soils of depths greater than 1 mm. The present study supports the observations of Hebert and Miller (*16*). They authors reported that although direct photolysis was limited to 0.2−0.3 mm, indirect photooxidation can extend to depths greater than 2 mm, and that the depth of singlet oxygen penetration is dependent upon moisture content as well as soil porosity and steepness of thermal gradients on sunlight-exposed surfaces. Our study showed less extractable radioactivity in the shallow samples, indicating that photolysis occurs only at these levels. If a photolysis study is performed on air-dried soil, there is much greater variance in pesticide half-life with soil depth due to reduced microbial activity, less chemical being available to penetrating photons, and the elimination of degradation by hydroxyl radical.

Although moisture and soil depth are important environmental factors in and of themselves, other observations reveal a complementary effect between the two. Although light penetrates deeper into air-dried soil, moisture provides mobility to chemicals applied deep in the soil through evaporation and condensation. The result is that moisture-maintained irradiated soils showed a consistent degradation pattern throughout all depths, whereas a photolysis study on air-dried soil could show different degradates depending on which soil depth was chosen. Optimal conditions for a soil photolysis study include moist soil at a depth of 2 mm. This is a reasonable penetration depth for the chemical upon application, allows for direct photolysis to be fully effective, and permits the investigation of mobility of the pesticide through the soil. Similar results have been obtained using the herbicide chloramben (*17*).

ABBREVIATIONS USED

CFU, colony-forming units; FMC, field moisture capacity; HPLC, high-performance liquid chromatography; LSC, liquid scintillation counting; TLC, thin-layer chromatography.

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