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CORPORATION

CYROMAZINE

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CYROMAZINE

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Accumulation and elimination of ^{14}C residues in bluegill sunfish (*Lepomis macrochirus*) exposed to ^{14}C-CGA-72662, prepared for Ciba-Geigy Corporation by EG + G, Bionomics, December, 1980, Report No. BW-80-12-805, Acc. No. 070914, Reference 2.

Procedure

Flow-through test. One hundred fifty bluegill sunfish (Lepomis macrochirus) were exposed to uniformly ring-labeled [^{14}C]cyromazine (CGA-72662, 1.0 ppm nominal concentration) in a flow-through test. A second group of 150 fish was maintained as a control in a clean aquarium. After 28 days, exposed fish were transferred to a clean aquarium for a 14-day depuration period.

Radioactive residues in the water of the control and test aquariums were measured on days 0, 1, 3, 7, 10, 14, 21, and 28 of the exposure period, and on days 1, 3, 7, 10, and 14 of the depuration period. Accumulation and elimination of ^{14}C residues in edible (muscle) and non-edible (viscera and carcass) tissues of four fish/aquarium were determined on the same days during exposure and depuration that water samples were taken.

Water quality characteristics were as follows: dissolved oxygen: 7.0-8.5 ppm; pH: 6.8-7.0; temperature: 20-23 C; and total hardness: 26-30 µg/ml as $CaCO_3$. Fish were fed a dry pelleted food ad libitum daily; there was 0.3% mortality during the 2-day period prior to test initiation.

In a second flow-through test 60 bluegill sunfish were exposed for 3 days. Experimental conditions were identical to those described previously. Water and fish samples were taken daily.

Static test. After 3 days of exposure in the second flow-through test, 30 bluegill sunfish were placed in 10 liters of water under static conditions. The fish were exposed to [^{14}C]cyromazine at a nominal concentration of 1.0 ppm. After 4 days, ^{14}C residues in the water and in tissues (muscle, viscera and carcass) from 20 fish were characterized.

Water quality characteristics were as follows: dissolved oxygen: 4.8-8.6 ppm; pH 6.6-7.1; and the temperature was 21 ± 1 C.

Methodology

Radioactivity of the water samples was determined by using liquid scintillation counting (LSC). Muscle tissue (edible) and viscera and carcass tissues (non-edible) from each fish were weighed, dried, and combusted, and evolved $^{14}CO_2$ was trapped and analyzed by using LSC.

The minimum detectable limits of ^{14}C residues in water was >0.0076< 0.0090 ppm, the minimum detectable limits in edible and non-edible tissues were >0.068 <0.42 and >0.067 <0.46 ppm, respectively. In all cases, limits of detectability varied due to differences in sample weights and counting efficiencies.

Results

Flow-through test. No mortality was observed in the exposure aquarium after the test began; one fish died in the control aquarium.

Table 1 summarizes ^{14}C residue data in the water, edible tissue, non-edible tissue, and whole fish during exposure and depuration. Radioactive residues in the test water, calculated as cyromazine, were 1.0 ± 0.14 ppm throughout the entire exposure period. The maximum accumulation of ^{14}C residues in edible tissue occurred on day 3 of exposure when 0.67 ppm was found; the half-life was 3-7 days. The maximum accumulation (2.1 ppm) in non-edible tissues occurred on the first day of exposure. The half-life was 1-3 days. In both fish tissues the rate of elimination was greater than the rate of accumulation for most of the exposure period. The mean maximum bioconcentration factor was <1 X for edible tissue for the first 3 days of exposure. The maximum bioconcentration factor for non-edible tissues was 2.5 X for the first day of exposure; the mean bioconcentration factor was <1 X for the 28-day exposure period. Whole fish data (calculated from data on both types of tissue) were similar to data on edible tissue.

Depuration data showed that ^{14}C residues were non-detectable (levels varied, see Table 1) for edible tissue and whole fish throughout the depuration period, and became non-detectable in non-edible tissues 10 days after depuration started. The depuration half-life for non-edible tissues was 3-7 days.

Radioactive residues in edible and non-edible tissues of fish exposed to [^{14}C]cyromazine for 3 days (the second flow-through test) were non-detectable (<0.16 ppm) and 0.22 ppm, respectively, on day 3 of exposure.

Radioactive residues were non-detectable (<0.14-<0.28 ppm) in edible and non-edible tissues of control fish sampled on days 1, 14, and 28 of exposure and day 14 of depuration.

Static test. Radioactive residues in the test water, calculated as cyromazine, throughout the 4-day exposure were 1.3 ± 0.04 ppm. Mean ^{14}C residues in bluegill muscle, viscera, carcass, and whole body were 0.057, 0.10, 0.15, and 0.090 ppm, respectively, on day 4 of exposure. Based on these data, the bioconcentration factor was <1 X.

Table 1. Measured ^{14}C residue concentrations, calculated as cyromazine, in the edible tissue (muscle) and non-edible tissues (viscera and carcass) of bluegill sunfish (Lepomis macrochirus) during 28 days of continuous aqueous exposure to [^{14}C]cyromazine at 1.0 ppm and during an additional 14 days depuration in flowing, uncontaminated water.

		^{14}C residues (ppm)			
Period	Day	Water[a]	Edible tissue[b]	Non-edible tissue	Whole fish[c]
Exposure	0	0.68	--	--	--
	1	1.0	<0.18[d]	2.1	<0.62[e]
	3	1.1	0.67	0.72	0.70
	7	1.1	<0.15[d]	0.51	<0.29[e]
	10	1.1	0.12	0.44	0.26
	14	1.0	0.081	0.27	0.15
	21	1.0	<0.20[d]	0.34	<0.25[e]
	28	1.0	<0.17[d]	0.35	<0.24[e]
Depuration	1	0.019	<0.26[d]	0.38	<0.31[e]
	3	0.023	<0.23[d]	0.21	<0.22[e]
	7	0.011	<0.14[d]	0.16	<0.15[e]
	10	<0.0078[d]	<0.15[d]	<0.28[d]	<0.21[d]
	14	0.0098	<0.19[d]	<0.17[d]	<0.17[d]

[a]Mean based on the radiometric analyses of triplicate water samples.

[b]Mean based on the radiometric analyses of triplicate samples of the pooled tissue homogenate from four fish.

[c]Concentration based on the summation of the mean measured concentration of ^{14}C residues from each tissue portion and the total weight of each tissue portion from four fish.

[d]Concentration below minimum detectable limits.

[e]Concentration of one portion below minimum detectable limits.

Conclusions

Cyromazine does not accumulate in tissues of bluegill sunfish exposed to ring-labeled [^{14}C]cyromazine at 1.0 ppm for 28 days under flow-through or 4 days under static conditions. The highest residue found was 2.1 ppm in non-edible (viscera and carcass) tissues on the first day of exposure corresponding to a bioconcentration factor of 2.5 X. Bioconcentration factors for all other residues found were <1 X. Radioactive residues in edible tissues were non-detectable (<0.14-<0.26 ppm) on day 21 of exposure and remained non-detectable throughout the 14-day depuration period. Radioactive residues in non-edible tissues were 0.38 ppm at the start of the depuration period and declined with a depuration half-life of 3-7 days, residues were non-detectable (<0.28 ppm) on day 10 of depuration.

The identity of ^{14}C residues in water and fish tissues was not reported.

Hydrolysis of CGA-72662 under laboratory conditions, Burkhard, N., Ciba-Geigy Limited, Basle, Switzerland, May 10, 1979, Project Report 17/79, Acc. No. 070914, Reference 5.

Procedure

Solutions of ring-labeled [^{14}C]cyromazine (CGA-72662) at 100 ppm in 0.1 N HCl, 0.1 N NaOH, and buffered solutions at pH 5, 7, and 9 were incubated for ≤28 days at 30, 50, and 70 C.

Methodology

Hydrolysis rates were determined by extracting the solutions with ethanol (the acidic solutions were neutralized with NaOH prior to extraction) followed by high pressure liquid chromatographic (HPLC) analyses of the extracts for cyromazine.

Results

Hydrolysis occurred only when [^{14}C]cyromazine was incubated in 0.1 N HCl at 50 or 70 C and in 0.1 N NaOH at 70 C (Table 1). Cyromazine was stable for at least 28 days in all other solutions and temperatures. Hydrolysis of cyromazine in acidic or basic solutions maintained at elevated temperatures followed first order reaction kinetics, facilitating half-life calculations. Half-lives were 106 and 7.7 days in 0.1 N HCl at 50 and 70 C, respectively, and 80 days in 0.1 N NaOH at 70 C.

HPLC analysis of the 0.1 N HCl solution maintained at 70 C for 21 days showed that 16% of the radioactivity was accounted for by cyromazine, and 76% was accounted for by "compound I" (2-amino-4-cyclopropylamino-6-hydroxy-s-triazine). HPLC analysis of the 0.1 N NaOH solution maintained at 70 C for 28 days showed three peaks: cyromazine accounted for 79% of the radioactivity, compound I accounted for 9%, and "compound II" (2-cyclopropylamino-4,6-dihydroxy-s-triazine) accounted for 4%.

Table 1. Hydrolysis of cyromazine in 0.1 N HCl and 0.1 N NaOH.

Hydrolysis period (days)	Cyromazine found (% of applied)			
	0.1 N HCl		0.1 N NaOH	
	50 C	70 C	50 C	70 C
0	100	100	100	100
7	92	58	100	95
14	84	29	101	93
16	87	25	--	--
21	89	16	98	83
28	81	8	99	79

Conclusions

Cyromazine does not hydrolyze in buffered solutions at pH 5, 7, or 9 maintained at 30-70 C for 28 days. Cyromazine hydrolyzes in 0.1 N HCl at 50 and 70 C with half-lives of 106 and 7.7 days, respectively. Cyromazine also hydrolyzes in 0.1 N NaOH at 70 C with a half-life of 80 days. HPLC analyses showed the presence of 2-amino-4-cyclopropylamino-6-hydroxy-s-triazine in both acidic and alkaline solutions at 70 C and the presence of 2-cyclopropylamino-4,6-dihydroxy-s-triazine in the alkaline solution at 70 C.

Photolysis of CGA-72662 in aqueous solutions under artificial sunlight conditions, Burkhard, N., Ciba-Geigy Limited, Basle, Switzerland, May 10, 1979, Project Report 18/79, Acc. No. 070914, Reference 6.

Procedure

A 100-ppm solution of ring-labeled[^{14}C]cyromazine (CGA-72662) in water with and without 1% acetone was exposed for ≤1 week to a mercury vapor lamp (173 ± 12 Langley/hr) with a Solidex glass filter that absorbed all light <290 nm. The solution temperature was maintained at 25 C, and the pH was 6.6 at the start of the experiment. A dark control was run.

Methodology

Aliquots of the photolysis solutions were analyzed after various exposure intervals by using high pressure liquid chromatography (HPLC).

Results

No degradation of cyromazine was observed in the non-sensitized solution at ≤168 hours of exposure. In the solution sensitized with 1% acetone, cyromazine levels declined from 99 ppm initially to 38 ppm after 24 hours of exposure; the half-life was 10 hours. HPLC analysis showed the presence of melamine at 54 ppm in the sensitized solution after 24 hours of exposure. By the end of the experiment, the pH of the sensitized solution had declined to 3.7.

Conclusions

Cyromazine does not photodegrade in aqueous solutions maintained at ~pH 7 and 25 C for 1 week. However, cyromazine photodegrades, with a half-life of 10 hours, in aqueous solutions sensitized with 1% acetone. The degradate, melamine, was detected at 54 ppm in the sensitized solution after 1 day of exposure.

Leaching characteristics of aged ^{14}C-CGA-72662 residues in two standard soils, Burkhard, N., Ciba-Geigy Limited, Basle, Switzerland, April 11, 1980, Project Report 14/80, Acc. No. 070914, Reference 7.

Procedure

Air-dried samples of Collombey sand (2.8% clay, 10.2% silt, 87.0% sand, pH 7.8, 2.2% organic matter, CEC 14.0 meq/100 g) and Les Evouettes silt loam (12.2% clay, 49.4% silt, 38.4% sand, pH 6.1, 3.6% organic matter, CEC 9.0 meq/100 g) soils were treated with ring-labeled [^{14}C]cyromazine (CGA-72662, >99% purity) in methanol to yield levels of 2.8 and 4.2 ppm, respectively. The soils were mixed in a ball mill for 1 hour after the methanol had evaporated. Distilled water was then used to moisten the soils to 50% of water-holding capacity. The soils were then aged for 30 days in an aerated system maintained in the dark at 25 ± 1 C. The treated, aged soil (containing 126 µg of cyromazine, equivalent to 1 kg ai/ha) was added as a 2-cm layer onto the top of 28 cm of untreated soil in 40-cm columns. The columns were eluted by adding 0.5 inches of distilled water daily for 45 days (22.5 inches total).

Methodology

The leachates from the soil columns were collected daily and aliquots were analyzed by using liquid scintillation counting (LSC). Eluates with radioactivity >0.1% of the applied dose were combined and analyzed by using thin-layer chromatography (TLC) on silica gel plates. The solvent used was acetonitrile:water (9:1) with and without 1% ammonium hydroxide.

The soil columns were divided into 15 2-cm sections at the end of the experiment. Each of these soil layers and a sample of the aged soil prior to leaching was Soxhlet-extracted first with acetone and then with methanol; aliquots of the extracts were analyzed by using LSC. The extracted soil samples were air dried, ball-milled, and combusted.

Results

After 30 days of aging, most of the ^{14}C residues were extractable with acetone and methanol, 82% for the sand and 70.5% for the silt loam soil; non-extractable residues accounted for 17.2 and 28.6% of the radioactivity in the respective soils.

The distribution of aged [^{14}C]cyromazine residues in columns of the two types of soil are shown in Table 1. None of the radioactivity leached from the silt loam soil, ∿80% of the applied dose was retained in the upper 10 cm of the column and ∿4% was found in the lower 10 cm. Approximately 44% of the applied dose was retained in the sand soil, ∿12% of which was retained in the upper 2 cm, the remainder was evenly distributed in the other 2-cm sections.

The distribution of the radioactivity in the leachate from the sand soil is shown in Table 2 (<0.1% of the applied dose was recovered in all leachate

samples collected from the silt loam soil). Approximately 51% of the applied dose leached through the column; ~28% leached after 14.5 inches of water was applied.

TLC analysis of the sand soil eluates showed that 18% of the radioactivity was unchanged cyromazine and 29% was the degradate melamine.

Table 1. Distribution of extractable and non-extractable radioactivity in columns of soil treated with [^{14}C]cyromazine, aged for 30 days, and then leached for 45 days with 22.5 inches of water (values are given in percent of the dose applied before aging).

Soil layer (cm)	Collombey sand			Les Evouettes silt loam		
	Extractable[c]	Non-extractable	Total	Extractable[a]	Non-extractable	Total
0-2	1.8	9.8	11.6	11.1	23.3	34.4
2-4	0.7	1.0	1.7	4.3	6.8	11.1
4-6	0.9	1.1	2.0	5.5	7.7	13.2
6-8	1.3	1.4	2.7	6.0	6.7	12.7
8-10	1.3	1.3	2.6	4.7	4.4	9.1
10-12	1.3	1.2	2.5	2.3	2.1	4.4
12-14	1.3	1.2	2.5	1.2	1.0	2.2
14-16	1.4	1.1	2.5	0.7	0.7	1.4
16-18	1.3	1.0	2.3	0.6	0.6	1.2
18-20	1.2	1.0	2.2	0.5	0.5	1.0
20-22	1.5	0.8	2.3	0.6	0.5	1.1
22-24	1.5	0.8	2.3	0.4	0.5	0.9
24-26	1.6	0.7	2.3	0.3[b]	0.5	0.8
26-28	1.7	0.8	2.5	0.2[b]	0.3	0.5
28-30	1.7	0.7	2.4	0.3[b]	0.3	0.6
Total	20.5	23.9	44.4	38.7	55.9	94.6

[a] Soxhlet extraction with acetone and then with methanol.

[b] Extraction with acetone only, <0.1% of radioactivity in methanol extracts.

Table 2. Distribution of radioactivity in leachate from columns of Collombey sand soil treated with [^{14}C]cyromazine, aged for 30 days, and then leached for 45 days with 22.5 inches of water (values are given in percent of the dose applied before aging).

Water applied (inches)	^{14}C activity (%)
0-9.5	0.12
9.5-10.5	0.45
10.5-11.5	1.16
11.5-12.5	6.31
12.5-13.5	11.18
13.5-14.5	8.56
14.5-15.5	4.96
15.5-16.5	2.97
16.5-17.5	3.20
17.5-18.5	2.51
18.5-19.5	2.62
19.5-20.5	2.39
20.5-21.5	2.15
21.5-22.5	2.41
Total	50.99

Conclusions

Cyromazine is mobile in sand and moderatively mobile in silt loam soil. After [^{14}C]cyromazine was aged for 30 days in two soils, ∿50% of the applied radioactivity leached through a 30-cm column of Collombey sand after 22.5 inches of water was applied over 45 days. None of the radioactivity leached through a Les Evouettes silt loam column under the same conditions. Radioactivity was present at the 28- to 30-cm depth; however, ∿80% of the applied radioactivity was found in the upper 10 cm of the column and only ∿4% was in lower 10 cm.

The degradate melamine was detected in the leachate of the sand column, ∿18 and 29% of the radioactivity was identified as unchanged cyromazine and melamine, respectively.

Photolysis of CGA 72662 on soil surfaces under artificial sunlight conditions, Burkhard, N., Ciba-Geigy Limited, Basle, Switzerland, June 26, 1980, Project Report 24/80, Acc. No. 070914, Reference 8.

Procedure

An aqueous solution of ring-labeled [^{14}C]cyromazine (CGA-72662) and unlabeled cyromazine was mixed with a sandy clay loam soil (reported as a sandy loam; 22.6% clay, 19.6% silt, 57.8% sand, pH 6.7, 5.6% organic matter) to give an initial concentration of 10 ppm. Treated and untreated soil samples (in layers of 0.6-0.7 cm, kept dry or moistened to 12%) were exposed for 24 hours to radiation emitted from a xenon arc lamp with an energy level of 940 ± 50 j/m^2/s. Soil samples were not cooled, and temperatures were 45 ± 5 C. Filters were used to filter out high IR radiation (no wavelength given) and UV radiation <290 nm. A dark control was run.

Methodology

Soil samples were Soxhlet-extracted first with acetone and then with methanol. Residues in the extracts were separated by using thin-layer chromatography with an acetonitrile:water:ammonium hydroxide (90:10:2) solvent. Cyromazine was then analyzed by using liquid scintillation counting.

Results

A materials balance resulted in a recovery of 97.7 ± 2% of the ^{14}C activity. Non-extractable residues ranged from 7 to 26%.

After 24 hours of irradiation, 68-78% of the extractable residues in dry and moist, exposed and covered (dark control) soils were identified as cyromazine. No other compounds were detected. Non-extractable residues were formed more rapidly in moist soils than in dry soils; values ranged from 23-26% to 18-20%, respectively. There was no difference between exposed and covered soil samples.

Conclusions

Cyromazine on soil surfaces does not photodegrade after being exposed to a xenon arc lamp for 24 hours. This study was not carried out long enough to adequately assess the photodegradation of cyromazine on soil.

Adsorption and desorption of CGA-72662 (Vetrazin) in various soil types, Burkhard, N., Ciba-Geigy Limited, Basle, Switzerland, October 13, 1981, Project Report 32-81, Acc. No. 070914, Reference 9.

Procedure/Methodology

The adsorption/desorption properties of [^{14}C]cyromazine (CGA-72662, analytical grade) were determined for four Swiss soils (Table 1). Oven-dry soil (20-100 g) was mixed with 100-ml aqueous solutions of [^{14}C]-cyromazine at 1.0, 2.5, 5.0, and 10.0 ppm and shaken overnight at 20 C. The suspensions were then vacuum filtered and aliquots of the filtrate were analyzed by using liquid scintillation counting (LSC). The amount of [^{14}C]cyromazine adsorbed onto soil particles was calculated from the difference between initial concentration and the calculated equilibrium concentration. The equilibrium concentration was obtained by regression analysis of disintegrations per minute and concentration. For desorption measurements, distilled water was added to the soils used for the adsorption determinations so that the volume was 100 ml. The soil-water suspensions were then shaken for 3 days at 20 C, filtered, and the filtrate was analyzed by using LSC. Desorption was calculated as described for adsorption calculations.

Results

Freundlich K and 1/n values for the four soils are given in Table 2. Adsorption K values ranged from 0.52 for the Collombey sand to 17.0 for the Illarsaz organic soil; desorption K values were higher and ranged from 1.35 to 26.9 for the Collombey and Illarsaz soils, respectively. The values of 1/n were <1.0 for all soils.

Table 1. Characteristics of four Swiss soils.

Soil	Mechanical analysis (%)			pH	Organic matter (%)	CEC (meq/ 100 g)
	Clay	Silt	Sand			
Collombey sand	2.8	10.2	87.0	7.8	2.2	14.0
Vetroz sandy clay loam	22.6	19.6	57.8	6.7	5.6	29.4
Les Evouettes silt loam	12.2	49.4	38.4	6.1	3.6	9.0
Illarsaz	--	--	--	7.5	22.9	68.0

Table 2. Constants from Freundlich adsorption and desorption isotherms for[^{14}C]cyromazine with various soils.

Soil	Adsorption		Desorption	
	K	1/n	K	1/n
Collombey	0.52	0.83	1.35	0.74
Vetroz	2.37	0.85	3.72	0.82
Les Evouettes	3.87	0.77	5.29	0.83
Illarsaz	17.00	0.81	26.90	0.89

Conclusions

Cyromazine shows slight to moderate adsorption to soils ranging from a sand with low organic matter to a soil high in organic matter. Freundlich K values ranged from 0.52 to 17.0 for adsorption and from 1.35 to 26.9 for desorption, generally increasing with increasing soil organic matter content.

Leaching model study with the insecticide/larvicide CGA-72662 in four different soils, Guth, J.A., Ciba-Geigy Limited, Basle, Switzerland, March 31, 1980, Project Report 13/80, Acc. No. 070915, Reference 27.

Procedure

Air-dried samples of four soils (see Table 1 for characteristics) were packed into columns to a height of 30-cm. [^{14}C] Cyromazine (aqueous solution of CGA-72662 50 SP and 10% [^{14}C]CGA-72662) was applied at 630 µg/column (equivalent to 5 kg ai/ha) to the top of each column. The columns were eluted by adding 8 inches of water within 2 days.

Methodology

The leachate from the soil columns was collected and analyzed by using liquid scintillation counting (LSC).

The soil columns were divided into 15.2-cm sections at the end of the experiment. Each of these soil layers was extracted by shaking it with methanol:water (8:2) for 4 hours. The extracts were concentrated and analyzed by using LSC. Non-extractable radioactivity was determined by combustion.

Results

[^{14}C]Cyromazine leached to a depth of >30, 16, 14, and 18 cm in Collombey sand, Lakeland sand, Les Evouettes silt loam, and Vetroz sandy clay loam soils, respectively (Table 2). Approximately 33% of the applied radioactivity was present in the leachate from the Collombey sand column. Leachability of cyromazine was not correlated with organic matter but was influenced by pH; cyromazine did not leach well in the slightly acidic Lakeland sand and Les Evouettes silt loam soils.

Table 1. Characteristics of four soils.[a]

Soil	Mechanical analyses (%)			pH	Organic matter (%)	CEC (meq/ 100 g)
	Clay	Silt	Sand			
Collombey sand	2.8	10.2	87.0	7.8	2.2	14.0
Lakeland sand	1.5	2.1	96.4	6.3	1.2	3.7
Les Evouettes silt loam	12.2	49.4	38.4	6.1	3.6	9.0
Vetroz sandy clay loam	22.6	19.6	57.8	6.7	5.6	29.4

[a]The Lakeland soil was from Florida, all other soils were from Switzerland.

Table 2. Distribution of total and non-extractable radioactivity in the leachate and in columns of soil treated with [^{14}C]cyromazine at 5 kg ai/ha and leached for 2 days with 8 inches of water (values are given in percent of applied dose).

Soil layer (cm)	Collombey sand		Lakeland sand		Les Evouettes silt loam		Vetroz sandy clay loam	
	Total	Non-extractable	Total	Non-extractable	Total	Non-extractable	Total	Non-extractable
0-2	3.4	1.9	26.6	11.2	8.9	4.6	6.6	3.3
2-4	2.4	2.0	21.0	5.8	13.2	4.7	4.4	1.2
4-6	2.2	1.6	13.7	3.7	20.1	6.2	8.8	2.1
6-8	1.5	0.8	8.6	2.5	23.2	6.0	14.8	3.2
8-10	1.7	0.8	4.4	1.3	24.5	6.9	28.1	5.9
10-12	1.9	0.9	3.1	0.8	10.2	2.7	25.4	5.4
12-14	2.1	0.8	3.9	0.8	0.9	--	10.1	1.9
14-16	2.3	0.7	0.8	--	<0.5	--	1.6	0.8
16-18	3.9	1.6	<0.5	--	<0.5	--	1.1	1.1
18-20	4.6	2.0	<0.5	--	<0.5	--	<0.5	--
20-22	4.5	0.8	<0.5	--	<0.5	--	<0.5	--
22-24	4.6	0.3	<0.5	--	<0.5	--	<0.5	--
24-26	5.6	0.3	<0.5	--	<0.5	--	<0.5	--
26-28	6.9	0.5	<0.5	--	<0.5	--	<0.5	--
28-30	10.1	1.0	<0.5	--	<0.5	--	<0.5	--
Total	57.7	16.0	82.1	26.1	101.0	31.1	100.9	24.9
Leachate	32.7	--	<0.5	--	<0.5	--	<0.5	--

Conclusions

Cyromazine is mobile in slightly alkaline sand and moderately mobile in silt loam, sandy clay loam, and slightly acidic sand soils. [^{14}C]-Cyromazine leached through a 30-cm column of Collombey sand soil when the column was eluted with 8 inches of water; ~33% of the applied radioactivity was present in the leachate. [^{14}C]Cyromazine leached to depths of 14-18 cm in Les Evouettes silt loam, Vetroz sandy clay loam, and Lakeland sand soil columns eluted with 8 inches of water.

The effects of 1, 10, and 100 ppm of CGA-72662 on soil nitrification, prepared for Ciba-Geigy Corporation by Mumma, R.O., and E.R. Bogus, Pesticide Research Laboratory and Graduate Study Center, The Pennsylvania State University, September 11, 1981, Acc. No. 070915, Reference 38.

Procedure

Samples of a Greenville Mississippi loam (50.8% sand, 35.2% silt, 14.0% clay, pH 6.7, 2.4% organic matter) and a York, Nebraska silt loam soil (20.8% sand, 59.2% silt, 20.0% clay, pH 6.4, 2.6% organic matter) were screened through a No. 4 and then a No. 10 sieve. The number of propagules of fungi, bacteria, and actinomycetes were determined by using dilution plate counts. The soils were then treated with cyromazine (CGA-72662, 93.7%) in methanol at 100 ppm and thoroughly mixed after the solvent evaporated. An aliquot of each of the two soil types was diluted with amended soil (made up of a 1:1 mixture of oven-dry soil and fine sea sand fortified with ammonium sulfate and calcium carbonate and then moistened to 60% of field capacity) to make a soil that contained cyromazine at 10 ppm. This process was repeated to make a 1-ppm treatment. Control soils were treated with methanol alone. The soils were placed in 400-ml beakers covered with aluminum foil perforated with five holes and incubated for 56 days at 28 C and 80% relative humidity. Twice weekly throughout the experiment the soils were moistened to maintain ~60% of field capacity and purged with air for 2 minutes.

Methodology

Soils were analyzed for nitrate levels at various intervals by extracting the soils with a solution made up of boric acid, aluminum sulfate, sulfamic acid, and ammonium sulfate. The soil:solution suspensions were shaken at 10-minute intervals for 1 hour. The water extract was removed by centrifugation and decantation. The nitrate-N level in the supernatant was measured with a nitrate specific-ion electrode of a microprocessor ionalyzer.

Results

The number of propagules of fungi, bacteria, and actinomycetes in samples of a Greenville loam were 1.8×10^4, 6.59×10^6, and 1.96×10^6, respectively. The respective numbers in a sample of a York silt loam were 4.03×10^4, 6.95×10^6, and 2.25×10^6.

The effect of cyromazine on soil nitrification is shown in Table 1. Cyromazine did not have an influence on nitrate-N levels found in the soils tested.

Table 1. Effects of cyromazine on soil nitrification.

Cyromazine applied (ppm)	NO_3-N (µg/g soil)								
	0 Day	1 Day	3 Days	7 Days	14 Days	21 Days	28 Days	42 Days	56 Days
					Greenville loam soil				
0	5.8	3.7	3.6	7.4	13.3	23.3	39.5	58.9	80.2
1	5.4	3.9	4.3	8.0	14.1	24.7	40.1	61.7	85.2
10	6.0	4.3	4.7	8.1	14.2	24.6	40.6	61.6	82.7
100	6.1	3.0	2.8	5.9	11.0	18.8	34.4	53.2	76.7
					York silt loam soil				
0	9.0	7.4	4.9	7.6	11.7	19.9	40.4	60.5	79.0
1	8.7	7.5	6.8	10.7	16.8	31.5	69.1	83.8	98.3
10	9.0	7.5	7.5	11.4	17.8	33.9	71.9	85.7	104.8
100	8.6	7.8	5.3	8.5	13.3	23.2	47.7	62.7	79.4

Conclusions

Cyromazine at ≤100 ppm does not influence the nitrification process in loam and silt loam soils. Nitrate-N levels were similar in control and treated soils incubated with cyromazine for 56 days.

Effects of CGA-72662 on the degradation of ^{14}C-cellulose, ^{14}C-protein, and ^{14}C-starch in soil, prepared for Ciba-Geigy Corporation by Mumma, R.O., and E.R. Bogus, Pesticide Research Laboratory and Graduate Study Center, The Pennsylvania State University, September 16, 1981, Acc. No. 070915, Reference 39.

Procedure

Samples of a Greenville, Mississippi loam (50.8% sand, 35.2% silt, 14.0% clay, pH 6.7, 2.4% organic matter) and a York, Nebraska silt loam soil (20.8% sand, 59.2% silt, 20.0% clay, pH 6.4, 2.6% organic matter) were screened through a No. 4 and then a No. 10 sieve. The soils were amended with 1% of either [^{14}C]cellulose, [^{14}C]protein, or [^{14}C]starch. The amended soils were treated with cyromazine (CGA-72662, 93.7%) in methanol at 1, 10, and 100 ppm. Control soils were treated with methanol alone. After treatment, the soils were moistened to 75% of field capacity, and incubated for 28 days in stoppered sample bottles equipped with gas-exchange tubes. The inlet air was first passed through a reservoir that contained a solution of sodium hydroxide and hydrochloric acid and then a reservoir that contained CO_2-free distilled water. The outlet air was connected to CO_2 trapping bottles.

Sterile controls were also run. Oven-dry soils in bottles were autoclaved at 251 C and 18 psi for 30 minutes. After the soils had cooled they were amended with 1% of either [^{14}C]cellulose, [^{14}C]protein, or [^{14}C]starch. The soils were then moistened to 75% of field capacity and incubated for 28 days in stoppered sample bottles equipped with gas-exchange tubes placed in a sterile hood.

Methodology

Soils were combusted and analyzed by using liquid scintillation counting (LSC). Carbon dioxide trapping bottles were removed from the air stream on days 1, 3, 7, 14, 21, and 28 and replaced with fresh bottles containing 0.2 N sodium hydroxide. Trapped CO_2 was analyzed by using LSC.

Results

Similar amounts of $^{14}CO_2$ evolved from control and cyromazine-treated loam and silt loam soils incubated with [^{14}C]cellulose, [^{14}C]protein, or [^{14}C]starch.

No $^{14}CO_2$ was trapped from sterile loam or silt loam soils incubated with [^{14}C]cellulose, [^{14}C]protein, or [^{14}C]starch.

Conclusions

Microbial degradation of cellulose, protein, and starch is not affected by cyromazine at $\leq$100 ppm in loam and silt loam soils. Similar amounts of $^{14}CO_2$ evolved from control and cyromazine-treated soils incubated for 28 days with [^{14}C]cellulose, [^{14}C]protein, and [^{14}C]starch.

The effects of 1, 10, and 100 ppm CGA-72662 on soil respiration, prepared for Ciba-Geigy Corporation by Mumma, R.O., and E.R. Bogus, Pesticide Research Laboratory and Graduate Study Center, The Pennsylvania State University, September 29, 1981, Acc. No. 070915, Reference 40.

Procedure

Samples of a Greenville, Mississippi loam (50.8% sand, 35.2% silt, 14.0% clay, pH 6.7, 2.4% organic matter) and York, Nebraska silt loam soil (20.8% sand, 59.2% silt, 20.0% clay, pH 6.4, 2.6% organic matter) were screened through a No. 4 and then a No. 10 sieve. The soils were then treated with cyromazine (CGA-72662, 93.7%) in methanol at 100 ppm and thoroughly mixed after the solvent evaporated. An aliquot of each of the two soil types was diluted with amended soil (made up of a 1:1 mixture of oven-dry soil and fine sea sand fortified with alfalfa meal and then moistened to 60% of field capacity) to make a soil that contained cyromazine at 10 ppm. This process was repeated to make a 1-ppm treatment. Control soils were treated with methanol alone. Sterile controls were also run. Soils were placed in sealed biometer flasks with sodium hydroxide in the sidearm and incubated at 25 C for 28 days. The biometer flasks were aerated for 3 minutes twice weekly throughout the experiment.

Methodology

The amount of CO_2 evolved was measured at various intervals by inserting a syringe into the biometer flasks and transferring the sodium hydroxide drawn up to a flask containing barium chloride. The sidearm was rinsed three times with CO_2-free water, recharged with sodium hydroxide, and resealed. The combined wash water and sodium hydroxide trapping solution was titrated with hydrochloric acid; phenolpthalein was used as an indicator. The milliequivalents of CO_2 evolved for each treatment was then calculated.

Results

The influence of cyromazine on soil respiration is shown in Table 1. Compared with control soils, cyromazine increased CO_2 evolution by 16-21% in Greenville loam soil and by 3-10% in York silt loam soil. Negligible amounts of $^{14}CO_2$ evolved from the sterile control soils.

Table 1. Effects of cyromazine on soil respiration.

Cyromazine applied (ppm)	CO_2 evolution (meq/50 g soil)						
	1 Day	3 Days	7 Days	14 Days	21 Days	28 Days	Total
			Greenville loam soil				
Sterile control	0.12	0.05	0.06	0.05	0.05	0.04	0.37
Control[a]	1.00	1.08	0.99	1.02	1.00	0.67	5.76
1	0.97	1.28	1.53	0.98	1.08	0.87	6.71
10	1.00	1.49	1.46	1.07	1.10	0.87	6.99
100	0.96	1.38	1.56	1.07	1.06	0.87	6.90
			York silt loam soil				
Sterile control	0.13	0.06	0.08	0.05	0.05	0.04	0.41
Control[a]	1.17	1.35	1.17	0.98	0.67	0.53	5.87
1	1.22	1.62	1.33	1.06	0.68	0.54	6.45
10	1.16	1.57	1.22	0.96	0.64	0.50	6.05
100	1.27	1.61	1.24	0.95	0.64	0.51	6.22

[a]Amended with alfalfa meal but not treated with cyromazine.

Conclusions

Cyromazine at ≤100 ppm slightly increases soil respiration. Cyromazine increased CO_2 evolution from Greenville loam and York silt loam soils by 16-21% and 3-10%, respectively, when incubated with the soils for 28 days.

Effects of 1, 10, and 100 ppm of CGA-72662 on nitrogen fixation in soil, prepared for Ciba-Geigy Corporation by Mumma, R.O., and E.R. Bogus, Pesticide Research Laboratory and Graduate Study Center, The Pennsylvania State University, October 12, 1981, Acc. No. 070915, Reference 41.

Procedure

Samples of a Greenville, Mississippi loam (50.8% sand, 35.2% silt, 14.0% clay, pH 6.7, 2.4% organic matter) and a York, Nebraska silt loam soil (20.8% sand, 59.2% silt, 20.0% clay, pH 6.4, 2.6% organic matter) were screened through a No. 4 and then a No. 10 sieve. Oven-dry samples of the soils were amended by adding 2% glucose, mixing thoroughly, and moistening to 60% of field capacity. The amended soils were treated with cyromazine (CGA-72662, 93.7%) in methanol at 100 ppm. An aliquot of each of the two soil types was diluted with amended soil to make a soil that contained cyromazine at 10 ppm. This process was repeated to make a 1-ppm treatment. Control soils were treated with methanol alone. Sterile controls were also run. The soils were placed in beakers and inoculated with Azotobacter vinelandii (10^7 propagules). The sterile control soils and some other control soils were not inoculated. The beakers were covered with aluminum foil perforated with five holes and incubated for 28 days at 26 C and 80% relative humidity. The soils were moistened twice weekly and purged with air for 1 minute once weekly throughout the experiment.

Methodology

At various intervals, the nitrogenase activity in each beaker was determined by the following method. Aliquots of soil were placed in serum bottles and then incubated for 2 days in a 10% acetylene in argon atmosphere. The amount of ethylene evolved, through nitrogenase-induced reduction of acetylene to ethylene, was measured by using a gas chromatograph equipped with a flame ionization detector.

Results

The effects of cyromazine on nitrogen fixation are shown in Table 1. No ethylene was evolved from any soil sample on days 0 and 28. Compared with inoculated controls, cyromazine increased ethylene evolution by $\leq$1,245% after 14 days incubation in Greenville loam soil and by $\leq$689% after 7 days in York silt loam soil.

Table 1. Effects of cyromazine on nitrogen fixation in soil.

Cyromazine applied (ppm)	Ethylene evolution (μM/g soil)				
	1 Day	3 Days	7 Days	14 Days	21 Days
	Greenville loam soil				
Sterile control	1.0	ND[a]	ND	ND	ND
Control	1.5	43.6	9.1	17.3	ND
Inoculated control[b]	2.5	73.9	29.5	46.5	ND
1	2.3	91.3	19.0	53.0	ND
10	2.6	65.6	33.6	81.9	ND
100	6.4	69.5	85.8	625.5	48.5
	York silt loam soil				
Sterile control	ND	ND	0.4	ND	ND
Control	ND	79.0	0.8	ND	ND
Inoculated control[b]	ND	175.7	0.9	ND	ND
1	ND	133.5	1.0	ND	ND
10	ND	161.2	1.2	ND	ND
100	2.4	180.1	7.1	4.7	ND

[a]ND = non-detectable, <0.007 μM/g soil.

[b]Inoculated with Azotobacter vinelandii.

Conclusions

Cyromazine at ≤100 ppm greatly increases soil nitrogen fixation. Cyromazine increased ethylene evolution from Azotobacter vinelandii-inoculated Greenville loam and York silt loam soils by ≤1,245% and ≤689%, respectively, when incubated with the soils for 28 days.

CGA-72662 Activated sludge metabolism, prepared for Ciba-Geigy Corporation by Olson, S., and W.C. Spare, Biospherics Incorporated, Protocol number 6-79-27, October 11, 1979, Acc. No. 070915, Reference 43.

Procedure

Activated sludge suspension (1.3 g solids/l, principally domestic wastes), synthetic sewage, and uniformly ring-labeled [^{14}C]cyromazine ([^{14}C]CGA-72662, 99.5% radiopurity) were incubated at 21 C for five 23-hour cycles in glass aeration chambers. Cyromazine was diluted with DMSO so that dosages of cyromazine for cycles 1, 2, 3, 4, and 5 were 0.1, 1.0, 10.0, 50.0, and 100.0 ppm, respectively. A DMSO control and a positive control with mercuric chloride were run. After each cycle, the following determinations were made: microbial and protozoa counts, suspended solids, percent settled solids, effluent turbidity, and radiocarbon analyses of solids and supernatant samples. Aeration was then discontinued for 30 minutes to allow solids to settle (dissolved oxygen, pH, and temperature were measured at this time) and an aliquot of supernatant was removed and replaced with fresh synthetic sewage and [^{14}C]cyromazine.

Methodology

Sludge solids were combusted and analyzed by using liquid scintillation counting (LSC). The filtrates and CO_2 traps were analyzed by using LSC. The filtrate was also analyzed by using thin-layer chromatography (TLC) with cyromazine and the cyromazine degradate melamine used as standards. The radioactive spots on the TLC plates were quantified by using LSC.

Results

Dissolved oxygen, pH, suspended solids, and turbidity were similar for cyromazine-treated sludge and the DMSO control. Microbial plate counts were similar for all levels of cyromazine and the DMSO control. In nutrient agar, for example, counts were 0.78-1.4 x 10^6 for cyromazine at 100 ppm and were 1.1-4.6 x 10^6 for the DMSO control at cycle 5. Mercuric chloride, however, severely damaged the sludge system and completely killed all protozoa, thus increasing bacterial, yeast, and actinomycete populations. Microbial counts in nutrient agar at cycle 5 were 3.9 x 10^8.

TLC showed no degradation of cyromazine to malamine.

Conclusions

Cyromazine at $\leq$100 ppm has little effect on a sewage sludge system. Cyromazine does not degrade in activated sewage sludge.

Pasture grass residue studies, Ciba-Geigy Corporation, October 30, 1981, AG-A 6443 I and AG-A 6457 I, Acc. No. 070915, References 45 and 46.

Procedure

Pasture grass field plots in Fresno, California (Reference 45, sandy loam soil) and in Lancaster, Pennsylvania (Reference 46, silt loam soil) were sprayed with cyromazine (CGA-72662, technical) at 18.0 g ai/A (15 gal/A in California, 40 gal/A in Pennsylvania) or treated with manure (amount not specified) from hens fed cyromazine (0.3% Pre Mix) at 5 ppm in their feed. Duplicate forage samples were analyzed immediately after treatment and 7 and 14 days later.

Methodology

Forage samples were extracted with HCl:methanol and analyzed by using gas chromatography (procedures described more fully in AG-346).

Results

Levels of cyromazine in forage samples from pastures sprayed with cyromazine declined from 4.8-10.5 ppm immediately after treatment to 0.19-0.32 ppm 14 days later; the half-life was <7 days. Levels in forage samples from pastures treated with cyromazine-containing manure were considerably lower. In California, levels declined from ∿1 ppm immediately after treatment to non-detectable levels (<0.05 ppm) 14 days later. In Pennsylvania, all values were non-detectable or just above the detection level (Table 1).

Table 1. Levels of cyromazine (ppm) in forage from a pasture treated with cyromazine at 18.0 g ai/A or treated with manure from hens fed cyromazine at 5 ppm.

Treatment	Days after treatment		
	0	7	14
		California	
Cyromazine spray	7.4, 10.5[a]	1.2, 1.5	0.27, 0.19
Cyromazine - manure	1.1, 0.78	0.06, 0.10	ND[b], ND
		Pennsylvania	
Cyromazine spray	4.8, 6.0	0.73, 1.1	0.22, 0.32
Cyromazine - manure	ND, 0.07	ND, 0.08	ND, ND

[a]Duplicate samples.

[b]ND = non-detectable, <0.05 ppm.

Conclusions

Cyromazine levels in forage dissipate rapidly when cyromazine is sprayed on pasture grass at 18 g ai/A. Cyromazine declined from 4.8-10.5 ppm immediately after treatment to 0.19-0.32 ppm 14 days later; the half-life was <7 days.

No conclusions can be drawn concerning cyromazine levels in forage from pastures treated with cyromazine-containing manure because the rate of application was not given.

Metabolism of topically applied Δ^{14}C-CGA-72662 in chicken manure, Simoneaux, B., and J.E. Cassidy, Ciba-Geigy Corporation, Greensboro, North Carolina, April 3, 1979, Report No. ABR-79024, Acc. No. 070915, Reference 49.

Procedure

Fresh chicken manure was treated topically with uniformly ring-labeled [^{14}C]cyromazine (CGA-72662, >99% radiopurity) as a 0.1% ai spray at 1 gallon/100 square feet and incubated for 21 days (temperature not given). Fresh manure was added daily to maintain biological activity. Samples were taken 1, 7, 14, and 21 days after treatment.

Methodology

Manure samples were combusted, extracted with acetonitrile:water (9:1), and co-chromatographed with a cyromazine standard on silica gel thin-layer chromatography (TLC) plates. The solvent mixture was chloroform:methanol:formic acid:water (75:20:4:2). Audioradiographs were then prepared and the separated compounds were analyzed by using liquid scintillation counting. Extracts were also chromatographed on a cation exchange column by using Method AG-248 (Reference 57).

Results

Levels of radioactivity in manure declined from 29.2 ppm (expressed as [^{14}C]cyromazine) at 1 day after the start of the experiment to 13.2 ppm 20 days later. This result is attributed mostly to the dilution effects of daily supplementing the samples with fresh manure. Nonextractable ^{14}C residues ranged from 4.0% to 6.1% of the applied radioactivity.

Characterization of radioactivity by TLC and by ion exchange chromatography showed that 97% and 90-94% of the extractable ^{14}C residues were cyromazine on days 1 and 21, respectively.

Conclusions

Cyromazine is stable for at least 21 days in chicken manure when it is applied as a 0.1% spray at 1 gallon/100 square feet. As much as 94% of the extractable radioactivity was identified as cyromazine 21 days after [^{14}C]cyromazine was applied to chicken manure supplemented daily with fresh manure. This study allows no conclusion regarding the environmental fate of cyromazine because an incubation temperature was not given.

Metabolism of topically applied Δ^{14}C-CGA-72662 in beef manure, Simoneaux, B., and J.E. Cassidy, Ciba-Geigy Corporation, Greensboro, North Carolina, May 15, 1979, Report No. ABR-79045, Acc. No. 070915, Reference 50.

Procedure

Fresh beef manure was treated topically with uniformly ring-labeled [^{14}C]cyromazine (CGA-72662, >99% radiopurity) as a 0.1% ai spray at 1 gallon/100 square feet and incubated for 21 days (temperature not given). Fresh manure was added daily to maintain biological activity. Samples were taken 1, 7, 14, and 21 days after treatment.

Methodology

Manure samples were combusted, extracted with acetonitrile:water (9:1), and co-chromatographed with a cyromazine standard on silica gel thin-layer chromatography (TLC) plates. The solvent mixture was chloroform: methanol:formic acid:water (75:20:4:2). Audioradiographs were then prepared and the separated compounds were analyzed by using liquid scintillation counting. Extracts were also chromatographed on a cation exchange column by using Method AG-248 (Reference 57).

Results

Levels of radioactivity in manure declined from 38.8 ppm (expressed as [^{14}C]cyromazine at 1 day after the start of the experiment to 11.3 ppm 20 days later. This result is attributed mostly to the dilution effects of daily supplementing the samples with fresh manure. Non-extractable ^{14}C residues increased from 5.3% of the applied radioactivity on day 1 to 13.1% on day 21.

Characterization of extractable radioactivity by TLC and by ion exchange chromatography showed that 87-92% and 64-69% of the ^{14}C residues were cyromazine on days 1 and 21, respectively. Two compounds, identified as Metabolites 1 and 2 accounted for 4.8% and 4.2% of the extractable ^{14}C residues, respectively, on day 21 (Table 1).

Table 1. Characterization of cyromazine and its metabolites in beef manure sprayed with ^{14}C cyromazine as a 0.1% ai spray at 1 gallon/100 square feet.

Compound	Percent of extractable radioactivity			
	1 Day	7 Days	14 Days	21 Days
Cyromazine[a]	86.7	82.5	78.4	68.6
Cyromazine[b]	92.2	83.6	76.4	63.6
Metabolite 1[b]	0.1	0.1	2.0	4.8
Metabolite 2[b]	0.6	0.9	1.9	4.2

[a]Characterized by using thin-layer chromatography.

[b]Characterized by using ion exchange chromatography.

Conclusions

Cyromazine degrades slowly in beef manure with a half-life of >21 days when applied as a 0.1% spray at 1 gallon/100 square feet. Levels of cyromazine declined from ∿90% of the extractable radioactivity at 1 day to ∿66% at 21 days after [^{14}C]cyromazine was applied to beef manure supplemented daily with fresh manure. Two unidentified metabolites were found at 4-5% of the extractable radioactivity on day 21. This study allows no conclusion regarding the environmental fate of cyromazine because an incubation temperature was not given.

The uptake of Δ-^{14}C-CGA-72662 and its degradation products from chicken manure amended soil by various indicator crops, Simoneaux, B., Ciba-Geigy Corporation, Greensboro, North Carolina, November 5, 1981, Report No. ABR-81049, Acc. No. 070915, Reference 51.

Procedure

A field plot in Mississippi containing silt loam soil (22.4% sand, 62.0% silt, 15.6% clay, pH 7.3, 0.9% organic matter, CEC 11.6 meq/100 g) was amended with chicken manure at 20 ton/A. The manure contained uniformly ring-labeled [^{14}C]-cyromazine (CGA-72662, >99% radiopurity) at 5 ppm; therefore, the treatment rate of [^{14}C]cyromazine was 0.2 lb ai/A. After the soil was aged for 30 days, lettuce, spring wheat, and carrots were planted. The crops failed to grow and 2 months later the top 3 inches of the treated soil was shipped to Florida for a greenhouse study, Buckets containing either 3 inches of treated soil on top of 5 inches of similar untreated soil, 8 inches of treated soil, or 8 inches of untreated soil were planted with the same three crops. Greenhouse conditions and plant maintenance schedules are referenced to an unavailable report. Lettuce, spring wheat, and carrots were grown for 70, 93, and 120 days, respectively (harvest was 165, 188, and 215 days post treatment, respectively).

Methodology

Soil and plant samples were combusted and then extracted with methanol. Extracts were purified by removing the organic solvent under vacuum and passing the aqueous phase through a column of Sephadex anion exchange resin. The aqueous eluate was lyophilized before redissolving the sample in methanol. The compounds in the sample were then characterized by cation exchange chromatography (Method AG-248, Reference 57) or by thin-layer chromatography [compounds were developed in toluene:dioxane:methanol:ammonium hydroxide (4:4:3:1)]. Separated compounds were analyzed by using liquid scintillation counting.

Results

Soil contained [^{14}C]cyromazine residues ranging from 0.12 to 0.18 ppm in the upper 3 inches, radioactivity was not detected below this depth for at least 27 weeks (last sampling date). Characterization of the radioactivity in soil can be found in Table 1. Extractable cyromazine declined from 80.8% of the total radioactivity at 0 weeks to 37.1% at 27 weeks; the half-life was ∿12 weeks.

The uptake of [^{14}C]cyromazine residues are shown in Table 2. Stalks of spring wheat and tops of carrots accumulated the greatest amounts of residues, as much as 0.76 and 0.27 ppm, respectively, when grown entirely in treated soil. Characterization of the extractable ^{14}C residues in spring wheat stalks showed that 21.7% of the total radioactivity was accounted for by cyromazine and 40.7% by melamine.

Table 1. Characterization of ^{14}C residues in the upper 3 inches of a silt loam soil amended with chicken manure at 20 ton/A containing [^{14}C]cyromazine at 5 ppm. The application rate is equivalent to 0.2 lb ai/A.

Compound	Percent of total radioactivity			
	0 Weeks	4 Weeks	12 Weeks	27 Weeks
Non-extractable	1.7	3.6	15.5	43.4
Extractable	99.1	82.3	84.5	81.9
Cyromazine[a]	80.8	--	45.4	37.1
Melamine[a]	14.9	--	25.0	40.5

[a]Identified by using thin-layer chromatography.

Table 2. Radioactivity present in lettuce, spring wheat, and carrots planted 95 days after a silt loam soil was amended with chicken manure at 20 ton/A containing [^{14}C]cyromazine at 5 ppm.

Treated Soil (inches)	^{14}C Residues (ppm)[a]				
	Lettuce leaves	Spring wheat		Carrot	
		Stalks	Grain	Tops	Roots
3	0.03	0.23	0.15	0.13	0.03
Non-extractable	--	(41.8%)	(69.4%)	--	--
Extractable	--	(68.6%)	(13.6%)	--	--
8	0.05	0.76[b]	0.17	0.27	0.06
Non-extractable	--	(41.5%)	(60.8%)	(28.5%)	--
Extractable	--	(71.3%)	(19.0%)	(60.1%)	--

[a]Lettuce, wheat, and carrots harvested 70, 93, and 120 days, respectively, after planting.

[b]Cyromazine accounted for 21.7% and melamine for 40.7% of the extractable radioactivity (identified by using ion exchange chromatography).

Table 2. Radioactivity present in lettuce, spring wheat, and carrots planted 95 days after a silt loam soil was amended with chicken manure at 20 ton/A containing [^{14}C]cyromazine at 5 ppm.

Treated Soil (inches)	^{14}C Residues (ppm)[a]				
	Lettuce leaves	Spring wheat		Carrot	
		Stalks	Grain	Tops	Roots
3	0.03	0.23	0.15	0.13	0.03
Non-extractable	--	(41.8%)	(69.4%)	--	--
Extractable	--	(68.6%)	(13.6%)	--	--
8	0.05	0.76[b]	0.17	0.27	0.06
Non-extractable	--	(41.5%)	(60.8%)	(28.5%)	--
Extractable	--	(71.3%)	(19.0%)	(60.1%)	--

[a]Lettuce, wheat, and carrots harvested 70, 93, and 120 days, respectively, after planting.

[b]Cyromazine accounted for 21.7% and melamine for 40.7% of the extractable radioactivity (identified by using ion exchange chromatography).

Conclusions

Uptake of ^{14}C residues occurs in lettuce, spring wheat, and carrots planted 95 days after a Mississippi silt loam soil was amended with chicken manure containing [^{14}C]cyromazine at 5 ppm (equivalent to 0.2 lb ai/A). Residues were as high as 0.03, 0.23, 0.15, 0.13, and 0.03 ppm in lettuce leaves, wheat stalks, wheat grain, carrot tops, and carrot roots, respectively, when these crops were grown under greenhouse conditions in buckets containing treated soil in the upper 3 inches.

Soil residues were stable at 0.12-0.18 ppm for the first 27 weeks; soil was not sampled at a later date. Cyromazine degraded in soil with a half-life of ~12 weeks, the remainder of the radioactivity was accounted for by the cyromazine degradate, melamine, and non-extractable compounds.

Melamine accounted for 40.7% of the radioactivity in wheat grain and cyromazine accounted for 21.7%. In soil at the same date, melamine accounted for 40.5% of the radioactivity and cyromazine accounted for 37.1%. Therefore, it appears that melamine is taken up by wheat plants and its presence in the plant is not a result of *in vivo* metabolism of cyromazine to melamine.

Biological report for greenhouse grown lettuce, sugar beets, and spring wheat grown in soil aged after treatment with Δ-^{14}C-CGA-72662 and amended with poultry manure, Seim, V., and G. Brown, Ciba-Geigy Corporation, Vero Beach, Florida, December 22, 1981, Report No. BIOL-81016, Acc. No. 070915, Reference 48.

The uptake of Δ-^{14}C-CGA-72662 and its degradation products by various greenhouse grown indicator crops in chicken manure amended soil, Simoneaux, B., Ciba-Geigy Corporation, Greensboro, North Carolina, February 19, 1982, Report No. ABR-82003, Acc. No. 070915, Reference 52.

Procedure

Buckets containing the following four media were prepared: Georgia sandy loam soil (78.8% sand, 13.2% silt, 8.0% clay, pH 8.3, 2.9% organic matter, CEC 9.5 meq/100 g), soil amended with poultry manure at 5 ton/A, soil treated with cyromazine (CGA-72662) at 0.05 lb ai/A, and soil amended with poultry manure at 5 ton/A and treated with uniformly ring-labeled [^{14}C]-cyromazine at 0.05 ai lb/A. The cyromazine application rate is equivalent to manure containing 5 ppm at the rate of manure applied. The treated and amended soils were placed as a 3-inch layer on top of untreated soil. 'Grand Rapids' lettuce, 'H-21' sugar beets, and 'Podax' spring wheat were planted in the buckets 33 days after the soil treatments were prepared. Plants were grown in a greenhouse at 75-85 F and a 15-hour photoperiod with supplemental light. Plants were fertilized with 20-20-20 or 18-6-12 and irrigated with 1-2 inches of water per week. Lettuce, wheat, and sugar beets were grown for 43, 87, and 98 days, respectively (harvest was 76, 120, and 131 days post treatment, respectively).

Lettuce samples were taken at 28 and 43 days after planting, wheat at 43 and 87 days, and sugar beets at 64 and 98 days.

Methodology

Soil and plant samples were combusted and then extracted with methanol. Extracts were purified by removing the organic solvent under vacuum and passing the aqueous phase through a column of Sephadex anion exchange resin. The aqueous eluate was by lyophilized before redissolving the sample in methanol. The compounds in the sample were then characterized by thin-layer chromatography [compounds were developed in toluene:dioxane:methanol:ammonium hydroxide (4:4:3:1)]. Separated compounds were analyzed by using liquid scintillation counting.

Results

[^{14}C]Cyromazine residues in the upper 3 inches of soil declined from 0.064 ppm at 30 days after treatment to 0.044 ppm at 121 days after treatment. Levels in the 3- to 6-inch and 6- to 8-inch layers were 0.020 and 0.002 ppm, respectively, at 30 days after treatment, and 0.016 and 0.003 ppm, respectively, at 121 days after treatment. Characterization of the radioactivity in soil can be found in Table 1. Extractable cyromazine declined from 72.3% of the total radioactivity at 30 days after treatment to 65.4% at 121 days after treatment.

[^{14}C]Cyromazine residues were not quantifiable (<0.009 ppm) in lettuce leaves and sugar beet roots at both sampling dates (61 and 76 days and 97 and 131 days after treatment, respectively). Residues in sugar beet tops declined from 0.011 ppm at 97 days after treatment to <0.009 ppm at 131 days after treatment. The uptake of ^{14}C residues by spring wheat was greater than that of the other two crops. Residues of 0.022 ppm were found in stalks 66 days after treatment. At 120 days after treatment, residues were 0.112, 0.078, and <0.009 ppm in wheat straw, hulls, and grain, respectively. Characterization of the extractable ^{14}C residues in wheat straw showed that 72.3% of the total radioactivity was accounted for by cyromazine and 6.5% by melamine (34.6% of the radioactivity was non-extractable).

No data were reported for soils that were amended only with chicken manure or treated with cyromazine alone.

Conclusions

Uptake of ^{14}C residues occurs in spring wheat and tops of sugar beets planted 33 days after a Georgia sandy loam soil was amended with chicken manure at 5 ton/A and treated with [^{14}C]cyromazine at 0.05 lb ai/A. Uptake of ^{14}C residues in quantifiable levels did not occur in lettuce, and residues were not found in sugar beet roots or wheat grain (<0.009 ppm). Residues were as high as 0.112 and 0.078 ppm in wheat straw and hulls, respectively, at 120 days after treatment. Residues in sugar beet tops declined from 0.11 ppm at 97 days after treatment to non-quantifiable levels 34 days later.

[^{14}C]Cyromazine residues in the upper 3 inches of soil declined from 0.064 ppm at 30 days after treatment to 0.044 ppm 91 days later. Little radioactivity was found at lower depths; 0.016 and 0.003 ppm were found in the 3- to 6-inch and 6- to 8-inch layers, respectively.

Melamine accounted for 6.5% of the radioactivity in wheat straw and cyromazine accounted for 72.3%. In soil at the same sampling date, melamine accounted for 17.8% of the radioactivity. Therefore, it appears that melamine is taken up by wheat plants, and its presence in the plant is not a result of *in vivo* metabolism of cyromazine to melamine.

Separation of polar triazine herbicide metabolites on an Aminex A-5 cation exchange column, Halama, P., and B. Simoneaux, Ciba-Geigy Corporation, Ardsley, New York, June 20, 1973, Method AG-248, Acc. No. 070915, Reference 57.

Procedure/Methodology

Biological materials are extracted with procedures described in Method AG-214. The two resulting organic phases are evaporated, and their aqueous phases are combined, and concentrated by azeotropic distillation with ethanol. This solution is evaporated to near dryness in a rotavapor apparatus, and then is transferred with a small amount of water to a separatory funnel. The residue in the flask is rinsed four times with hexane; each rinse is added to the separatory funnel. The solution is shaken, allowed to stand until an aqueous and organic phase separate, and then each phase is radioassayed. The aqueous phase is transferred to a flask containing ethanol and evaporated to near dryness in a rotavapor. The residue is dissolved in a 0.1 N buffer solution (pH 4.0) in an ultrasonic bath and then placed in a flask. This process is repeated twice. An aliquot of the combined solutions is then radioassayed. An additional aliquot with L-histidine and L-tyrosine markers is placed on an Aminex column.

The Aminex column is prepared by pouring a slurry containing 20-25 g Aminex A-5 resin and pH 4.0 buffer solution into a glass column to a height of 15 cm with pressure from nitrogen gas.

The column is eluted with 300 ml each of pH 4.0 and pH 6.0 buffer solutions and then 300 ml 0.1 N ammonium hydroxide solution; 180 5-ml fractions are collected and radioassayed.

The location of the tyrosine and histidine markers is determined by spotting on silica gel plates aliquots from five fractions on either side of the assumed elution peak of each amino acid. The location is then related to elution volume of radioactive peaks. Identification of unknown peaks is made by measurement of their elution volume in relation to elution volumes of the two amino acids and previously measured hydroxy triazine standards.