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Effects of Fertilizer and Soil Components on Pesticide Photolysis

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An environmental fate study was performed analyzing the effects of soil composition on the soil photolysis of a chemical. The study was conducted in two phases in which both moist and air-dried soils were fortified with either the common fertilizer sodium nitrate or the natural soil components iron or humic acid and dosed with niclosamide. The soils were photolyzed under a xenon lamp for 7 days. Increasing concentration of sodium nitrate did not affect the degradation pattern but did produce a lower concentration of aminoniclosamide. Soils fortified with iron displayed an unknown, which was not observed in other experiments, and the degradation of niclosamide from these soils was slower than from the sodium nitrate-fortified soils. There were no extractable degradates from any of the soils fortified with humic acid. In irradiated moist soils, the half-life of niclosamide increased when sodium nitrate was present at 20 ppm, and the half-lives of niclosamide in iron- and humic acid-fortified soil were increased slightly over that in unfortified soil. The effect of the nitrate and iron on the half-lives in dark control moist soils was minimal, but humic acid increased the dark control half-life from 420 to 611 h. No transformation of niclosamide was observed in the dark control air-dried soils. Soils with higher organic or iron contents or exposed to fertilizers do not affect as dramatically the half-life of pesticides as does the presence of moisture in the soil. Soil photolysis samples that were not maintained with moisture exhibited differences in half-life and degradation pattern. The maintenance of moisture was found to be more crucial to the reliability of soil photolysis studies than soil composition.

KEYWORDS: Niclosamide; soil photolysis; environmental fate; fertilizer; moisture; half-life

INTRODUCTION

Pesticides are an important part of everyday life. Pesticide use ensures that crop yields are abundant and of high quality. Hand in hand with pesticide application is the use of fertilizers, which provide soil with sufficient nutrients to promote healthy and fully grown crops. However, the introduction of materials into the environment raises concerns as to their effect on ecological balance.

One of the environmental concerns is the anthropogenic alteration of the nitrogen cycle. The increased use of nitrogenous fertilizers has led to an increase in anthropogenic nitrogen fixation, with the possible result of altering the ability of ecosystems to respond to carbon dioxide (*1*). Nitrogen salts contained in fertilizers are also species that could react directly with the pesticide, forming degradation products with the potential to be transported outside the area of application. The degradation products thus formed may themselves have important toxicological properties. For instance, urea has been shown to have detrimental effects on amphibians (*2*).

Adding to the formation of ionic species is the effect of sunlight on soil components and additives. Foremost of these reactants is the hydroxyl radical, the most reactive oxidant in the environment (*3*). The hydroxyl radical is formed by direct photolytic breakdown of nitrates and nitrites at wavelengths >290 nm (*4*) and also from photolysis of humic substances (*5*). The hydroxyl radical has also been found to be formed by irradiation of hydrogen peroxide (*3*), which itself is generated by irradiation of humic acids (*6*). The hydroxyl radical derived from humic acids has been attributed to the degradation of pesticides (*7*) and organic pollutants (*8*). Often soil constituents can have a cumulative effect. The addition of nitrate was shown to geometrically increase the production of hydroxyl radical and alkyl peroxides derived from the irradiation of humic acids (*8*), the hypothesis being that nitrate-produced hydroxyl radicals react with humic acid components to indirectly produce more of the radicals.

Sunlight-induced reactions involving iron also are an important factor in soil chemistry. Iron is thought to be a catalyst in the production of chlorophyll, and normal chlorophyll development cannot proceed until enough residual iron has accumulated in the leaf (*9*). Photochemical reactions reduce Fe(III) to Fe(II), which can lead to augmented iron concentrations in natural

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waters through increased solubility. Many biological and chemical processes are dependent upon and correlated to iron in atmospheric and surface waters. Iron(III) catalyzes the aqueous-phase oxidation of S(IV) species such as HSO_3^- and SO_3^{2-} by atmospheric oxygen, which can be an important pathway for the oxidation of SO_2 to H_2SO_4. A cyclic effect occurs as H_2SO_4 lowers the pH of atmospheric water and increases the solubility of Fe(III) (*10*). Iron is also a basic nutrient for phytoplankton in the ocean, which aids in the global uptake of CO_2. The ability of phytoplankton to use iron is limited by the availability of monomeric Fe(III)−hydroxy species (*11*).

Iron also plays a role in the formation of many reactive species, including the hydroxyl radical and hydrogen peroxide. Soil contains iron in many forms, such as hydrous oxides, associated complexes with humic substances (*10*) and at clay mineral surfaces. Hydrous oxides of Fe(III) undergo photoreduction reactions resulting in important pathways for the introduction of iron solids into surface waters (*11*). The hydroxyl radical can be formed directly from the photolysis of iron hydrous oxides, and soil receives sufficient solar energy to transform Fe(III)−ligand compounds to free Fe(II) and a ligand-free radical. The photolysis of Fe(III)−oxalate complexes results in the rapid formation of H_2O_2 (*10*). Almost all clay minerals contain iron, and clay surface properties vary with structural iron oxidation state, possibly affecting the behavior of pesticides. Pesticides have been found to both adsorb and degrade in reduced smectite, but little interaction and only small quantities of degradation products have been observed upon exposure to oxidized clays (*12*). Xu et al. report that in the case of alachlor this reaction with Fe(II) and Fe(III) does not occur in aqueous solutions, that is, outside the clay mineral structure. There is a unique behavior exhibited toward herbicides by the binding of iron to clays that is absent when iron is solubilized. Biological and microbiological activity and climate may subject soils to natural reducing conditions at various times of the year (*13*−*15*). With such an extensive variety of reactive species generated by soil iron content, the role of iron in the photodegradation of pesticides should be examined.

Plants do not directly use organic materials. Plants use inorganic compounds for growth, producing organic compounds in the process. The organic materials are used as food by soil microorganisms. This symbiosis of organic compound breakdown by microorganisms, forming inorganic compounds, and the buildup of new organic matter by higher plants lend fertility to the soil (*9*). Organic substances in soil are believed to be involved in the formation of singlet oxygen (*16*), although no correlation of the photolysis rates of singlet oxygen traps to soil organic content has been found (*17*). Several pesticides have been found to have decreased degradation rates in natural waters containing humic substances compared with that in distilled water (*18*). This decrease was found to be a result of the strongly absorbing humic substances filtering the incident light, not one of sensitization by the organic material. On the other hand, humic substances can also enhance pesticide degradation (*19*). Transport of pesticides into and out of the photolytic zone of the soil is also dependent upon soil composition. The organic matter content as well as moisture content, particle size (surface area to volume ratios), mineralogy, pH, and the presence of other organics all influence the adsorption of organic molecules onto the soil (*16*). Organic matter in soil also normally increases the content of soluble iron (*9*).

In this paper, the direct influence of nitrate ions, iron, and humic acid on the soil photolysis of niclosamide is examined through the use of a specially designed apparatus capable of maintaining temperature and soil moisture content throughout the course of the study. Niclosamide is used in the control of sea lamprey in the Great Lakes. It is also widely used as a molluscide in tropical regions for the control of fresh water snails (*20*).

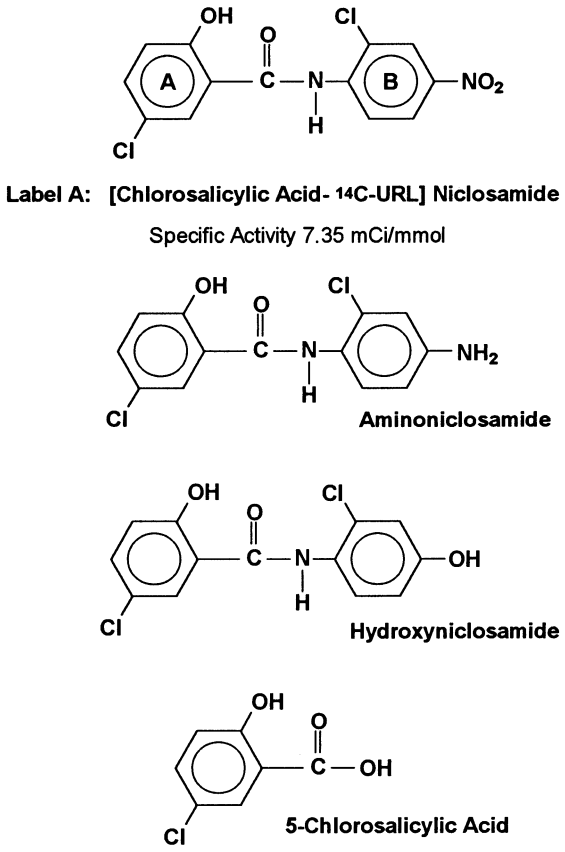

Figure 1. Structures of test and reference substances.

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 Co. (St. Louis, MO), aminoniclosamide (2′,5-dichloro-4′-aminosalicylanilide) synthesized by Derse and Schroeder Associates (Madison, WI), hydroxyniclosamide (2′,5-dichloro-4′-hydroxysalicylanilide) produced by Natland International (Morrisville, NC), and 5-chlorosalicylic acid obtained from Aldrich (Milwaukee, WI). All reference substances were used without further purification. Stock solutions of reference substances were prepared in methanol and were stored refrigerated. Prior to use, a solution of [^{14}C]niclosamide in methanol was prepared and analyzed by HPLC and found to be 99.5% radiochemically pure.

Soil Acclimation. Soil was obtained from Sauk County, Wisconsin, and classified by Agvise Laboratories (Northwood, ND) as loamy sand. The soil classification is given in **Table 1**. The moisture content of 75% at 0.33 bar field moisture capacity (FMC) was determined by saturating a 50 g portion of soil 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 0.33 bar. Multiplication by 0.75 resulted in the weight of 50 g of natural soil at 75% FMC at 0.33 bar. Prior to use, the soil was brought to 75% FMC at 0.33 bar and incubated at 25 °C. The soil weight was checked daily and adjusted with water to maintain the moisture. Prior to initiation, the soil composition was fortified as required for each of the experiments. Humic acid (Aldrich, St. Louis, MO) was added at 3 wt % to increase the organic content, and iron oxide or a solution of sodium nitrate (both from Fisher Scientific,

Table 1. Characteristics of Soil

organic matter		2.7%
pH		5.4
sand		87%
silt		08%
clay		05%
USDA textural class		loamy sand
cation exchange capacity		7.2 mequiv/100 g
bulk density		1.26 g/cm^3
base saturation data		
cation		
calcium	34.7%	500 ppm
magnesium	11.6%	100 ppm
sodium	2.1%	35 ppm
potassium	2.9%	81 ppm
hydrogen	48.8%	35 ppm

Pittsburgh, PA) was applied to obtain concentrations of 10 or 20 μg/g of soil. Portions of the fortified soils were also air-dried overnight.

Microbial Population. Soil microbial viability was determined for the sodium nitrate experiment at the end of the moist soil exposure and at the end of the dry soil exposure. A 1−2 g aliquot of the reference soil was extracted with 10 mL of sterile calcium chloride solution. Serial dilutions (1−5-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 (Becton-Dickinson, Cockeysville, MD). Sterile calcium chloride solution was also plated as controls. After 24, 48, and 72 h of plate incubation at 25 °C, the colonies were counted, and the colony-forming units per gram (CFU/g) of soil was calculated.

Test Systems. The irradiated test system has been described previously (*21*). The consistency of the lamp intensity was verified before and after each exposure phase with a radiometer and photodetector assembly (International Light, Inc., Newburyport, MA) using 280, 365, and 440 nm sharp cut (high pass) filters and a wide eye quartz diffuser. The lamp intensity was found to be consistent throughout the study. The photolysis apparatus was designed to continuously monitor and maintain soil temperature and moisture at preset values. A soil tray containing undosed reference soil is equipped with probes to monitor temperature and moisture. Temperature is controlled via a circulating water bath. An automated water spray nozzle next to each soil tray dispensed 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 0.33 bar). The water spray period per cycle for a soil tray was ∼1 s (40−60 μL of water/s). The soil depth in each experiment was 2 mm.

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 °C was 3.1 V. Soil temperature and moisture values were recorded every 3 min and were analyzed by spreadsheet application software. 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.

The volatiles were trapped with 80 mL of 1 N NaOH, 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 at 25 ± 1 °C (Precision Scientific, Cleveland, OH). 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 Packard (Meriden, CT) TriCarb liquid scintillation analyzer. 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 (∼25 cpm) and efficiency (95.8 ± 0.17%; $n = 15$).

Dosing Procedure and Study Initiation. The dosing solutions were prepared from an 800 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 0.33 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 syringe to yield a concentration of 2.5 μg/g. 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 ∼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 ∼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 18−20 °C to prevent overheating, equilibrated under the lamp to 25 °C within ∼30 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 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 (<0.1%) was calculated in the same manner.

A dark control experiment was conducted on moist and air-dry soils of 2 mm depth. Dosing was performed in the same manner as the irradiated soils. The moist and air-dry 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 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 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 Corp., 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 on the basis of the recovery of radioactivity from cellulose containing a known quantity of [^{14}C]niclosamide. The average efficiency of the biological oxidizer was 93.5% during the course of the study.

High-Pressure Liquid Chromatography (HPLC). Extract concentrates were analyzed in duplicate by HPLC. A Waters (Milford,

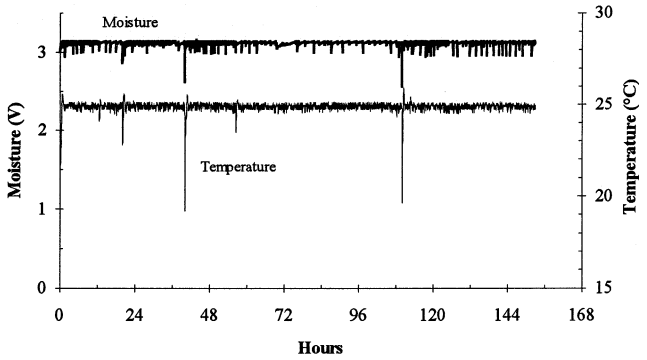

Figure 2. Representative environmental control of moisture-maintained experiments under continuous irradiation. Data are from the iron-fortified experiment.

MA) 501 HPLC system, configured with a Waters model 715 WISP autosampler, a Waters 484 tunable UV detector, and a Packard FLO-ONE/*beta* model BD radioflow detector using FLO-SCINT II (Packard) scintillant at 3 mL/min was used for the analysis. The HPLC used an Advanced Chromatography Technologies Ace 5 4.6 × 250 mm C_{18} column and guard column and a mobile phase of 0.1% trifluoroacetic acid (TFA) and acetonitrile. The gradient began with a 3 min hold at 10% acetonitrile, ramped to 80% acetonitrile at 15 min, and 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 non-radiolabeled reference standards were analyzed with UV detection at 265 nm. Typical retention times were 28 min for niclosamide, 23 min for aminoniclosamide, 24.5 min for 5-chlorosalicylic acid, and 25 min for hydroxyniclosamide. The quantitation of the test substance was carried out from beta detection area response. Identification of degradates was made by coelution of UV 265 nm peaks of non-radiolabeled standards with radioactive peaks.

Thin Layer Chromatography (TLC). TLC was used as a second method of analysis for confirmation 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 the radioactive areas were imaged using an Ambis (San Diego, CA) radioanalytical scanner. Identification was confirmed when radioactive areas overlapped positions corresponding to reference standards.

RESULTS AND DISCUSSION

Environmental Control. A representative graph of the performance of the moisture and temperature control system is shown in **Figure 2**. Moisture and temperature values were recorded every 3 min. The soil moisture was maintained at the sensor value to which the system equilibrated after each sampling. Values greater than the setpoint indicate drying of the soil. The soil moisture level was consistently maintained at or slightly above 75% water holding capacity at 0.33 bar during irradiation. At no time during the irradiation did the soils become dry.

In the unfortified soil experiment, the moisture reading equilibrated to the initial value of 3.31 V after the first two samplings, but at the 110 h sampling the setpoint required adjustment to avoid constant spraying. The initial setpoint of 3.1 V in the 10 ppm of $NaNO_3$ experiment required adjustment at the 40 and 110 h samplings, and the 20 ppm of $NaNO_3$ experiment setpoint was adjusted from 3.1 to 3.6 V at the 110 h sampling. This may be due to the nitrate ions. In the iron-fortified soils, the system equilibrated to the original moisture setpoint of 3.1 V after each sampling.

Humic acid had the most variable effect on the soil moisture reading. The addition of organic matter to soils increases the amount of available water that the soil will hold (*9*). Whereas the initial moisture value in each of the other experiments was 3.1 V, for the humic acid experiment this value dropped to 2.6 V. At the 20 h sampling, the moisture value equilibrated to 3.1 V. After 80 h of irradiation in the humic acid experiment, a brief power outage caused the lamp to shut off. The moisture value was reset to the original 2.6 V.

Soil temperature was maintained at 25 ± 1 °C during irradiation. Short-term temperature spikes occurred when the xenon lamp was ignited after sampling, but temperature control was quickly reestablished.

Microbial Population. Because the test system was able to maintain moisture in the soils, microbial activity was maintained during the course of the moist soil experiments. There was little change in the viabilities of soils fortified with various concentrations of sodium nitrate, ranging from 4.8×10^7 to 6.6×10^7 CFU/g. Previous results (*22*) have shown there is an order of magnitude decrease in the microbial populations of dry soil. This may be a contributing factor to prolonged half-lives in soil when moisture is not maintained.

Distribution of Radioactivity. Material balance was determined from the sum of radioactivity in the acetonitrile/1 N H_3PO_4 extract, in the 1 N NaOH volatile trapping solution, and bound to the soil. **Table 2** summarizes the distribution of radioactivity and material balance for the experiments. Material balance was maintained throughout the study, averaging 97.0 ± 4.5% for the photolysis experiments and 98.3 ± 3.8% for the dark controls.

Sodium Nitrate Experiments. The presence of sodium nitrate in irradiated and dark control soils generally resulted in an overall increase in the amount of extractable radioactivity and a corresponding decrease in bound material (**Table 2**). Increasing the concentration of nitrate from 10 to 20 ppm produced little difference in the extractable and bound radioactivities. Volatile material exhibited an inverse relationship to sodium nitrate concentration in moist irradiated samples. In the moist dark control samples, volatile material decreased only slightly with the increasing sodium nitrate concentration. Less than 1% of the applied radioactivity was recovered as volatile material in the irradiated air-dried soils, and no volatiles were detected in air-dried dark controls. The more important factor affecting the distribution of radioactivity in the test systems was not the increasing concentration of sodium nitrate, but the presence and maintenance of moisture, as indicated in Frank et al. (*22*). The presence of moisture greatly increased the amount of bound radioactivity for both irradiated and dark control soils. Extractable radioactivity in the irradiated experiments was ∼30% less in the moisture-controlled soil than in the air-dried soil ($p = 0.00128$). Seven to 10% of the radioactivity in irradiated air-dried soils was unextractable, compared to 22−35% in moist soils ($p = 0.0139$). Moist dark control soils contained 5−6 times as much bound material as air-dried dark controls.

The presence of fertilizer chemicals can affect the degradation of pesticides. Sodium nitrate reduced the rate of transformation of niclosamide in both irradiated and dark control samples (**Figure 3**). The decline of niclosamide and the degradation products observed in the studies are listed in **Table 3**. Moist irradiated samples fortified with sodium nitrate contained three degradates: aminoniclosamide, hydroxyniclosamide, and 5-chlorosalicylic acid, each in approximately equal concentrations. Whereas the degradation patterns of the moist irradiated samples

Table 2. Distribution of Radioactivity as Percent of Applied

hour	extracted	volatile	bound	total	hour	extracted	volatile	bound	total
	Irradiated Moist Soil, Unfortified					Irradiated Air-Dried Soil, Unfortified			
0	95.7	N/A[a]	4.9	100.5	0	101.8	N/A	1.4	103.1
20	70.6	1.2	16.6	88.4	20	90.4	0.0	7.2	97.6
40	73.5	1.3	16.5	91.3	40	92.2	0.5	6.6	99.4
110	62.9	2.9	21.0	86.9	110	80.0	2.1	8.8	91.0
153	52.3	3.3	35.3	90.9	153	82.5	2.2	10.3	95.0
	Irradiated Moist Soil, 10 ppm of NO_3					Irradiated Air-Dried Soil, 10 ppm of NO_3			
0	96.9	N/A	3.1	100.0	0	101.4	N/A	2.1	103.5
20	86.8	0.2	11.7	98.7	20	97.2	0.0	6.2	103.3
40	78.9	0.5	13.2	92.6	40	92.8	0.1	6.1	99.0
110	69.1	1.9	22.4	93.4	110	90.6	0.5	7.5	98.5
153	64.6	2.5	26.3	93.4	153	89.6	0.7	7.5	97.9
	Irradiated Moist Soil, 20 ppm of NO_3					Irradiated Air-Dried Soil, 20 ppm of NO_3			
0	96.8	N/A	3.8	100.6	0	103.1	N/A	2.7	105.8
20	87.0	0.1	10.5	97.6	20	98.8	0.1	5.7	104.6
40	80.8	0.3	13.6	94.8	40	94.3	0.2	5.6	100.1
110	71.0	1.1	19.7	91.8	110	89.7	0.7	7.6	98.0
153	63.8	1.1	22.3	87.2	153	91.3	0.8	7.5	99.7
	Irradiated Moist Soil, 20 ppm of Fe					Irradiated Air-Dried Soil, 20 ppm of Fe			
0	100.9	N/A	4.0	104.8	0	96.9	N/A	2.3	99.2
20	90.9	0.1	10.3	101.3	20	94.4	0.0	4.1	98.5
40	86.1	0.4	13.4	99.9	40	91.6	0.2	4.5	96.4
110	78.6	1.7	17.1	97.4	110	90.2	0.3	5.1	95.5
153	70.5	2.3	25.9	98.8	153	87.7	0.3	7.3	95.2
	Irradiated Moist Soil, 3% Humic Acid					Irradiated Air-Dried Soil, 3% Humic Acid			
0	95.3	N/A	02.6	97.9	0	97.9	N/A	2.2	101.1
20	84.6	0.0	10.5	95.0	20	95.5	0.0	3.0	98.5
40	79.8	0.1	15.5	95.4	40	93.2	0.0	4.2	97.4
110	72.2	0.6	22.2	95.0	110	89.1	0.1	4.9	94.0
153	57.6	0.7	30.9	89.2	153	88.9	0.1	6.0	95.1
	Dark Control Moist Soil, Unfortified					Dark Control Air-Dried Soil, Unfortified			
0	96.8	N/A	03.0	99.8	0	95.1	N/A	2.2	97.3
20	83.4	1.4	07.3	92.1	20	94.9	0.1	2.5	97.5
40	81.9	1.6	07.9	91.4	40	91.3	0.1	2.3	93.7
112	70.9	2.4	15.5	88.8	112	91.9	0.1	2.5	94.5
160	68.3	2.9	17.9	89.2	160	93.7	0.1	2.5	96.3
	Dark Control Moist Soil, 10 ppm of NO_3					Dark Control Air-Dried Soil, 10 ppm of NO_3			
0	96.9	N/A	3.1	100.0	0	101.4	N/A	2.1	103.5
20	90.6	0.2	5.7	96.4	20	98.5	0.0	2.5	101.0
40	87.2	0.3	7.2	94.8	40	97.1	0.0	2.8	99.8
112	81.7	1.2	13.6	96.5	112	97.6	0.0	3.5	101.1
160	75.1	2.0	19.0	96.2	160	98.3	0.0	2.9	101.2
	Dark Control Moist Soil, 20 ppm of NO_3					Dark Control Air-Dried Soil, 20 ppm of NO_3			
0	96.8	N/A	3.8	100.6	0	103.1	N/A	2.7	105.8
20	92.9	0.1	5.8	98.9	20	100.8	0.0	2.2	103.0
40	89.1	0.3	7.3	96.7	40	100.4	0.0	2.6	103.0
112	83.4	1.0	14.1	98.6	112	101.4	0.0	2.3	103.7
160	79.0	1.7	15.6	96.4	160	101.7	0.0	2.7	104.3
	Dark Control Moist Soil, 20 ppm of Fe					Dark Control Air-Dried Soil, 20 ppm of Fe			
0	100.9	N/A	4.0	104.8	0	96.9	N/A	2.3	99.2
20	97.1	0.1	6.0	103.3	20	98.0	0.0	2.0	100.0
40	91.4	0.3	8.5	100.2	40	96.6	0.0	2.1	98.7
112	85.5	0.9	12.2	98.6	112	97.1	0.0	2.4	99.5
160	80.5	1.3	14.1	96.0	160	94.5	0.0	2.4	96.9
	Dark Control Moist Soil, 3% Humic Acid					Dark Control Air-Dried Soil, 3% Humic Acid			
0	95.3	N/A	2.6	97.9	0	97.9	N/A	2.2	100.1
20	91.7	0.0	5.4	97.0	20	98.4	0.0	1.4	99.8
40	88.4	0.0	7.0	95.3	40	97.0	0.0	1.7	98.7
112	83.3	0.0	13.1	96.3	112	95.8	0.0	1.8	97.7
160	78.5	0.0	13.3	91.8	160	96.2	0.0	2.4	98.6

[a] Not applicable.

were similar, the sodium nitrate-fortified soils exhibited a greater concentration of parent niclosamide, which explains the increased extractability of these samples. The addition of sodium nitrate to moist irradiated soils increased the concentration of extractable niclosamide by a relative 20 and 30% compared to unfortified moist irradiated samples.

In the absence of light, the only degradate formed in moist soils was hydroxyniclosamide. At the end of each moist dark

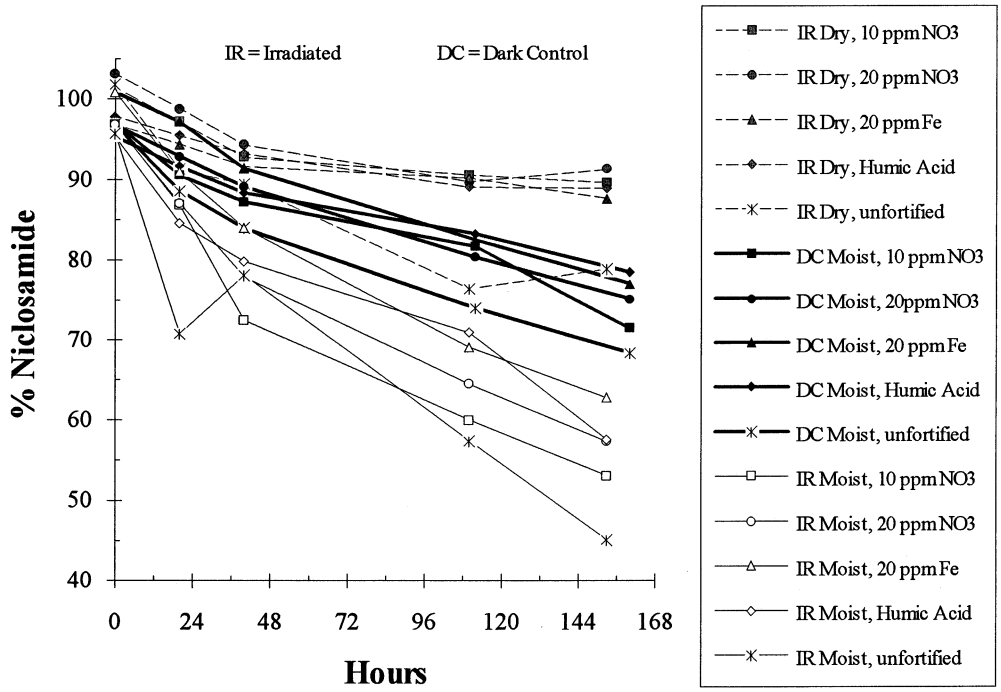

Figure 3. Decline of niclosamide concentrations in soils fortified with various amounts of fertilizer and soil components.

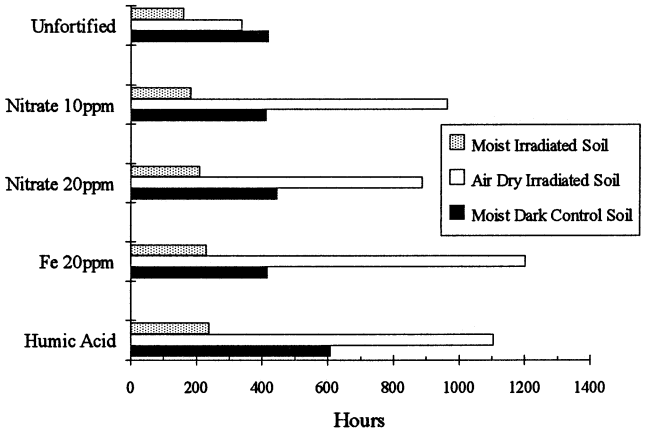

Figure 4. Half-lives of niclosamide in moist and air-dried soil from the irradiated and dark control experiments. There was no degradation of niclosamide observed in the air-dried dark control soils.

control experiment, the concentration of hydroxyniclosamide was the same at 4%. However, the addition of sodium nitrate again resulted in an increase of niclosamide recovered.

As was the case with the distribution of radioactivity throughout the system, the degradation of niclosamide was less affected by the concentration of fertilizer than it was by the maintenance of moisture in the soil. Compared to the three degradates and parent extracted from irradiated moist soils, extracts of air-dried soils (both irradiated and dark control) contained only niclosamide. Irradiated moist soils showed the greatest amount of niclosamide degradation, but irradiated air-dried soils showed less degradation than even the moist dark control samples ($p < 0.01$).

Moisture showed an influence on degradate formation in the dark control samples as well. Hydroxyniclosamide was formed in the later samplings of moist dark control samples, but no degradation was observed in air-dried dark control samples.

With regard to half-life, the presence of sodium nitrate fertilizer tended to increase only slightly the half-life of niclosamide due to the reduction in degradation rate (**Table 3**). The increase in half-life in the irradiated moist soil fortified with 10 μg/g $NaNO_3$ by 20 h was insignificant compared to that in nonfortified moist soil (Student's $t = 1.041$), but photolysis of niclosamide in moist soil containing 20 μg of $NaNO_3$ per gram of soil resulted in a half-life of 211 h ($r^2 = 0.978$), an increase of 50 h over the unfortified soil half-life ($t = 3.536$).

In the dark control samples, only the 20 ppm concentration showed an effect on the half-life of niclosamide. A half-life of 447 h ($r^2 = 0.994$) was reported in the nitrate-fortified soils, whereas the unfortified soils yielded a half-life of 420 h ($t = 7.300$).

When soil moisture is not maintained, the half-lives of pesticides overall are greatly increased. The half-life of niclosamide in unfortified irradiated air-dried soils was twice that of unfortified moist soils. However, in the presence of sodium nitrate, air-dried irradiated samples yielded half-lives 4−5 times greater than the moist irradiated soils (**Table 3**). Even in the dark, a measurable half-life was obtained for moist samples, but the half-life in air-dried soil could not be calculated because the niclosamide did not degrade. Soil composition has a pronounced effect only on the half-lives of compounds in air-dried soil. Nitrate-fortified irradiated air-dried soils had half-lives ~3 times greater than those of unfortified irradiated air-dried soil.

Iron Experiments. The distribution of radioactivity in the 20 ppm iron test system is presented in **Table 2**. As in the nitrate experiments, a higher soil iron content led to an increase in the amount extractable and a decrease in the amount bound (both by 35%) in moist irradiated samples. Volatile radioactivity decreased slightly from 3.3% from unfortified soils to 2.3%. This pattern held true in the dark control soils as well. However, moisture was the determining factor that influenced the distribution of radioactivity. Air-dried soils fortified with iron produced 25% more extractable radioactivity ($p = 0.000365$) and a 73% decrease in the amount of bound material ($p = 0.000166$).

Iron also decreased the extent of degradation of niclosamide in the irradiated experiments (**Figure 3** and **Table 3**). Moist irradiated iron samples showed the same three degradates as

Table 3. Effect of Fertilizer and Soil Components on Niclosamide Degradation and Half-Life

soil	% niclosamide[a]	half-life (h)	degradation product(s)[b]	soil	% niclosamide	half-life (h)	degradation product(s)
	Irradiated, Moisture-Maintained Soils				Irradiated, Air-Dried Soils		
unfortified	45.1	162	Am, CSA, OH	unfortified	078.9	340	none
10 ppm of NO_3	53.1	183	Am, CSA, OH	10 ppm of NO_3	089.6	965	none
20 ppm of NO_3	57.3	211	Am, CSA, OH	20 ppm of NO_3	091.3	890	none
20 ppm of Fe	62.8	231	unknown 1, Am, CSA, OH	20 ppm of Fe	087.7	1204	none
humic acid	57.6	239		humic acid	088.9	1106	none
	Dark Control, Moisture-Maintained Soils				Dark Control, Air-Dried Soils		
unfortified	68.4	420	OH	unfortified	098.4	N/A[c]	none
10 ppm of NO_3	71.5	413	OH	10 ppm of NO_3	098.3	N/A	none
20 ppm of NO_3	75.2	447	OH	20 ppm of NO_3	101.7	N/A	none
20 ppm of Fe	77.1	418	OH	20 ppm of Fe	094.5	N/A	none
humic acid	78.5	611		humic acid	096.2	N/A	none

[a] Percent of applied niclosamide remaining after final sampling. [b] Am = aminoniclosamide; CSA = 5-chlorosalicylic acid; OH = hydroxyniclosamide. [c] Not applicable—niclosamide not degraded.

the unfortified moist soils (aminoniclosamide, hydroxyniclosamide, and 5-chlorosalicylic acid) plus one other, unknown 1, eluting just before aminoniclosamide by HPLC. This compound coeluted with salicylic acid by HPLC, but it did not match salicylic acid using the TLC method. Hydroxyniclosamide and 5-chlorosalicylic acid were present in approximately the same amounts as were formed in the unfortified control study, 2.5 and 2%, respectively. Aminoniclosamide declined slightly in the 153 h iron sampling to 1.6%, compared to the 5% remaining at the end of the unfortified study. Unknown 1 reached 2% in the final irradiated sampling. The result is a half-life of niclosamide in iron-fortified soils of 231 h ($r^2 = 0.983$), compared to 162 h in unfortified soil ($t = 5.559$).

In moist dark control soil, hydroxyniclosamide was the only degradate, as was the case in the sodium nitrate experiment. The maximum concentration of hydroxyniclosamide occurred at the final sampling, attaining 3.5% of applied. The concentrations of hydroxyniclosamide in each of the iron and nitrate moist dark control experiments were approximately the same. The half-life calculated from the moist dark control samples was equal to those from the 10 ppm nitrate and unfortified experiments.

As with the sodium nitrate experiments, moisture was more important to the degradation of niclosamide than was soil composition. If moisture was not maintained, less transformation of niclosamide through degradation or binding to the soil occurred. As a result, an irradiated half-life of 1204 h was calculated ($r^2 = 0.906$). The dark control air-dried half-life could not be calculated due to insignificant degradation of niclosamide, resulting in a rate of reaction indistinguishable from 0 ($t = 1.900$).

Humic Acid Experiment. Humic acid showed the most pronounced effects of the soil additives. The extractable and bound radioactivities were approximately equal to those from the unfortified soils (**Table 2**). However, no volatiles evolved from the moist dark controls as in the other experiments. The degradation profile was significantly changed with the higher soil organic content (**Figure 3** and **Table 3**). The other moist irradiated experiments produced at least three degradates, but only aminoniclosamide was observed in the humic acid fortified moist samples, and that at only 110 h of irradiation. We attribute this to the filter effect of humic substances due to their strong light-absorbing properties. Even in the dark controls, no hydroxyniclosamide was observed. Whereas the half-life was only nominally increased compared to those of the 10 ppm nitrate and unfortified irradiated moist soil experiments ($t = 3.781$), the moist dark control half-life was increased from an average of 425 to 611 h ($t = 5.621$), with $r^2 = 0.979$.

The effect of moisture observed in the other experiments was also observed in the humic acid experiments. In the studies conducted on air-dried soils, there was an increase in the amount of extractable radioactivity, a corresponding decrease in bound material, and negligible volatiles compared to the moist soils. When moisture is not maintained, the irradiated half-life of niclosamide rose from 239 to 1106 h ($r^2 = 0.916$), a 4.6-fold increase in line with the increases observed by the nitrate- and iron-fortified experiments ($t = 1.387$). The levels of niclosamide recovered at each sampling of the air-dried dark control experiment did not indicate a significant decline to permit a meaningful calculation of the half-life ($r^2 = 0.7395$). The probability that the rate of reaction is 0 is 0.0433.

CONCLUSION

The expected enhancement of niclosamide degradation due to increased reactive species generation with varying soil composition or soil additives was not observed in moist irradiated samples. In fact, the irradiated half-lives of niclosamide in moist soil with the various additives were marginally increased over the half-life of niclosamide in unfortified moist soil. Organic content has an effect on niclosamide soil photolysis, more so than iron content or the presence of fertilizer. Moist irradiated soil with a higher organic content exhibited more bound material and a different degradation pattern. The result was a half-life approximately equal to moist soil with higher iron content ($t = 0.280$). It has been shown, however, that the influence of humic substances is dependent upon the pesticide under study (*18*, *19*). Only in the dark control moist soil was the effect of soil composition (humic acid or iron) significant. Increased humic acid imparted a 44% longer half-life on niclosamide under these conditions.

Independent of the organic composition of the soil, aminoniclosamide, hydroxyniclosamide, and 3-chlorosalicylic acid were formed in each of the irradiated moist soil experiments. The concentration of each degradate ranged from 1.7 to 7.8%. Failure to maintain moisture in irradiated experiments, however, yielded no extractable degradates. In the dark control when moisture was maintained, only hydroxyniclosamide was formed regardless of the nitrate or iron composition of the soil. In the air-dried soils, niclosamide was not degraded at all. This illustrates that moisture is the single most important variable in a soil photolysis study.

Iron-rich soil produced a compound not observed in the other moist irradiated experiments. Unknown 1 may result from transformation of aminoniclosamide in the presence of iron, on the basis of the decrease in aminoniclosamide concentration in the iron-fortified soils compared to the unfortified samples. Soil composition is thus shown to influence the pesticide degradation pattern.

Whereas soil composition exhibited only a slight effect on the irradiated half-life of niclosamide when soil moisture was maintained, the presence of fertilizer material or increased iron or humic acid content greatly influenced the irradiated half-life of niclosamide in air-dried soil. Calculated half-lives of niclosamide in air-dried soil varied up to 3-fold, depending on soil composition. The irradiated half-life in iron-fortified air-dried soils was ~33% longer than that in the irradiated air-dried soils containing nitrate and 4 times that of the unfortified irradiated air-dried experiment. Dry soil samples also produced none of the degradates that were observed in the moist experiments.

Clearly from these experiments moisture is the single most critical factor regulating the degradation of agrochemicals by soil photolysis. This is particularly emphasized by the various degradation patterns exhibited by the moist and air-dried experiments. To accurately predict the fate of chemicals in the environment, experimental conditions must accurately reflect and maintain the environmental conditions to which pesticides are exposed.

ABBREVIATIONS USED

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

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LITERATURE CITED

(1) Vitousek, P. M. Beyond Global Warming: Ecology and Global Change. *Ecology* **1994**, *75*, 1861−1876.

(2) Hatch, A. C.; Belden, L. K.; Scheessele, E.; Blaustein, A. R. Juvenile Amphibians Do Not Avoid Potentially Lethal Levels of Urea on Soil Substrate. *Environ. Toxicol. Chem.* **2001**, *10*, 2328−2335.

(3) Buxton, G. V.; Greenstock, C. L. Critical Review of Rate Constants for Reactions of Hydrated Electrons, Hydrogen Atoms, and Hydroxyl Radicals (•OH/•O$^-$) in Aqueous Solution. *J. Phys. Chem. Ref. Data* **1988**, *17*, 513.

(4) Barnes, A. R.; Sugden, J. K. The Hydroxyl Radical in Aqueous Media. *Pharm. Acta Helv.* **1986**, *61*, 218

(5) Takahashi, N. M. I.; Mikami, N.; Matsuda, T.; Miyamoto, J. Identification of Reactive Oxygen Species generated by Irradiation of Aqueous Humic Acid Solution. *J. Pestic. Sci.* **1988**, *13*, 429.

(6) Draper, W. M.; Crosby, D. G. The Photochemical Generation of Hydrogen Peroxide in Ground Waters Exposed to Sunlight. *Science* **1983**, *220*, 711.

(7) Khan, S. U.; Gamble, D. S. Ultraviolet Irradiation of an Aqueous Solution of Prometryn in the Presence of Humic Acids. *J. Agric. Food Chem.* **1983**, *31*, 1099.

(8) Kotzias, D.; Huster, K.; Wieser, A. Formation of Oxygen Species and Their Reactions with Organic Chemicals in Aqueous Solution. *Chemosphere* **1987**, *16*, 1463.

(9) Berger, K. C. *Introductory Soils*, MacMillan: New York, 1965.

(10) Faust, A. Review of the Photochemical Redox Reactions of Iron-(III) Species in Atmospheric, Oceanic, and Surface Waters: Influences on Geochemical Cycles and Oxidant Formation. In *Aquatic and Surface Photochemistry*; Helz, G. R., Zepp, R. G., Crosby, D. G., Eds.; Lewis Publishers: Chelsea, MI, 1994; pp 3−37.

(11) Finden, D. A. S.; Tipping, E.; Jaworski, G. H. M.; Reynolds, C. S. Light-Induced Reduction of Natural Iron(III) Oxide and Its Relevance to Phytoplankton. *Nature (London)* **1984**, *309*, 783.

(12) Xu, J. C.; Stucki, J. W.; Wu, J.; Kostka, J. E.; Sims, G. K. Fate of Atrazine and Alachlor in Redox-Treated Ferruginous Smectite. *Environ. Toxicol. Chem.* **20**, *12*, 2717−2724.

(13) Stucki, J. W.; Komadel, P.; Wilkinson, H. T. Microbial Reduction of Structural Iron(III) in Smectites. *Soil Sci. Soc. Am. J.* **1987**, *51*, 1663−1665.

(14) Wu, J.; Roth, C. B.; Low, P. F. Biological Reduction of Structural Iron in Sodium Nontronite. *Soil Sci. Soc. Am. J.* **1988**, *52*, 295−296.

(15) Kostka J. E.; Stucki, J. W.; Nealson, K. H.; Wu, J. Reduction of Structural Fe(III) in Smectite by a Pure Culture of *Shewanella putrefaciens* Strain MR-1. *Clays Clay Minerals* **1996**, *44*, 522−529.

(16) Miller, G. C.; Donaldson, S. G. Factors Affecting Photolysis of Organic Compounds on Soils. In *Aquatic and Surface Photochemistry*; Helz, G. R., Zepp, R. G., Crosby, D. G., Eds.; Lewis Publishers: Chelsea, MI, 1994; pp 97−109.

(17) Gohre, K.; Scholl, R.; Miller, G. C. Singlet Oxygen Reactions on Irradiated Soil Surfaces. *Environ. Sci. Technol.* **1986**, *20*, 934.

(18) Frimmel, F. H.; Hessler, D. P. Photochemical Degradation of Triazine and Anilide Pesticides in Natural Waters. In *Aquatic and Surface Photochemistry*; Helz, G. R., Zepp, R. G., Crosby, D. G., Eds.; Lewis Publishers: Chelsea, MI, 1994; pp 137−147.

(19) Vialaton, D.; Pilichowski, J.-F.; Baglio, D.; Paya-Perez, A.; Larsen, B.; Richard, C. Phototransformation of Propiconazole in Aqueous Media. *J. Agric. Food Chem.* **2001**, *49*, 5377−5382.

(20) Muir, D. C. G.; Yarechewski, A. L. Degradation of Niclosamide (2′,5-Dichloro-4′-nitroslicylanilide) in Sediment and Water Systems. *J. Agric. Food Chem.* **1982**, *30*, 1028−1032.

(21) Misra, B.; Graebing, P. W.; Chib, J. S. Photodegradation of Chloramben on a Soil Surface: A Laboratory-Controlled Study. *J. Agric. Food Chem.* **1997**, *45*, 1464−1467.

(22) Frank, M. P.; Graebing, P. W.; Chib, J. S. Effect of Moisture and Sample Depth on Soil Photolysis. *J. Agric. Food Chem.* **2002**, *50*, 2607−2614.

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