Simultaneous Derivatization and Trapping of Volatile Products from Aqueous Photolysis of Thiamethoxam Insecticide

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An aqueous photolysis study was conducted with radiolabeled thiamethoxam, 4*H*-1,3,5-oxadiazin-2-imine, 3-[(2-chloro-5-thiazolyl)methyl]tetrahydro-5-methyl-*N*-nitro, to establish the relevance of aqueous photolysis as a transformation process for ^{14}C-[thiazolyl]-thiamethoxam. ^{14}C-[thiazolyl]-thiamethoxam was applied to sterile sodium acetate pH 5 buffer solution at a dose rate of approximately 10 ppm. The resulting samples were incubated for up to 30 days at 25 °C under irradiated and nonirradiated conditions. The irradiated samples were exposed to a 12-hour-on and 12-hour-off light cycle. Volatile fractions accounted for up to an average of 56.76% of the total dose for the irradiated incubations and <0.08% for the nonirradiated incubations. These fractions were proposed to be a mixture of carbonyl sulfide (COS) and isocyanic acid (CONH). Verification of these components was accomplished by trapping with cyclohexylamine and formation of the thiocarbamate and the isocyanic acid derivatives. A similar method of trapping thiocarbamate metabolites was reported (Chen and Casida, 1978) where filter paper saturated with isobutylamine in methanol was arranged to trap ^{14}COS and $^{14}CO_2$ under a positive flow of O_2 at 25 °C. Mass spectroscopy of the derivatized components confirmed the presence of carbonyl sulfide as the cyclohexylamine thiocarbamate and of isocyanic acid as its cyclohexylamine derivative. Evidence from this study indicates that thiamethoxam degrades significantly under photolytic conditions.

Keywords: *Pesticides, photolysis; volatiles; derivatization*

INTRODUCTION

Thiamethoxam insecticide, 4*H*-1,3,5-oxadiazin-2-imine, 3-[(2-chloro-5-thiazolyl)methyl]tetrahydro-5-methyl-N-nitro, is a member of the chemical class called the neonicotinoids. Thiamethoxam systematically controls a broad spectrum of chewing pests and sucking pests and is a developmental insecticide to be distributed for worldwide use in a variety of agricultural crops, seed treatment, turf, ornamental, and pet use.

MATERIALS AND METHODS

Chemicals. ^{14}C-[Thiazolyl]-thiamethoxam (specific activity = 43.4 μCi/mg or 1.61 MBq/mg, radiochemical purity = 98.5%, chemical purity = >99.9%), carbonyl sulfide gas (purity = 97.5+%), cyclohexylamine (purity = 99+%), and the following nonlabeled reference standards were used for cochromatography purposes only: component A, thiamethoxam, 4*H*-1,3,5-oxadiazin-2-imine, 3-[(2-chloro-5-thiazolyl)methyl]tetrahydro-5-methyl-*N*-nitro, (purity 98.9%); component F, 4*H*-1,3,5-oxadiazin-4-one, 3-[(2-chloro-5-thiazolyl)methyl]tetrahydro-5-methyl-, (purity 99.9%); and component J, urea, *N*-[(2-chloro-5-thiazolyl)methyl]-*N*′-methyl-, (purity > 99.9%). The nonlabeled reference standards were dissolved in acetonitrile and stored at less than −2 °C. The radiolabeled ^{14}C-[thiazolyl]-thiamethoxam was dissolved in acetonitrile as a cosolvent for application purposes but was stored as a neat solid when not in use at less than −2 °C. The radiochemical purity and chemical purity for the thiamethoxam were determined by thin-layer chromatography (TLC) prior to application.

Apparatus. Borosilicate photolysis vials, 2 × 2 × 5 cm, were custom-made with seven threads/cm and sealed with open-top screw caps and Teflon-coated (10 μm) silicon (125 μm thickness) septa (20 mm diameter). The artificial sunlight was simulated with a Suntest CPS+ photolysis unit (Suntest accelerated exposure unit, W. C. Heraeus, Hanau, Germany). The light source was a xenon arc lamp equipped with a quartz glass dish with a selective reflecting coating and a UV glass filter. These filters and a Pyrex plate placed over the samples absorb wavelengths below 290 nm to simulate natural sunlight. Total intensity natural sunlight measurements were made at Novartis, Greensboro, NC using an International Light IL-1700 with a SUD400 UV/vis detector and a Heraeus Radialux equipped with a Radialux global sensor. Natural and artificial spectral distributions were made using an International Light IL-1700, an International Light IL-760 power supply, and an International Light IL-790 Kratos double monochromator.

Light Intensity Determination. The average daily irradiation from the sun is 75% of the peak intensity over a 12-hour period (Parker and Leahey, 1988). The maximum or peak natural sunlight intensity was measured at Novartis, Greensboro, NC, at 36°5.86′N latitude and 79°56.24′W longitude. The peak intensity was 534 W/m^2; therefore, 534 × 75% is an average day intensity of 400.5 W/m^2. A spectral distribution was measured when the total intensity was 410 W/m^2. The natural sunlight spectral distribution was measured from 200 to 700 nm, whereas the artificial light spectral distributions were measured from 250 to 700 nm. Each spectral distribution was measured in 10 nm increments The natural sunlight spectral distribution from 300 to 400 nm, the most photolytically relevant range, was used to set the xenon lamp intensity of the Suntest CPS+ instruments. A ratio of natural sunlight to artificial light of 1.0 was used for each 10 nm increment as an ideal state. Spectral distribution of each Suntest Unit was measured prior to and after sample incubation. During the Suntest set up and spectral distribution measurements, a borosilicate filter made from sample vials was utilized to account for the transmission characteristics of the glass.

Determination of Radioactivity. The radioactivity in solution was determined by liquid scintillation counting (LSC,

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Beckman model 6000 or 6500) using either Fisher Scintisafe or Scintisafe Gel scintillation cocktail with a background value determined by assaying an equivalent volume of cocktail in absence of sample. Fisher Scintisafe Gel (10 mL) scintillation cocktail was used as part of the background for TLC quantitation. Aliquots of the cyclohexylamine volatile trap solutions were mixed with 5 mL of Fisher Scintisafe scintillation cocktail. Aliquots of the potassium hydroxide volatile trap solutions were mixed with 10 mL of Packard Hionic scintillation cocktail. Counting efficiencies were determined by external standardization. Limits of quantitation and detection were established by statistical methods of radioactivity counting.

The radiolabel material balance was determined for each sample on the day of harvest. The aqueous fractions and volatile traps were radioassayed by LSC. The total radiochemical balance for each sample is equivalent to the sum of the percent total dose for each of the aqueous and the combined volatile fractions.

Thiamethoxam Application. An aliquot of the ^{14}C-[thiazolyl]-thiamethoxam in acetonitrile was added to the pH 5, 0.01 M sodium acetate buffer to prepare a 9.41 ppm dose solution. The dose rate was calculated based on nine radioassays at pre-, mid-, and postdose. The dose solution was filtered through a sterile 0.2 μm disposable filtering unit for sterilization. The dosing area was sterilized by spray with an ethanol/water (70:30) solution. All sample vials, glassware, and any utensils needed for dosing were autoclaved prior to use. The dose solution (10 mL) was aseptically pipetted into each sample vial. Aseptic conditions were monitored at selected times throughout the experiment by inoculation with treated surrogate samples onto plate count agar (Difco) Petri dishes.

Sample Incubation. Irradiated sample vial caps were sealed with a glue gun (All Purpose Hot Melt Glue Stix, Arrow Fastener Company, Inc.) and wrapped with Parafilm to ensure a tight seal. The sample vials were placed on their sides in a water bath to allow maximum exposure to the artificial light source. A recirculating pump to maintain the temperature at 25 ± 1 °C fed the water bath. The temperature of the water bath was monitored by an Omega thermocouple, an Omega data logger, and computer support. The Omega thermocouple was inserted into a surrogate vial containing 10 mL of sodium acetate buffer. The samples were irradiated for 12 h per day, for up to 30 days.

Dark control (nonirradiated) samples were sealed in the same manner as the irradiated set and wrapped in aluminum foil. The samples were placed in an aluminum foil lined box to ensure no light reached the samples. The nonirradiated samples were incubated up to 30 days in the Environmental Specialties Incubation Chamber (model 9-19 TC/GC). The temperature and humidity were monitored with a Honeywell hydrothermograph, and the temperature was also monitored by an Omega thermocouple, an Omega data logger, and computer support.

Sample Harvest. Duplicate irradiated and nonirradiated samples were harvested at 0, 6, and 12 h and on days 1, 2, 3, 5, 7, 14, 21, and 30. The duplicate irradiated and nonirradiated samples were purged for approximately 20 min postharvest to collect volatiles. Following volatile collection, the volumes of each sample were measured and each sample was radioassayed. The volumes of the volatile traps were measured and each volatile trap was radioassayed. The radiochemical balance was calculated. The volumes were measured by drawing the entire sample into a sterile 10 mL disposable glass serological pipet. Aliquots (1 mL) of each sample were transferred to a 7 mL vial in order to determine the pH of each sample. Samples were stored below −2 °C when not in use.

Volatile Collection. Zero hour samples were not purged for volatile collection. All other samples were purged immediately after harvest using the purge system shown in Figure 1. The purge system consisted of a gang valve with four ports, the sample vial, an empty trap, two to five cyclohexylamine traps, and two KOH traps (10% aqueous). Nitrogen was sparged through the headspace of the sample and into the trap solutions. The flow rate ranged from 115 to 175 mL/min.

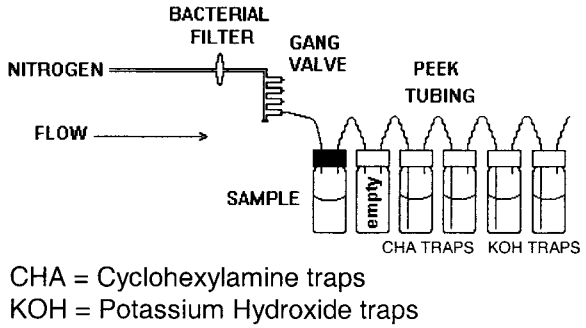

Figure 1. Representative Volatile Trapping System: CHA = cyclohexylamine traps, KOH = potassium hydroxide traps

The derivatization traps were either a 1% or a 10% cyclohexamine solution in absolute ethanol. In this solution, the volatile photolysis products, isocyanic acid and carbonyl sulfide, are derivatized and trapped as the cyclohexyl thiourea and urea derivatives. The 6 and 12 h and 1−3 day samples were purged into 1% solutions of the cyclohexylamine. The 6 and 12 h and 1 day samples were purged into two cyclohexylamine traps in series followed by the two potassium hydroxide traps. A third cyclohexylamine trap was added in line for purging the headspace of the 2 day samples. A fourth cyclohexylamine trap was added in line for purging the headspace of the 3 day samples. On the fifth day, the concentration of the cyclohexylamine traps was increased to 10% in absolute ethanol, and a fifth cyclohexylamine trap was added to the series of traps. From days 7 through 30, four traps with 10% cyclohexylamine in absolute ethanol were followed by two KOH traps in series for the trapping system. The additional traps and increasing the cyclohexylamine concentration ensured maintenance of material balance above 90%.

Between the nitrogen gas line and the sample was a sterile Bactivent filter (0.1 μm pore size, 37 mm diameter, Gelman Sciences) to ensure the incoming nitrogen was sterile. Filters were replaced every 7 days or less just prior to collecting volatiles. The cyclohexylamine traps consisted of a 40 mL vial containing 20 mL of the 1% or 10% v/v absolute ethanol solution. The KOH traps consisted of two 40 mL vials containing 20 mL of a 10% w/v aqueous KOH solution. Each volatile trap was sealed with an open-top screw cap and a Teflon (Tuf-Bond Disks, Pierce Chemical Co.)-coated septum. These volatile traps were connected by Peek (poly ether ether ketone, Alltech Associates, Inc.) 0.02" ID tubing from the headspace of the first trap into the solution of the next consecutive trap. The 18 gauge needle was inserted through the septum of the final KOH trap to provide an outlet for the positive flow of nitrogen through the trapping system.

Chromatographic Methods. The aqueous fractions from both the irradiated and nonirradiated set of samples were assayed by TLC and LSC on the day of harvest. Aliquots (25 μL) of each sample were applied to the origin of two TLC plates (0.25 mm thick silica gel, 60F fluorescence indicator, 254 λ, 20 cm × 20 cm, Merck). One plate was prepared for quantitation, and the second plate was prepared for cochromatography with reference standards. An aliquot (2−3 μL) of reference standard solution was applied to the origin and the margins of the second TLC plate. Each plate was developed in dichloromethane/methanol (90:10, v/v) (system 1) and chloroform/methanol/ammonium hydroxide/water (80:30:4:2, v/v/v/v) (system 2). The R_f values of thiamethoxam were 0.31 in system 1 and 0.46 in system 2. Radioactive zones were visualized with a bioimaging analyzer, BAS 2000 (Fuji, Inc.). The mass spectral analysis was performed on a Finnigan TSQ-7000 LC/MS in the infused electrospray positive ionization mode. The mobile phase started at 95% water with a linear gradient to 100% MeOH (0.1% HCOOH) at a flow rate of 0.4 mL/min in a 2 × 150 mm Inertsil C8 column.

RESULTS AND DISCUSSION

Material Balance. Tables 1 and 2 contain the irradiated and nonirradiated radiolabel material bal-

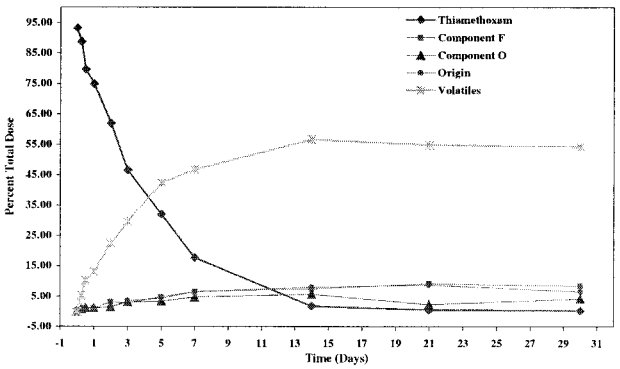

Figure 2. Decline of Parent and Accumulation of Degradates in the Irradiated Sample Set

Table 1. Radiolabel Material Balance for the Irradiated Sample Set

irradiated	total dose aq. fraction	total dose volatile fraction	total percent material balance.
0 h	96.22	N/A	96.22
6 h	93.40	5.23	98.63
12 h	86.80	10.22	97.03
1 day	82.44	13.06	95.50
2 day	73.19	22.40	95.59
3 day	62.37	29.60	91.97
5 day	53.22	42.39	95.62
7 day	44.92	46.75	91.68
14 day	34.93	56.75	91.68
21 day	38.40	54.87	93.26
30 day	35.50	54.29	89.70
av mater. bal.			94.27 ± 2.74%

Table 2. Radiolabel Material Balance for the Nonirradiated Sample Set

nonirradiated	total dose aq. fraction	total dose volatile fraction	total percent material balance
0 h	97.08	N/A	97.08
6 h	98.57	0.06	98.62
12 h	96.91	0.07	96.97
1 day	95.26	0.05	95.31
2 day	96.73	0.05	96.78
3 day	96.62	0.05	96.67
5 day	97.38	0.04	97.42
7 day	96.98	0.06	97.04
14 day	98.18	0.05	98.23
21 day	98.12	0.05	98.17
30 day	96.05	0.05	96.11
av mater. bal.			97.13 ± 0.97%

ances for each sample set, respectively. The material balance was maintained at >90% for both sets of samples.

The amount of radioactivity corresponding to thiamethoxam and the major degradates in the aqueous fraction were calculated based upon TLC analysis. Graphical representations of the thiamethoxam and its degradates in the irradiated and nonirradiated sample sets are shown in Figures 2 and 3, respectively.

Major Degradates. The volatile fraction contained the major degradate for the irradiated set of samples (Figure 2). The volatiles accumulated to a maximum average of 56.76% and plateaued after 14 days of incubation. The percent of total dose at each sampling of the aqueous fractions was observed to decline while the volatile fraction percent of total dose increased. After 30 days of incubation, less than 0.5% of the total dose remains as undegraded parent in the irradiated set of samples, whereas greater than 90% of the total dose remained as parent in the nonirradiated set of samples. Several components in the aqueous fraction were minor,

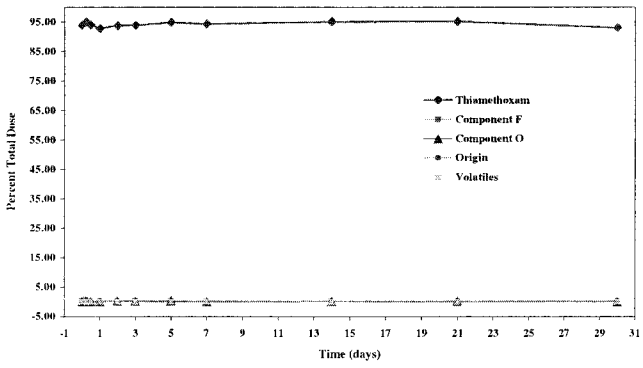

Figure 3. Decline of Parent and Accumulation Of Degradates in the Nonirradiated Sample Set

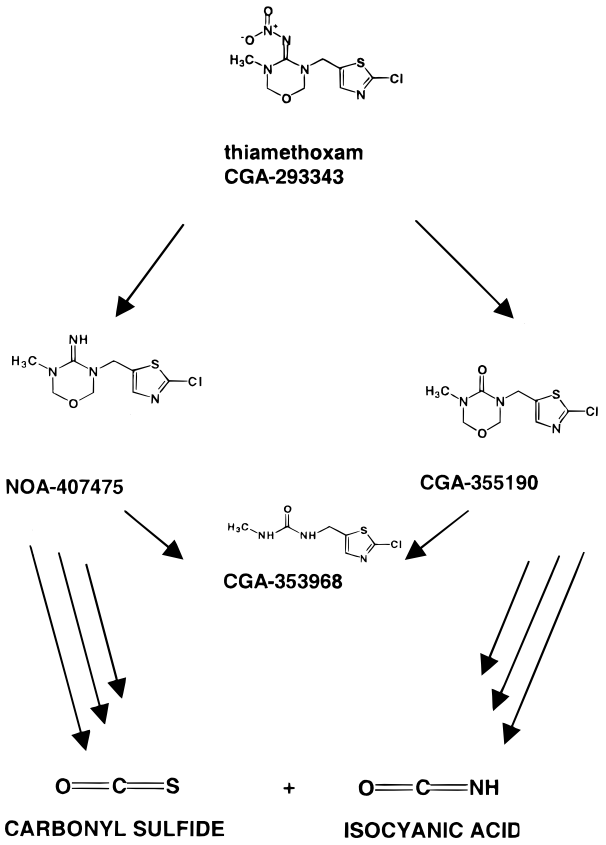

Figure 4. Pathway for the Photodegradation of ^{14}C-[Thiazolyl]-thiamethoxam in pH 5 Buffered Solution under Artificial Light

representing less than 10% of the total dose (components E and J). Minor components that accumulated between 2% and 10% of the total dose in the aqueous fraction include components F and O and Origin. Degradates of the aqueous fraction were identified by two-dimensional thin-layer chromatography (components A, E, F, and J) and cochromatographed with reference standards. Components A and F were isolated and identified by mass spectral analysis in the study of the photolytic degradation of ^{14}C-[guanidine]-CGA-293343 (Sparrow, 1997). The proposed pathway for the degradation of ^{14}C-[thiazolyl]-thiamethoxam is shown in Figure 4.**Mass Spectroscopy of the Volatile Component.** The volatile fraction has been proposed to be a mixture of carbonyl sulfide (COS) and isocyanic acid (CONH). The volatile components were trapped by derivatization with cyclohexylamine. Carbonyl sulfide gas was derivatized in cyclohexylamine traps to syn-

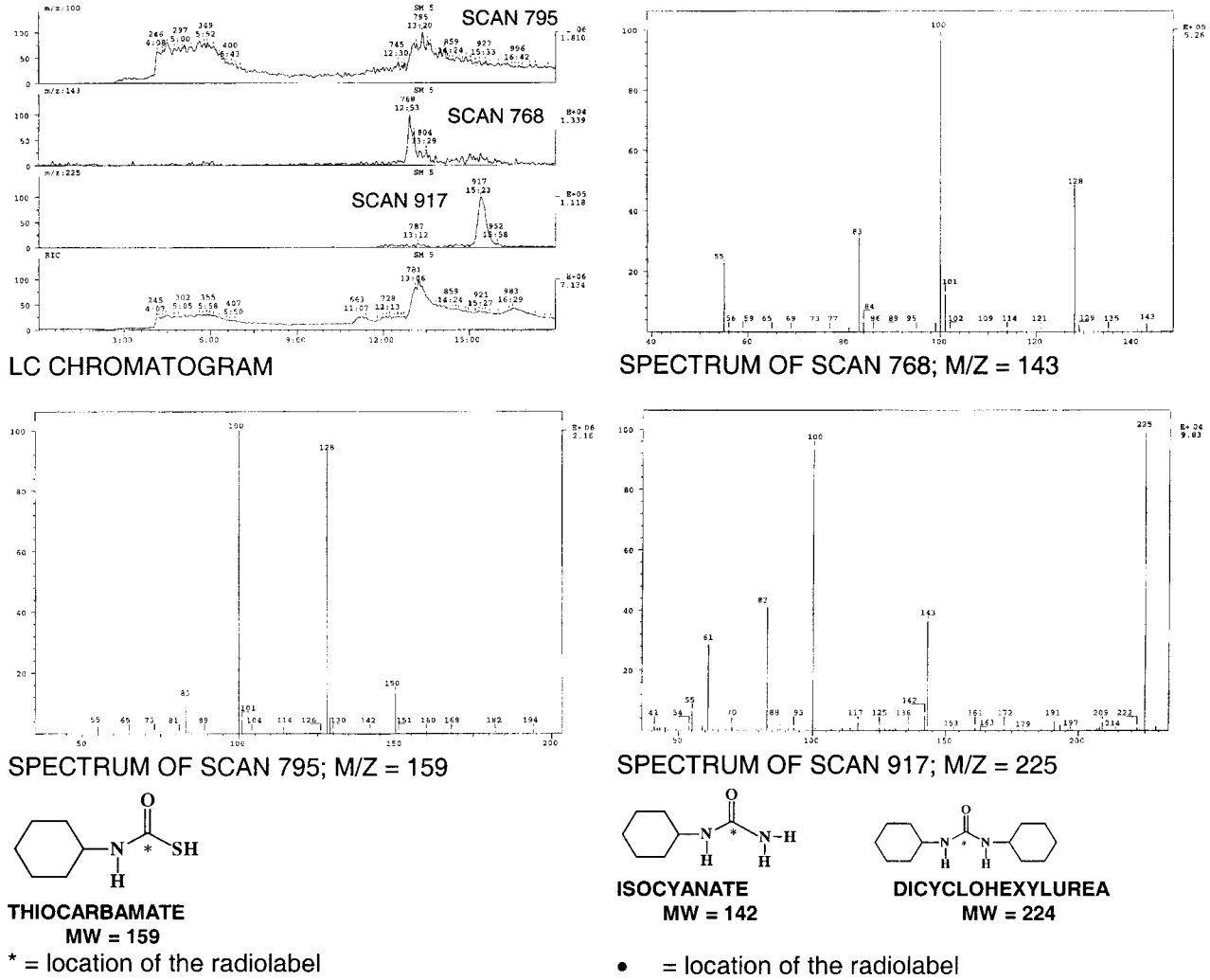

Figure 5. LC Chromatogram and Mass Spectra of the Derivatized Volatile Components Thiocarbamate, Isocyanate, and Dicyclohexylurea

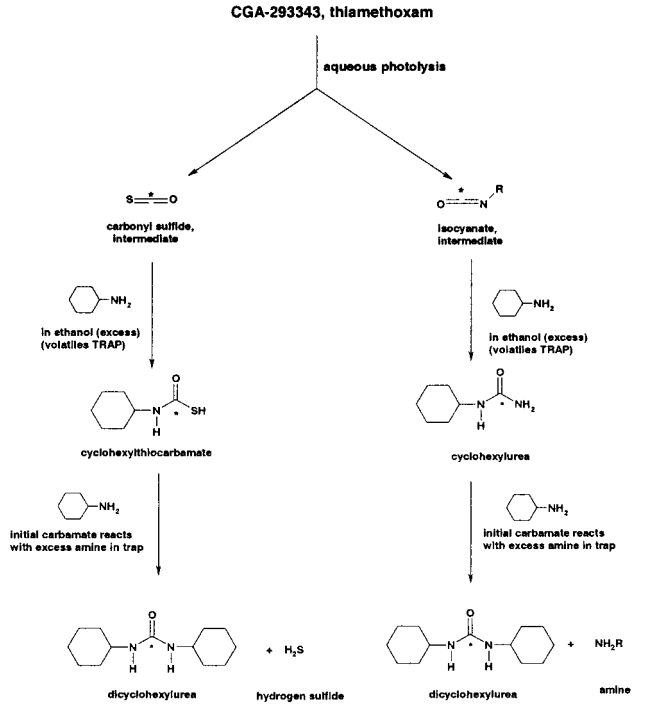

Figure 6. Proposed Route of Volatile Trapping and Decomposition

thesize a standard for use in the mass spectral analysis of the volatile component. Mass spectroscopy of the derivatized volatile component confirms the presence of carbonyl sulfide as the cyclohexylamine thiocarbamate and of isocyanic acid as its cyclohexylamine derivative (Figure 5). Figure 6 represents the proposed route of volatile trapping and decomposition in the cyclohexylamine traps.

CONCLUSION

Evidence from this study and the photolytic degradation of the ^{14}C-[guanidine]-thiamethoxam (Sparrow, 1997) indicate that this insecticide degrades significantly under photolytic conditions. Conventional trapping methods (ethylene glycol, foam plugs, potassium hydroxide) did not provide radiolabel material balance under artificial photolytic conditions. The volatile components of ^{14}C-[thiazolyl]-thiamethoxam were simultaneously trapped and derivatized by a series of room-temperature traps containing cyclohexylamine. Perhaps this system can be utilized in other investigations of volatile chemicals.

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