4-AMINOPYRIDINE

Task 1: Review and Evaluation of Individual Studies

Contract No. 68-01-5830

Final Report

April 4, 1980

SUBMITTED TO:

Environmental Protection Agency
Arlington, Virginia 22202

SUBMITTED BY:

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4-Aminopyridine

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CASE GS0015 4-AMINOPYRIDINE STUDY 1 PM 320 09/19/79

CHEM 069201 4-Aminopyridine

BRANCH EFB DISC 30 TOPIC 0505

FORMULATION 00 - ACTIVE INGREDIENT

FICHE/MASTER ID 00004030 CONTENT CAT 01

Sullivan, J.B. (1970) Final Report: Evaluation of 4-Aminopyridine, Evaluation of the Sponsor's Analytical Method, and Analysis of Field Samples. Includes method dated May 27, 1970. (Unpublished study received Jan 3, 1973 under 1F1013; prepared by Hazleton Laboratories, Inc., submitted by Phillips Petroleum Co., Bartlesville, Okla.; CDL:091757-C)

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
PRIM: RCBR-25-10125005 RCBR-35-05200030
SEC: EFB -30-051015 EFB -30-051515

DIRECT RVW TIME = 13 (MH) START-DATE END DATE

REVIEWED BY: D. Harper
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SIGNATURE: Daniel Harper DATE: Dec. 18, 1979

APPROVED BY:
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CONCLUSIONS:

Physical/Chemical Transformation - Photolysis

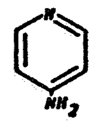
This portion of the study is scientifically invalid because dark controls were not run and a quantitative analysis was not performed.

Field Dissipation - Terrestrial

This part is scientifically invalid because the application rate of 4-aminopyridine to the field samples, the date that samples were collected, and the texture of the samples were not given.

MATERIALS AND METHODS:

AVITROL



4 - Aminopyridine

Physical/Chemical Transformation - Photolysis

4-Aminopyridine (formulation and purity not reported) was placed in a petri dish and exposed to direct sunlight for about 6 weeks. Also, two samples of [^{14}C]4-aminopyridine were placed in petri dishes with concentrated HCl. One of the dishes was exposed to sunlight for several hours, and the other sample was placed in a UV cabinet under short- and long-wave UV light for the same length of time. Butyl acetate was added to each dish, and the dishes were shaken. After the phases separated, the butyl acetate fraction was removed. The solution was reextracted with butyl acetate, and the butyl acetate fraction was removed and combined with the first butyl acetate fraction. The butyl acetate fraction was dried over an anhydrous Na_2SO_4 column. The eluate was evaporated almost to dryness with a stream of N_2. Small amounts of the solution were spotted on alumina thin-layer chromatography (TLC) plates along with standards. The plates were developed in 80% isopropyl alcohol, 4% NH_4OH, and 16% distilled water (SS-1) or in 3% NH_4OH and 97% acetone (SS-2). Developed plates were placed in a beta camera and a betagram was prepared.

The TLC plates of the nonradiolabeled 4-aminopyridine that had been exposed to sunlight were visualized with a UV system.

Field Dissipation - Terrestrial

Air-dried control soil samples were spiked with 4-aminopyridine at 0.4 ppm (formulation and purity not reported) and placed in a flask containing an NH_4OH-acetonitrile solution. The flask was shaken and filtered.

The filtrate was dried, and the residue was dissolved in butyl acetate. The solution was partitioned with 0.1 N HCl. The acid phase was separated and washed with butyl acetate. The phases were allowed to separate, and the aqueous phase was collected, adjusted to pH 11 with NaOH, and extracted with butyl acetate. The butyl acetate phase was dried over anhydrous Na_2SO_4. The eluates were combined and reduced to dryness with a flash evaporator. The residues of half of the samples were dissolved in acetone, and the solution was streaked on an alumina TLC plate. The plates were developed in SS-1 and SS-2 and analyzed by an unspecified liquid scintillation counting (LSC) method. The other half of the samples were analyzed directly by gas chromatography (GC).

REPORTED RESULTS:

Physical/Chemical Transformation - Photolysis

The R_f values of the 4-aminopyridine exposed to sunlight or UV light reportedly were identical to the R_f value of standard 4-aminopyridine. Therefore, it was concluded that 4-aminopyridine was not photodegraded.

Field Dissipation - Terrestrial

The residue levels measured in all control and treated corn and soil samples were less than 0.1 ppm.

DISUCSSION:

1. All of the methods used were presented in an unclear and confusing manner. The period of time that the 4-aminopyridine-HCl mixture was exposed to UV light was not reported. The author did not report the location of the ^{14}C label on the 4-aminopyridine molecule. The reviewer assumes that since GC was used to analyze the field samples, it was also used to analyze the spiked samples. The author did not describe the texture and characteristics of the soil samples or the depths to which the soils were sampled. The application rate of 4-aminopyridine to the field samples and the date that soil samples were collected were not provided. The quantity or concentration and the formulation and purity of the 4-aminopyridine used in the various experiments were not specified. In addition, the reviewer questions the acceptability of using petri dishes in a solvent extraction method.

2. Dark controls were not run in the photolysis study, and a quantitative method was not employed in the analysis for photolysis products.

3. Method sensitivity and raw GC and LSC data were not provided; therefore, the reviewer is unable to verify the accuracy of the methods used.

4. The photographs of the betagrams presented in the study did not show any streaks from the movement of the compounds on the TLC plates. Some of the copies of the gas chromatograms were not legible.

5. The photolysis study was conducted in petri dishes containing HCl. The reviewer believes the 4-aminopyridine should have been added to distilled water instead of HCl.

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CHEM 069201 4-Aminopyridine

BRANCH EFB DISC 30 TOPIC 05

FORMULATION 00 - ACTIVE INGREDIENT

FICHE/MASTER ID 00004007 CONTENT CAT 02

Sullivan, J.B. (1970) Progress Report: Evaluation of 4-Aminopyridine. Includes method entitled: Thin-layer chromatography (Betagram O). (Unpublished study received on unknown date under 224-EX-3; prepared by Hazleton Laboratories, Inc., submitted by Phillips Petroleum Co., Bartlesville, Okla.; CDL:122744-N)

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
PRIM: RCBR-35-05200032
SEC: EFB -30-052010 EFB -30-051510

DIRECT RVW TIME = 1 (MH) START-DATE END DATE

REVIEWED BY: S. Gould
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SIGNATURE: Sandra Gould DATE: Dec. 18, 1979

APPROVED BY:
TITLE:
ORG:
LOC/TEL:

SIGNATURE: DATE:

CONCLUSION:

An independent assessment of this study is not provided because the identical information is included in Study 1 (MRID 00004030), which was reviewed and evaluated in its entirety.

CASE GS0015 4-AMINOPYRIDINE STUDY 3 PM 320 09/19/79

CHEM 069201 4-Aminopyridine

BRANCH EFB DISC 30 TOPIC 050525 GUIDELINE 40 CFR 163.62-9b/c/d

FORMULATION 00 - ACTIVE INGREDIENT

FICHE/MASTER ID 05003407 CONTENT CAT 01

Betts, P.M.; Giddings, C.W.; Fleeker, J.R. (1976) Degradation of 4-aminopyridine in soil. Journal of Agricultural and Food Chemistry 24(3):571-574.

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
SEC: EFB -30-05052005 EFB -30-05052010

DIRECT RVW TIME = 8½ (MH) START-DATE END DATE

REVIEWED BY: B. Karnofsky
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CONCLUSIONS:

Metabolism - Soil

1. This portion of the study is scientifically valid.

2. Based on this study, 4-aminopyridine is gradually metabolized in soil under both aerobic and anaerobic conditions. In clay, loam, and sandy soils maintained under aerobic conditions, about 6, 20, and 24%, respectively, of the applied [^{14}C]4-aminopyridine was recovered as $^{14}CO_2$ after 60 days. Under flooded conditions, between 21 and 24% of the applied compound was recovered as $^{14}CO_2$ over the same period. No degradation products were detected in soil extracts from either of these studies.

3. A 20-day lag in $^{14}CO_2$ production occurred under aerobic conditions but not under flooded conditions.

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4. This portion of the study partially meets metabolism data requirements in Sections 163.62-8(b,c) of EPA's Proposed Guidelines for Registering Pesticides (July 1978) by providing data on the rate of aerobic and anaerobic soil metabolism.

Metabolism - Effects of Microbes on Pesticides

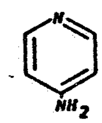
1. This portion of the study is scientifically valid.

2. None of several pure cultures of microbes (*Pseudomonas fluorescens*, *Enterobacter aerogenes*, *Aspergillus niger*, *Streptomyces griseus*, and *Agrobacterium tumefaciens*) were able to metabolize 4-aminopyridine to detectable metabolites within 120 hours.

3. This portion of the study partially meets the microbiological data requirement in Section 163.62-8(f)(2) by providing some data on the ability of pure cultures of soil microbes to metabolize 4-aminopyridine.

MATERIALS AND METHODS:

AVITROL



4 - Aminopyridine

Metabolism - Soil

Air-dried samples of Barnes sandy loam (64% sand, 18% silt, 18% clay, 4.2% organic matter, pH 7.4), Towner loamy fine sand (85% sand, 10% silt, 5% clay, 1.1% organic matter, pH 7.2), and Fargo clay (1% sand, 33% silt, 66% clay, 3.4% organic matter, pH 7.7) were placed in bio-meter flasks and treated with [2-^{14}C]4-aminopyridine (U.S. Fish and Wildlife Service, >98% purity) to yield 10 ppm. For the measurement of CO_2 evolution, 0.4 N CO_2-free NaOH was placed in the sidearms of the flasks. The flasks were sealed and incubated at 20 C. The NaOH was periodically removed and assayed for $^{14}CO_2$ by liquid scintillation counting. After incubation, the treated soils were extracted with 1 N HCl and ethanol and analyzed with thin-layer chromatography (TLC).

For the study of degradation in soil, chopped corn was impregnated with 4-aminopyridine, and the soil in biometer flasks was scattered with treated or untreated corn. The flasks were incubated and sampled as described above. Degradation in flooded soil was studied by covering fresh soil samples in biometer flasks with water and incubating them at 25 C for 60 days. The $^{14}CO_2$ and soil residues were analyzed as described above.

Metabolism - Effects of Microbes on Pesticides

Some common soil microbes (*Pseudomonas fluorescens*, *Enterobacter aerogenes*, *Aspergillus niger*, *Streptomyces griseus*, and *Agrobacterium tumefaciens*) were studied to determine their ability to utilize 4-aminopyridine as a carbon source. Pure cultures were incubated with 4-aminopyridine at 0, 10, and 100 µg/200 ml media at 24-26 C on a rotary shaker for 120 hours. The cultures were centrifuged for 20 minutes, and the supernatant was analyzed for ^{14}C and chromatographed on TLC plates. The cells were extracted with hot ethanol, and the extracts were chromatographed on TLC plates. Growth of *P. fluorescens*, *E. aerogenes*, and *A. tumefaciens* was followed by a change in absorbence at 650 nm. Growth of *S. griseus* and *A. niger* was followed by dry weight increase of the cells.

REPORTED RESULTS:

Radioactive compounds other than 4-aminopyridine were not detected on chromatographs of the extracts from any of the studies. Labeled CO_2 from the NaOH traps was the only degradation product detected. In moist soil under anaerobic conditions, a lag period of approximately 20 days was observed before the evolution of CO_2 was detected. It was suggested that the lag period was needed for an increase in populations of microbes capable of degrading the pesticide. Depending on soil type, between 6 and 24% of the applied radioactivity was recovered as CO_2 over a 60-day period. The level of CO_2 formation was different for each soil type (Fargo>Towner>Barnes); however, it was not related to the organic matter content or any other reported characteristic of the soils.

When the stability of 4-aminopyridine was tested in moist soil treated with the corn impregnated with the compound, a lag time before $^{14}CO_2$ evolution similar to that found in directly treated soil was observed, and 16% of the radioactivity was recovered as $^{14}CO_2$. No lag time was observed in the production of $^{14}CO_2$ from untreated chopped corn; however, a 10-day lag time was observed with the treated chopped corn. Again, the lag was believed to reflect a period of population increase for organisms tolerant to the high 4-aminopyridine levels on the bait.

None of the organisms tested were able to utilize 4-aminopyridine as a carbon source or to metabolize the compound to detectable metabolites. All of the radioactivity remaining in the culture media and ethanol extracts chromatographed as 4-aminopyridine. At the tested concentrations, no growth inhibition was observed in any of the microbes assayed.

Under flooded conditions, the lag period experienced under aerobic conditions was not observed. However, degradation of the compound remained slow. The loss of extractable radioactivity ranged from 21 to 24% over a 60-day period.

DISCUSSION:

1. The pyridine ring was not uniformly labeled; therefore, release of $^{14}CO_2$ should not be interpreted to mean complete oxidation of all the carbons of 4-aminopyridine. Because of the position of the label (^{14}C-2), the finding that no radioactive degradation products were present should be accepted with caution.

2. The level of extraction recovery was not reported. Although no degradation products were discovered in the extracts, some may have remained in the soil as bound residues.

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CHEM 069201 4-Aminopyridine

BRANCH EFB DISC 30 TOPIC 0505

FORMULATION 00 - ACTIVE INGREDIENT

FICHE/MASTER ID 05003185 CONTENT CAT 01

Starr, R.I.; Cunningham, D.J. (1975) Leaching and degradation of 4-aminopyridine-14C in several soil systems. Archives of Environmental Contamination and Toxicology 3(1):72-83.

SUBST. CLASS = S.

DIRECT RVW TIME = 12 (MH) START-DATE END DATE

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CONCLUSIONS:

Mobility - Leaching

1. This part of the study is scientifically valid.

2. Virtually no leaching occurred in any of the soils tested after an application of 7 inches of water. The pesticide was strongly adsorbed to the soil in the upper 1-inch layer.

3. Data from this part of the study partially satisfy the requirements in Section 163.62-9(b) of EPA's Proposed Guidelines for Registering Pesticides (July 1978) by providing information on leaching after an application of 7 inches of water.

Metabolism - Soil

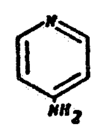
1. This portion of the study is scientifically valid.

2. Aerobic [^{14}C]4-aminopyridine soil metabolism rates increased with increasing organic matter and pH. Of two slightly acidic (pH 5.6-5.8) loam soils containing 16% clay, the soil with the greater level of organic matter (5%) degraded to $^{14}CO_2$ almost 3 times faster ($t_{½}$ = 8 months) than a similar soil with 2.9% organic matter ($t_{½}$ = 22 months). Soil pH also influenced 4-aminopyridine metabolism. Recovered $^{14}CO_2$ was minute (0.35% over 3 months) for a highly acidic loam (pH 4.1) but much greater (4.88%) for an alkaline sandy clay loam with similar levels of organic matter and clay.

3. Data from this portion of the study partially fulfill aerobic and anaerobic soil metabolism data requirements in Sections 163.62-8(b,c) of EPA's Proposed Guidelines for Registering Pesticides (July 1978). This portion of the study would have completely fulfilled data requirements if residues comprising more than 10% of initial application (or 0.01 ppm) had been identified, a material balance had been provided, and the experiment had been continued until a 90% loss of the pesticide was seen.

MATERIALS AND METHODS:

AVITROL



4 - Aminopyridine

Mobility - Leaching

Triplicate soil samples (160 g) (Table 1) in 15-cm columns were surface treated with 1 ml (1,600 μg; 2 μCi) of α-labeled [^{14}C]4-aminopyridine in methanol. The [^{14}C]4-aminopyridine had a specific activity of 0.15 mCi/mM, and the unlabeled compound was analytical grade. A single control was treated with methanol only. Distilled water was periodically added to each column to produce 10 ml of leachate. Throughout the experiment, the columns were kept in a growth chamber maintained at 27 C (day) or 21 C (night), 40% relative humidity, and 26,900 lux light intensity. Radioactivity in each leachate was assayed by liquid scintillation counting (LSC). Twenty days later, the soil columns were removed by air pressure, sectioned (1 inch), and frozen. Each section was mixed with acetone and vacuum filtered, and the activity in the extract was analyzed by LSC. The soil from each extracted section was air dried and pulverized, and 0.1-g subsamples were oxidized by Schoniger oxygen flask combustion. The combustion products were absorbed in ethanolamine in methanol, and the solution was counted by LSC.

Metabolism - Soil

Ten-gram samples of each of three alkaline soils (A-C, Table 1) were wetted to 50% water holding capacity and placed in stainless steel wire (64 mesh) cylinders (aerobic) and in 10-ml beakers (anaerobic). The cylinders and beakers were incubated for 1 week in enclosed jars maintained in an oven at 29 C. The aerobic samples were flushed with O_2, and the soils were moistened periodically. Each soil was then surface treated with methanol (0.1 ml) containing 100 µg (0.2 µCi) of [^{14}C]4-aminopyridine; 1 g of untreated soil was placed over the treated soil to yield a concentration of 10 ppm. A beaker containing 3 ml of 1 N NaOH was placed in each jar as an absorbent solution. The jars were then sealed and returned to the oven for up to 2 months. Anaerobic samples were maintained under a layer of water and not disturbed; aerobic samples were flushed with O_2 and remoistened every 3 or 4 days. The absorbent solutions were removed and replaced with fresh solutions at 7, 29, 43, and 56 days after treatment. Radioactivity in the solutions was assayed by LSC.

In a second degradation experiment, 8-g samples of six soils (A-E, G, Table 1) were wetted to 50% water holding capacity, maintained under aerobic conditions for 3 months at 30 C (in addition, soils A and E were maintained at 15 C, 50% water, and 30 C, 25% water, respectively) and treated with [^{14}C]4-aminopyridine by procedures reported previously for aerobic soil samples (except there was no mention of O_2 flushing). In addition to the NaOH absorbent solution, 3 ml of 1 N H_2SO_4 was placed in each jar of soil A that was moistened to 50% water holding capacity to absorb possible volatilized compounds. Alkaline and acidic absorbents were removed and replaced with fresh solution at 2-week intervals. Radioactivity in the solutions was assayed by LSC. Samples for each treatment were combined and blended with acetone after the 3-month incubation period. Each slurry was vacuum filtered, and the resulting soil filter cakes were rinsed with acetone. Radioactivity in these extracts was analyzed by LSC and later by thin-layer chromatography (TLC)-autoradiography. The filter cakes were analyzed for radioactivity as reported for the leaching portion of this study.

Soil A filtrate was analyzed by TLC-autoradiography before and after purification, and the results were compared with those for [^{14}C]4-aminopyridine standards. After the autoradiograms were developed, the radioactivity in the TLC adsorbents of both crude and purified soil A filtrates was assayed by LSC.

REPORTED RESULTS:

Mobility - Leaching

Radioactivity was detected in the leachate from all of the alkaline soils (A-C) and one acidic soil (D) after 7 inches of simulated rainfall over a 20-day period. Maximum cumulative detectable leachate radioactivity was 0.18, 0.02, 0.09, and <0.01% of the initial ^{14}C application for soils A, B, C, and D, respectively.

Most of the adsorbed radioactivity in three soil sections was in the upper 1-inch soil layer; it ranged from 77% (soil A) to 91-99% (soils B-G) of the radioactivity in the three soil sections. Recovery percentages ranged from 42 to 60% of the initial application (Table 2). The authors concluded that 4-aminopyridine was strongly adsorbed to soil and that adsorption was pH dependent.

Metabolism - Soil

Under aerobic incubation in alkaline soils, degradation of [^{14}C]4-aminopyridine to $^{14}CO_2$ was greater than it was under anaerobic conditions, with virtually no degradation occurring before 1 week for both treatments (Table 3).

The results of the second degradation experiment are presented in Table 4. These data show that 4-aminopyridine was strongly adsorbed to soils tested and was virtually nonextractable by acetone. After 3 months of incubation, acetone extracts of acidic soil G gave only 0.5% of the initial applied ^{14}C, the lowest recovery for the soils tested. The authors suggested that a better method be developed to overcome the strong adsorption of the pesticide.

The investigators suggested that in soil the degradation of 4-aminopyridine proceeds by deamination to produce 4-hydroxypyridine followed by formation of either of the pyridine diols, 3,4-dihydroxypyridine or 3,4,6-trihydroxypyridine. The ring is then ruptured, and aliphatic compounds are metabolized to CO_2.

Figures 1 and 2 show the effects of soil temperature and moisture on breakdown of 4-aminopyridine in two soils. Increased temperature and moisture caused more pesticide breakdown in both soils, but the rate of degradation was greater in soil A (Figure 1), which contained less clay than soil E (Figure 2).

No radioactivity was detected in the acidic adsorption solution, which indicated an absence of volatilization of [^{14}C]4-aminopyridine or of basic radiolabeled metabolites.

DISCUSSION:

1. EPA's Proposed Guidelines for Registering Pesticides (July 1978) require that 20 acre-inches of water be used in leaching studies. The authors only reported results after an application of 7 inches of water, thus underestimating leaching potential.

2. The authors stated that the leaching study showed that adsorption of [^{14}C]4-aminopyridine is pH dependent. Although there is a trend toward an inverse relationship (pH range 4.1-7.8, adsorption 60.2-41.8), the authors did not provide statistical support for their statement that adsorption is pH dependent.

3. There was no statement of variability or statistical analysis, although the authors mentioned the use of more than one replication.

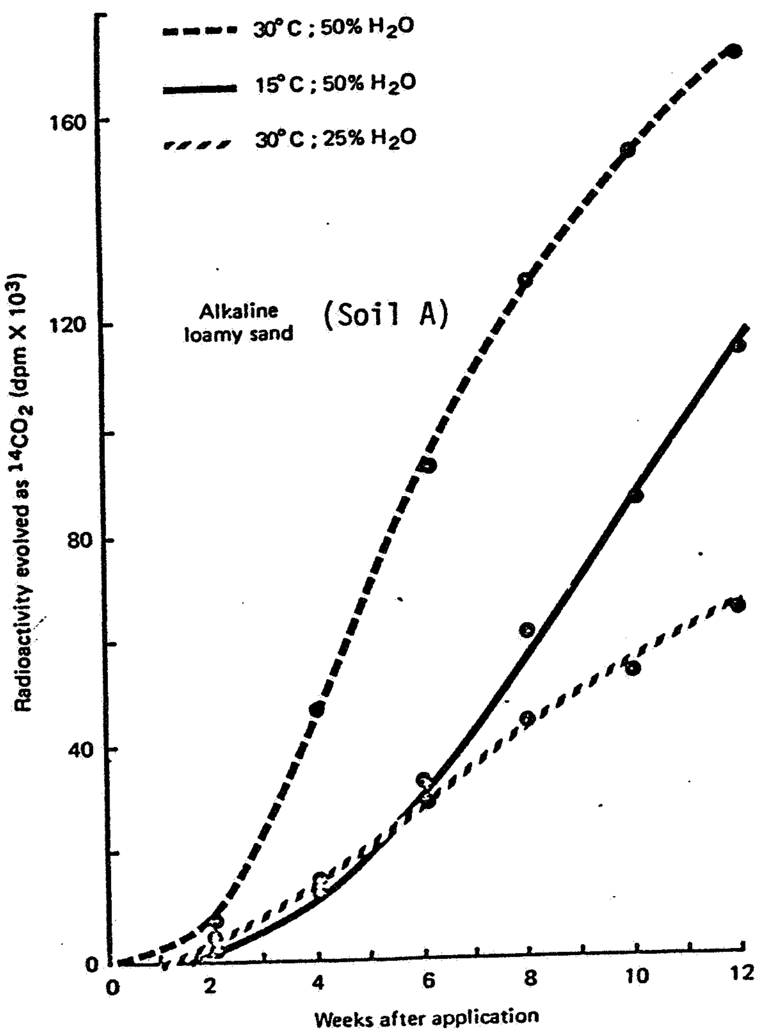


Figure 1. Effect of temperature and moisture on the degradation of [^{14}C]4-aminopyridine at 10 ppm in an alkaline loamy sand soil (A) over a 3-month period.

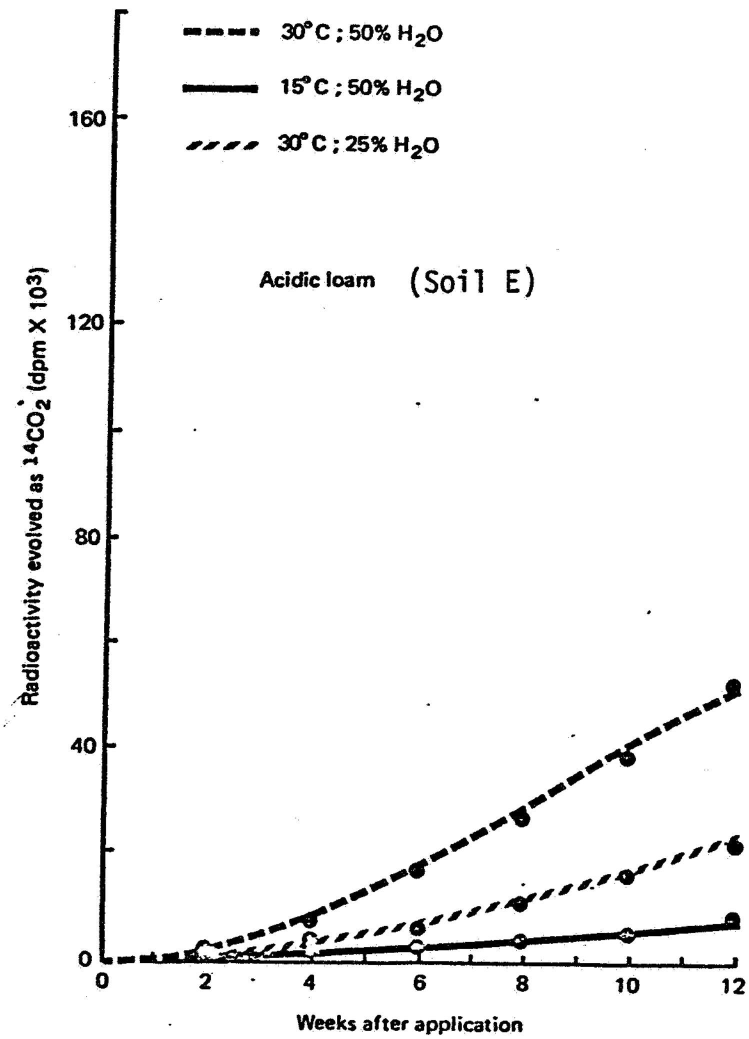


Figure 2. Effect of temperature and moisture on the degradation of [^{14}C]4-aminopyridine at 10 ppm in an acidic loam soil (E) over a 3-month period.

Table 1. Characteristics of soils used in leaching and degradation studies.

Soil	Soil type	pH (paste)	Organic matter (%)	Sand (%)	Silt (%)	Clay (%)
			Alkaline soils			
A	Loamy sand	7.8	2.9	77	16	7
B	Sandy clay loam	7.7	4.0	61	19	20
C	Sandy clay loam	7.6	1.9	47	27	26
			Acidic soils			
D	Loam	5.8	2.9	46	38	16
E	Loam	5.6	5.0	50	34	16
F	Sandy loam	5.6	4.0	53	33	14
G	Loam	4.1	1.4	31	44	25

Table 2. Distribution of radioactivity in soils containing [^{14}C]4-aminopyridine at 10 ppm after application of 7 inches of simulated rainfall over 20 days.

		Mean radioactivity[a]			
		Percent of ^{14}C recovered from sections of soil columns			
Soil	Section	Acetone extractable	Non-extractable	Total	Percent of initial application
		Alkaline soils			
A	Upper	18.35	76.83	95.18	41.82
	Middle	0.27	2.92	3.19	
	Lower	0.08	1.55	1.63	
B	Upper	7.39	91.42	98.81	53.51
	Middle	0.03	0.74	0.77	
	Lower	0.03	0.39	0.42	
C	Upper	6.47	91.06	97.53	62.22
	Middle	0.05	2.16	2.21	
	Lower	0.03	0.23	0.26	
		Acidic soils[b]			
D	Upper	2.91	97.09	-	66.96
E	Upper	5.04	94.96	-	68.86
F	Upper	3.58	96.42	-	63.38
G	Upper	0.55	99.45	-	60.17

[a] Mean values for three samples per soil type.

[b] Radioactivity was not detected in acetone extracts of the middle or lower section of any acidic soil.

Table 3. Degradation of [^{14}C]4-aminopyridine at 10 ppm in alkaline soils incubated for 2 months under aerobic and anaerobic conditions at 30 C and 50% moisture.[a]

Soil	Soil aeration	Mean radioactivity recovered as $^{14}CO_2$[b] (% of initial application)			
		7 days	29 days	43 days	56 days
A	Aerobic	0.29	20.94	41.37	41.83
	Anaerobic	0.04	0.28	0.15	--
B	Aerobic	0.29	2.69	17.88	44.45
	Anaerobic	0.05	0.25	0.40	--
C	Aerobic	0.45	0.07	23.38	59.68
	Anaerobic	0.04	0.47	0.31	--

[a] Moisture expressed as 50% of the maximum capillary capacity.

[b] Mean values for four samples per soil type.

Table 4. Degradation of [^{14}C]4-aminopyridine at 10 ppm in soils incubated for 3 months under aerobic conditions at 30 C and 50% moisture.

	Mean radioactivity (% of initial application)[a]				
		Residual			
Soil	Degraded to $^{14}CO_2$	Acetone extractable	Non-extractable	Total	Half-life (months)[b]
		Alkaline soils			
A	54.16	4.54	26.60	85.30	3
B	10.74	5.66	47.15	63.55	11
C	4.88	11.23	45.32	61.43	32
		Acidic soils			
D	5.95	1.87	51.78	59.60	22
E	16.52	3.83	46.84	67.19	8
F	0.35	0.46	42.00	42.81	>22

[a] Mean values for three samples per soil type.

[b] Approximate time required for 50% of parent compound to degrade to CO_2.

CASE GS0015 4-AMINOPYRIDINE STUDY 5 PM 320 09/19/79

CHEM 069201 4-Aminopyridine

BRANCH EFB DISC 30 TOPIC 050525 GUIDELINE 40 CFR 163.62-9b/c/d

FORMULATION 00 - ACTIVE INGREDIENT

FICHE/MASTER ID 00004009 CONTENT CAT 01

Starr, R.I.; Cunningham, D.J. (1969) Absorption and Translocation of 4-Aminopyridine by Corn Plants: Progress Report No. 3--September 29, 1969. (Unpublished study received Oct 20, 1969 under 224-EX-3; prepared by U.S. Fish and Wildlife Service, Denver Wildlife Research Center, submitted by Phillips Petroleum Co., Bartlesville, Okla.; CDL:122744-P)

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
PRIM: RCBR-20-15
SEC: EFB -20-150515 RCBR-20-150505

DIRECT RVW TIME = 1 (MH) START-DATE END DATE

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CONCLUSION:

This study on the fate of 4-aminopyridine in soil and corn has been updated and resubmitted; it is reviewed in detail in Study 6 (MRID 00004001).

CASE GS0015 4-AMINOPYRIDINE STUDY 6 PM 320 09/19/79

CHEM 069201 4-Aminopyridine

BRANCH EFB DISC 20 TOPIC

FORMULATION 00 - ACTIVE INGREDIENT

FICHE/MASTER ID 00004001 CONTENT CAT 01

Starr, R.I.; Cunningham, D.J. (1970) Translocation and Degradation of 4-Aminopyridine in Corn Plants--Its Movement and Degradation in Soil Systems: ¢Third Periodic Report, Avitrol Concentrate|. (Unpublished study received Apr 24, 1970 under 224-EX-3; prepared by U.S. Fish and Wildlife Service, Denver Wildlife Research Center, submitted by Phillips Petroleum Co., Bartlesville, Okla.; CDL: 122744-H)

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
PRIM: EFB -30-05
SEC: EEB -20-201028005 EFB -20-150515 RCBR-20-150505
EFB -30-05052010 EFB -30-05101510 EFB -30-051020
RCBR-20-1510

DIRECT RVW TIME = 9½ (MH) START-DATE END DATE

REVIEWED BY: D. Harper
TITLE: Staff Scientist
ORG: Enviro Control, Inc., Rockville, MD
LOC/TEL: 468-2500

SIGNATURE: Daniel Harper DATE: Dec. 20, 1979

APPROVED BY:
TITLE:
ORG:
LOC/TEL:

SIGNATURE: DATE:

CONCLUSIONS:

The part of this study dealing with the phytotoxicity, translocation, and degradation of 4-aminopyridine in corn does not contain environmental chemistry data. Therefore, a full review of this part of the study was not conducted.

Physical/Chemical Transformation, Metabolism - Microbiological, Mobility

1. This portion of the study is scientifically valid.

2. Approximately 14, 15, and 18% of the applied radioactivity (10 ppm [^{14}C]4-aminopyridine) was lost after 7 days from nonaerated plant nutrient solution, aerated nutrient solution, and aerated water, respectively. This loss was due to either microbiological degradation, volatilization, hydrolysis, or oxidation.

Metabolism - Soil

The part of this study dealing with the metabolism of 4-aminopyridine in soil has been published; it is reviewed in detail in Study 4 (MRID 05003185).

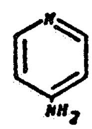
Mobility - Leaching

1. This study is scientifically valid.

2. Less than 0.03% of the applied radioactivity leached through the soil in 7 days. Approximately 97% of the radioactivity remaining in the soil was in the top inch of the soil column; therefore, 4-aminopyridine did not leach under the conditions of this experiment.

MATERIALS AND METHODS:

AVITROL



4 - Aminopyridine

Samples of four soils (for soil characteristics see Table 1) were packed in chromatographic columns (4 cm in diameter and 15 cm long). A corn seedling was planted in each column. Ten days later, the column was treated with [^{14}C]4-aminopyridine at 10 ppm. Water was added to move the compound into the soil. The plants were watered every 1-2 days with a nutrient solution and enough water to produce 10 ml of leachate. The total amount of water added was 5.8 inches. At each sampling period, an aliquot of each leachate solution was added to scintillation solution and Triton X-100. The radioactivity was determined by liquid scintillation counting (LSC).

The soils were removed from the columns. One of the sandy loam soils (4% organic matter) was cut into 1-inch sections and extracted in a blender with water and acetone. The solution was filtered, and the soil filter cake was rinsed twice with acetone. The soil extract was analyzed by LSC and thin-layer chromatography.

The soil remaining after extraction was air dried and weighed. A portion was placed in a counting vial with water, and an ethanolamine-methanol solution and a scintillation solution were added. The solution was mixed and counted.

As part of the control for the corn translocation study, the loss of [^{14}C]4-aminopyridine from aerated water, an aerated plant nutrient solution (adapted from Hoagland and Arnon, 1938), and nonaerated nutrient solution was studied. These media were treated with alpha-labeled [^{14}C]4-aminopyridine at 10 ppm and maintained in the dark. Radioactivity was measured initially and at 7 days by LSC.

REPORTED RESULTS:

Less than 0.03% of the applied radioactivity leached through the soils in the 7 days following treatment. Approximately 97% of the radioactivity found in the sandy loam soil (4% organic matter) remained in the top inch of the soil column at an unknown period of time after treatment. At 7 days posttreatment, 73.5% of the applied radioactivity remained in the sandy loam soil.

Approximately 14, 15, and 18% of the applied radioactivity was lost after 7 days from the nonaerated nutrient solution, aerated nutrient solution, and aerated water, respectively. These losses were attributed to volatilization and/or microbial metabolism.

DISCUSSION:

1. The reviewer believes that the soil was not eluted with enough water to approximate field conditions; therefore, the leaching potential of 4-aminopyridine was underestimated.

2. Judging from the results of the mechanical analyses of the soil used, identification of the soil textures was not consistent with the USDA soil classification system. One of the loam soils was actually a sandy loam and the other loam soil was a sandy clay loam.

3. Losses from the aqueous and nutrient solutions were attributed to either volatilization or microbial metabolism. These losses may have also been caused by hydrolysis or oxidation.

Table 1. Characteristics of soils used to study the degradation of 4-aminopyridine.

Soil type	pH	Organic matter (%)	Sand (%)	Silt (%)	Clay (%)
Loamy sand	7.8	2.9	77	16	7
Sandy loam	7.6	2.5	59	30	11
Sandy clay loam	7.7	4.0	61	19	20
Sandy clay loam	7.6	1.9	47	27	26

CASE GS0015 4-AMINOPYRIDINE STUDY 7 PM 320 09/19/79

CHEM 069201 4-Aminopyridine

BRANCH EFB DISC 30 TOPIC 050520

FORMULATION 00 - ACTIVE INGREDIENT

FICHE/MASTER ID 00004117 CONTENT CAT 02

Phillips Petroleum Company (19??) Results of a Laboratory Test to Determine the Rate of Degredation of 4-Aminopyridine in Soil: Good-67-66R. (Unpublished study received May 16, 1966 under 224-EX-3; CDL:122746-D)

SUBST. CLASS = S.

DIRECT RVW TIME = 3 (MH) START-DATE END DATE

REVIEWED BY: B. Karnofsky
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SIGNATURE: Brian Karnofsky DATE: Dec. 18, 1979

APPROVED BY:
TITLE:
ORG:
LOC/TEL:

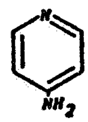
SIGNATURE: DATE:

CONCLUSION:

This study is scientifically invalid because of inadequate experimental design and protocols. No sterile controls were employed and no attempts were made to identify potential degradation products. Soil characteristics were not reported, and it was not clear whether the soil was maintained under light or dark conditions.

MATERIALS AND METHODS:

AVITROL



4 - Aminopyridine

The hydrochloride of 4-aminopyridine (5 g) was thoroughly mixed with a sifted moist topsoil in a glass bottle to yield an initial concentration of 0.5%. The bottle was capped and stored at room temperature. Soil samples were taken 5, 35, 69, and 144 days after treatment and analyzed with a UV spectrophotometric method designated as "PK-62R."

REPORTED RESULTS:

Refer to Table 1.

DISCUSSION:

The design of this study was so poorly described that no acceptable conclusions may be drawn from the data. Soil characteristics and sieve mesh size were not reported. The authors did not specify whether the soil was maintained in the light or dark and whether or not the soil was sterile. No controls were described, and no reference was made to the accuracy or availibility of method "PK-62R." Furthermore, the objective of the study was to investigate 4-aminopyridine degradation, but no attempts were made to identify potential degradation products.

Table 1. Loss of 4-aminopyridine from moist topsoil.

Days	4-Aminopyridine (%)
0	0.5
5	0.39
35	0.31
69	0.10
144	<0.03[a]

[a]Near detection limit.

TDMS0030 DATA EVALUATION RECORD PAGE 1 OF

CASE GS0015 4-AMINOPYRIDINE STUDY 8 PM 320 09/19/79

CHEM 069201 4-Aminopyridine

BRANCH EFB DISC 30 TOPIC 050520

FORMULATION 00 - ACTIVE INGREDIENT

FICHE/MASTER ID 00004004 CONTENT CAT 01

Starr, R.I.; Cunningham, D.J. (1969) Absorption, Translocation and Metabolism of 4-Aminopyridine by Corn Plants: Progress Report No. 4--December 23, 1969. (Unpublished study received Jan 20, 1970 under 224-EX-3; prepared by U.S. Fish and Wildlife Service, Denver Wildlife Research Center, submitted by Phillips Petroleum Co., Bartlesville, Okla.; CDL:122744-K)

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
PRIM: RCBR-20-15
SEC: RCBR-20-1510 EFB -20-150515

DIRECT RVW TIME = 2½ (MH) START-DATE END DATE

REVIEWED BY: S. Gould
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SIGNATURE: Sandra Gould DATE: Dec. 17, 1979

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ORG:
LOC/TEL:
SIGNATURE: DATE:

<u>CONCLUSIONS:</u>

1. This study was not reviewed because it contains data on phytotoxicity, translocation, and degradation of 4-aminopyridine in corn.

2. The portion of this study on the fate of 4-aminopyridine in corn is part of Study 6 (MRID 00004001) of this package. A full review can be found there under mobility - leaching.

CASE GS0015 4-AMINOPYRIDINE STUDY 9 PM 320 09/19/79

CHEM 069201 4-Aminopyridine

BRANCH EFB DISC 30 TOPIC 0505

FORMULATION 00 - ACTIVE INGREDIENT

FICHE/MASTER ID 00004132 CONTENT CAT 02

Starr, R.I.; Cunningham, D.J. (1972) Fate of 4-Aminopyridine in Corn, Sorghum, and Soil Systems: A Summary of Research Findings. (Unpublished study received Apr 16, 1973 under 3G1320; prepared by U.S. Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, submitted by Avitrol Corp., Tulsa, Okla.; CDL:093548-A)

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
PRIM: RCBR-20-15
SEC: RCBR-20-1515 RCBR-20-1520 RCBR-20-1510
EEB -20-201028005 EEB -20-201028019 EFB -30-050515
EFB -30-050520 EFB -30-050530

DIRECT RVW TIME = 2 (MH) START-DATE END DATE

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SIGNATURE: Sandra Gould DATE: Dec. 10, 1979

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CONCLUSIONS:

1. The part of this study dealing with the fate of 4-aminopyridine in soil has been published; it is reviewed in detail in Study 4 (MRID 05003185) of this package.

2. A full review of the part of this study dealing with the phytotoxicity of 4-aminopyridine to corn and sorghum was not conducted.

TDMS0030 DATA EVALUATION RECORD PAGE 1 OF

CASE GS0015 4-AMINOPYRIDINE STUDY 10 PM 320 09/19/79

CHEM 069201 4-Aminopyridine

BRANCH EFB DISC 30 TOPIC 050520

FORMULATION 00 - ACTIVE INGREDIENT

FICHE/MASTER ID 00003993 CONTENT CAT 02

Phillips Petroleum Company (19??) Residue Data: 4-Aminopyridine (Avitrol 200; DRC-1327). (Unpublished study received Apr 22, 1969 under 224-EX-3; CDL:122747-G)

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
PRIM: RCBR-20-150505

DIRECT RVW TIME = 5½ (MH) START-DATE END DATE

REVIEWED BY: S. Gould
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SIGNATURE: Sandra Gould DATE: Dec. 11, 1979

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ORG:
LOC/TEL:

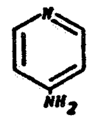
SIGNATURE: DATE:

CONCLUSION:

This study is scientifically invalid because procedures and protocols were not provided.

MATERIALS AND METHODS:

AVITROL



4 - Aminopyridine

Whole corn (100 g) containing 1.44% 4-aminopyridine was mixed with 1 kg moist garden soil. The source, purity, and formulation of the chemical were unspecified. The mixture was kept in a jar with a loose-fitting cap for 98 days. Both the soil and corn were sampled at periodic intervals; the percentage of moisture and the 4-aminopyridine content were determined at each sampling date. Water was added to the soil after 26 days to restore lost moisture.

REPORTED RESULTS:

Data in Table 1 show that the amount of 4-aminopyridine in the corn decreased at each sampling date and that a corresponding increase in the corn mixture was observed up to day 26. The 4-aminopyridine content in the soil and corn mixture decreased to 0.02% (below the accuracy of the analytical method) by day 98.

DISCUSSION:

1. Essential information regarding the experimental protocol was not provided. The extraction procedures and method of quantitative analysis were not described. No data concerning the number of samples assayed were provided. The reviewer assumes that the corn containing 4-aminopyridine (Materials and Methods) was whole grain corn because the purpose of the study was to show that 4-aminopyridine applied on grain was decomposed in moist soil.

2. Although the objective of the study was to investigate the degradation of 4-aminopyridine, the investigator did not discuss possible mechanisms of degradation in soil, such as photodegradation, hydrolysis, and soil metabolism.

3. Due to insufficient information, the reviewer cannot validate the investigator's results.

Table 1. Degradation of 4-aminopyridine in corn and soil.

	Corn				Soil			
Days	Weight of corn (g)	Water (%)	4-Amino-pyridine (%)	4-Amino-pyridine (g)	Weight soil and corn (g)	Water (%)	4-Amino-pyridine (%)	4-Amino-pyridine (g)
0	100	10	1.44	1.44	1,100	17	0	0
7	147	42	0.31	0.45	950	13	0.013	0.12
26	87	8	0.091	0.09	826	2.2	0.108	0.89
98	Decomposed	--	--	0.0	1,008	22	0.02	0.20

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