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DATA EVALUATION RECORD

STUDY 5a

CHEM 030703 Naptalam sodium salt §162-2

FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 41427202
Cohen, S.P., and A.M. Levine. 1990b. Anaerobic soil metabolism of alanap. Uniroyal Project No. 8967. CHMR Project No. 008/005/005/89. Unpublished study performed by Center for Hazardous Materials Research, Pittsburg, PA, and submitted by Uniroyal Chemical Company, Inc., Middlebury, CT.

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CONCLUSIONS:

Metabolism - Anaerobic Soil

1. The study is acceptable and fulfills the 162-2 data requirement.

2. Naptalam degraded with a calculated half-life of 246 days in sandy loam soil that was incubated anaerobically (nitrogen atmosphere) in the dark at 25 C for 60 days following 30 days of aerobic incubation. Two nonvolatile degradates were identified: N-1-naphthylphthalimide and 1-naphthylamine. Carbon dioxide was the only volatile degradate identified.

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METHODOLOGY:

Air-dried, sieved (2-mm) sandy loam soil (62.0% sand, 28.0% silt, 10.0% clay, 5.0% organic matter, pH 6.5, CEC 6.6 meq/100 g) was placed in a beaker and moistened to 75% of 0.33 bar. The soil was incubated aerobically for 34 days, then transferred to a 1-L flask that was designed with top ports that allowed for the removal of subsamples of soil as well as continuous purging with humidified air. The soil was then treated at 6.43 ppm with the sodium salt of [1-^{14}C]naptalam (radiochemical purity 98%, specific activity 37.9 uCi/mg, Uniroyal) dissolved in propanol:water (70:30, v:v), and mixed by rotation and with a spatula for approximately 15-20 minutes to obtain homogeneity. The flask was connected, in series, to tubes of ethylene glycol, 1 M sulfuric acid, and 5% sodium hydroxide for trapping volatiles (Figure 4). The rate of air flow through the system was 45-50 mL/minute. The sealed flask was placed in a water bath maintained at 25 $\pm$ 0.1 C, and the flask and water bath were covered to exclude light. Prior to each sampling, the rate of air flow was increased 3-fold for about 20-30 minutes to force volatiles into the trapping solutions. The air flow was then stopped, and duplicate portions (approximately 5 g each) of soil and trapping solutions were sampled immediately posttreatment and at 30 days. The trapping solutions were replaced with fresh trapping solution at various intervals.

Following the 30-day aerobic incubation, a 100-g aliquot of the aerobically-aged test soil was transferred to a second flask, and the flask was connected to a second set of trapping solutions. The soil was incubated as described previously, except that the system was continuously purged with humidified nitrogen gas rather than air. The establishment of anaerobic conditions was confirmed by using a dissolved oxygen probe. Duplicate portions of the soil and the trapping solutions were sampled after 31 and 60 days of anaerobic incubation (61 and 90 days posttreatment).

Portions of each soil sample were analyzed for total radioactivity by LSC following combustion; the detection limit was 0.005 ppm. Additional portions of soil were extracted immediately after sampling; the extraction procedure is outlined in Figure 5. The samples were sequentially extracted three times each with acetonitrile, propanol:water (70:30, v:v), and 1 N sodium hydroxide solution. Each extraction was done for 20 minutes using occasional vortexing at 65 C or on a rotator at room temperature. Each set of extracts was pooled, and aliquots of each extract were analyzed for total radioactivity by LSC. Additional aliquots of the acetonitrile and propanol:water extracts were evaporated to dryness under nitrogen, resuspended in water, and filtered (0.45 nm). Aliquots of the 1 N sodium hydroxide soil extracts were partitioned three times with acetonitrile, and the acetonitrile was evaporated to dryness under nitrogen, resuspended in water, neutralized with 1% trifluoroacetic acid, and filtered. All filtered extracts were

analyzed for naptalam and its degradates by HPLC and TLC. HPLC was conducted using a mobile phase of 0.1% trifluoroacetic acid in water:acetonitrile with a linear gradient in the water:acetonitrile from 90:10 (v:v) to 50:50 (v:v); detection was by UV absorbance (280 nm) and radioactivity monitoring. The detection limit was 0.005 ppm. One-dimensional TLC was done with nonradiolabeled reference standards on silica gel plates developed in chloroform:acetone:formic acid (70:30:0.5, v:v:v). Radioactive zones were visualized using a TLC linear analyzer and UV light. The radioactive areas were located and quantified using radioscanning and were identified by comparison to the Rf of the reference standards. Unextracted [^{14}C]residues in the extracted soil were analyzed for total radioactivity by LSC following combustion.

Duplicate aliquots of the trapping solutions were analyzed for total radioactivity by LSC.

Portions of the soil collected at each sampling interval were stored frozen at -20 C for an unspecified period of time for future degradate identification. Aliquots of each extract were stored at 4-6 C for possible future analysis.

DATA SUMMARY:

The sodium salt of [1-^{14}C]naptalam (radiochemical purity 98%), at 6.430 ppm, degraded with a registrant-calculated half-life of 245.8 days in sandy loam soil that was incubated under anaerobic conditions (nitrogen atmosphere) in the dark at 25 C for 60 days following 30 days of aerobic incubation at 75% of 0.33 bar moisture. Naptalam declined from 5.98 ppm immediately posttreatment to 4.14 ppm at 30 days (immediately prior to establishing anaerobic conditions), 3.95 ppm at 61 days (31 days after establishing anaerobic conditions), and 3.48 ppm at 90 days (60 days of anaerobic incubation) (Table 7). Two nonvolatile degradates,

N-1-naphthylphthalimide (alanap imide) and

1-naphthylamine (alpha-naphthylamine, 1-NA),

were isolated at a maximum of 0.42 ppm and 0.12 ppm, respectively, following 31 days of anaerobic incubation. After 60 days of anaerobic incubation, unextracted [^{14}C]residues were 0.98 ppm and $^{14}CO_2$ totaled 0.05 ppm (Table 5). During the study, material balances ranged from 97.8 to 103.9% of the applied radioactivity (Table 4).

COMMENTS:

1. The study is acceptable and fulfills the 162-2 data requirement.

2. In this study, the sample extracts were analyzed using HPLC with two solvent systems. The identities of the compounds were confirmed using one-dimensional TLC and a single solvent system. Radioactive areas on the TLC plates were identified only by comparison with the location of known reference standards chromatographed on the same plates.

   EFGWB prefers that [^{14}C]residues in samples be analyzed by an additional chromatographic methods (such as HPLC, or GC), and that specific compounds isolated by chromatography be identified using a confirmatory method such as MS in addition to comparison with the R_f of reference standards.

FIGURE 4. SCHEMATIC DIAGRAM OF ANAEROBIC SOIL METABOLISM INCUBATION APPARATUS.

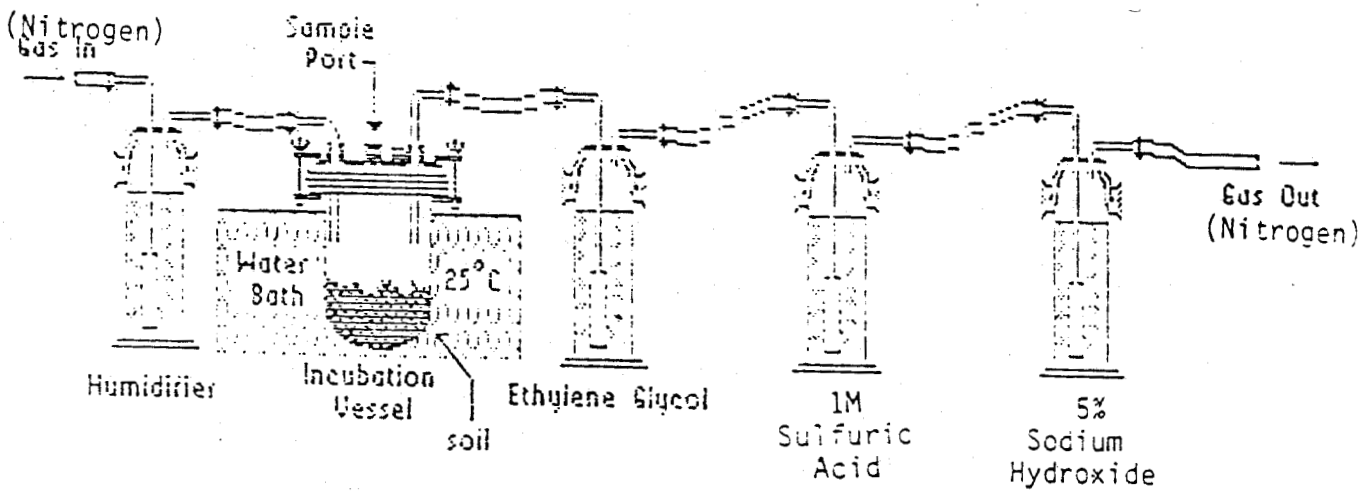

FIGURE 5. EXTRACTION PROCEDURE FOR SOIL SAMPLES.

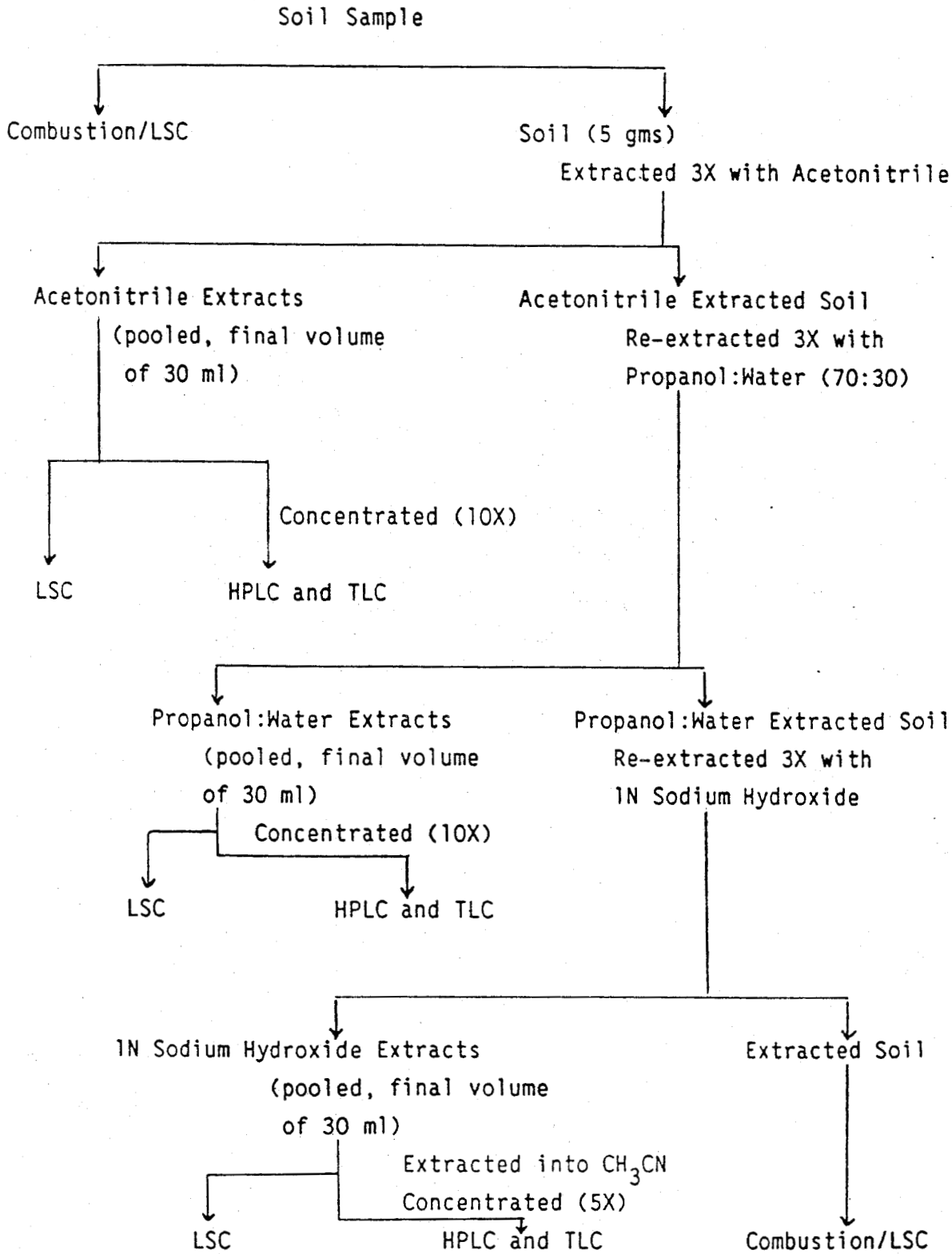

TABLE 1. DISTRIBUTION OF ^{14}C-ALANAP RESIDUES (PERCENT OF INITIALLY-APPLIED RADIOACTIVITY[1]) AS DETERMINED BY LSC ANALYSIS IN SANDY LOAM SOIL TREATED WITH ^{14}C-ALANAP AND INCUBATED FOR 30 DAYS UNDER AEROBIC CONDITIONS IN THE DARK AT 25.1±0.2°C.

Recovery of ^{14}C-Alanap Residues[2]	Incubation Time in Days	
	0[3]	30
Total ^{14}C-Alanap Residues Determined By Combustion Prior to Extraction[4]	100.0	85.5
Extractable ^{14}C-Alanap Residues		
Acetonitrile Extract	45.1	27.2
Propanol/Water Extract	43.0	13.7
Sodium Hydroxide Extract	7.5	28.8
Total Extractable ^{14}C-Alanap Residues	95.6	69.7
Unextractable ^{14}C-Alanap Residues	1.3	13.9
Total Extractable + Unextractable ^{14}C-Alanap Residues Recovered	96.9	83.6
Cumulative Volatiles		
$^{14}CO_2$ (5% Sodium Hydroxide) Trap	NA[5]	6.5
Ethylene Glycol Trap	NA	0.0
1M H_2SO_4 Trap	NA	0.0
Total Cumulative Volatiles	NA	6.5
Total ^{14}C-Alanap Residues Recovered After Extraction (Extractable + Unextractable + Volatiles)	96.9	90.1

1. Initial alanap concentration in the test soil immediately post-treatment was 6.43 ppm (5.38×10^5 dpm/gm test soil; Specific Activity = 37.7 uCi/mg).

2. Average of two measurements at each sampling time.

3. Samples were taken immediately after treatment of the soil (Day 0 of aerobic incubation).

4. Soil samples were combusted and analyzed by LSC.

5. Not Analyzed; detection limit of LSC was 0.005 ppm.

TABLE 2. DISTRIBUTION OF RADIOACTIVITY (PPM[1]) AS DETERMINED BY LSC ANALYSIS IN SANDY LOAM SOIL TREATED WITH ^{14}C-ALANAP AND INCUBATED FOR 30 DAYS UNDER AEROBIC CONDITIONS IN THE DARK AT 25.1±0.2°C.

Recovery of ^{14}C-Alanap Residues[2]	Incubation Time in Days	
	0[3]	30
Total ^{14}C-Alanap Residues Determined By Combustion Prior to Extraction[4]	6.43	5.50
Extractable ^{14}C-Alanap Residues		
Acetonitrile Extract	2.90	1.75
Propanol/Water Extract	2.76	0.88
Sodium Hydroxide Extract	0.48	1.85
Total Extractable ^{14}C-Alanap Residues	6.14	4.48
Unextractable ^{14}C-Alanap Residues	.08	0.89
Total Extractable + Unextractable ^{14}C-Alanap Residues Recovered	6.22	5.37
Cumulative Volatiles		
$^{14}CO_2$ (5% Sodium Hydroxide) Trap	NA[5]	0.42
Ethylene Glycol Trap	NA	0.0
1M H_2SO_4 Trap	NA	0.0
Total Cumulative Volatiles	NA	0.42
Total ^{14}C-Alanap Residues Recovered After Extraction (Extractable + Unextractable + Volatiles)	6.22	5.79

1. Initial alanap concentration in the test soil immediately post-treatment was 6.43 ppm (5.38 x 10^5 dpm/gm test soil; Specific Activity = 37.7 uCi/mg).

2. Average of two measurements at each sampling time.

3. Samples were taken immediately after treatment of the soil (Day 0 of aerobic incubation).

4. Soil samples were combusted and analyzed by LSC.

5. Not Analyzed; detection limit of LSC was 0.005 ppm.

TABLE 3. SUMMARY OF ^{14}C-RESIDUES AS DETERMINED BY HPLC ANALYSIS[1] OF EXTRACTS OF SANDY LOAM SOIL TREATED WITH ^{14}C-ALANAP AND INCUBATED UNDER AEROBIC CONDITIONS IN THE DARK AT 25.1±0.2°C.

Compound	Incubation Time (Days) 0[2]	30[3]
	Percent of Initially-Applied Alanap	
Alanap	93.0	64.4
1-Naphthylamine	2.2	0.3
Alanap Imide	0.2	4.7
	PPM[4]	
Alanap	5.98	4.14
1-Naphthylamine	0.14	0.02
Alanap Imide	0.01	0.30

1. Detection limit was 0.005 ppm using a radioactive flow detector.
2. Samples were taken immediately after treatment of the soil (Day 0 of aerobic incubation).
3. Samples were taken immediately prior to establishing anaerobic conditions (30 days after aerobic incubation).
4. Initial alanap concentration in the test soil immediately post-treatment was 6.43 ppm.

TABLE 4. DISTRIBUTION OF ^{14}C-ALANAP RESIDUES (PERCENT OF DAY 0 OF ANAEROBIC INCUBATION[1]) AS DETERMINED BY LSC ANALYSIS IN SANDY LOAM SOIL TREATED WITH ^{14}C-ALANAP AND INCUBATED FOR 30 DAYS UNDER AEROBIC CONDITIONS FOLLOWED BY 60 DAYS UNDER ANAEROBIC CONDITIONS IN THE DARK AT 25.1±0.2°C.

Recovery of ^{14}C-Alanap Residues[2]	Incubation Time in Days		
	0[3]	31	60
Total ^{14}C-Alanap Residues Determined By Combustion Prior to Extraction[4]	100.0	98.5	105.2
Extractable Radioactivity			
Acetonitrile Extract	31.8	37.9	28.6
Propanol/Water Extract	16.0	12.9	10.7
Sodium Hydroxide Extract	33.7	38.8	41.2
Total Extractable Radioactivity	81.5	89.6	80.5
Unextractable Radioactivity	16.3	13.6	17.8
Cumulative Volatiles			
5% NaOH ($^{14}CO_2$) Trap	NA[5]	0.7	0.9
Ethylene Glycol Trap	NA	0.0	0.0
H_2SO_4 Trap	NA	0.0	0.0
Total Cumulative Volatiles	NA	0.7	0.9
Total ^{14}C-Alanap Residues Recovered After Extraction (Extractable + Unextractable + Cumulative Volatiles)	97.8	103.9	99.2

1. Concentration of alanap residues remaining in the test soil at Day 0 of anaerobic incubation (30 days after aerobic aging) was 5.50 ppm (see Table 2).

2. Average of two measurements at each sampling time.

3. Samples were taken immediately prior to establishing anaerobic conditions (30 days after aerobic incubation).

4. Soil samples were combusted and analyzed by LSC.

5. Not Applicable.

TABLE 5. DISTRIBUTION OF ^{14}C-ALANAP RESIDUES (PPM[1]) AS DETERMINED BY LSC ANALYSIS IN SANDY LOAM SOIL TREATED WITH ^{14}C-ALANAP AND INCUBATED FOR 30 DAYS UNDER AEROBIC CONDITIONS AND 60 DAYS UNDER ANAEROBIC CONDITIONS IN THE DARK AT 25.1±0.2°C.

Recovery of ^{14}C-Alanap Residues[2]	Incubation Time in Days		
	0[3]	31	60
Total ^{14}C-Alanap Residues Determined By Combustion Prior to Extraction[4]	5.50	5.42	5.79
Extractable ^{14}C-Alanap Residues			
Acetonitrile Extract	1.75	2.08	1.57
Propanol/Water Extract	0.88	0.71	0.59
Sodium Hydroxide Extract	1.85	2.13	2.27
Total Extractable Radioactivity	4.48	4.92	4.43
Unextractable ^{14}C-Alanap Residues	0.90	0.75	0.98
Cumulative Volatiles			
5% NaOH ($^{14}CO_2$) Trap	NA[5]	0.04	0.05
Ethylene Glycol Trap	NA	0.00	0.00
H_2SO_4 Trap	NA	0.00	0.00
Total Cumulative Volatiles	NA	0.04	0.05
Total ^{14}C-Alanap Residues Recovered After Extraction (Extractable + Unextractable + Cumulative Volatiles)	5.38	5.71	5.46

1. Initial concentration of alanap and its metabolites in the test soil on Day 0 of anaerobic incubation was 5.50 ppm (4.60×10^5 dpm/gm test soil; Specific Activity = 37.7 uCi/mg).

2. Average of two measurements at each sampling time.

3. Samples were taken immediately prior to establishing anaerobic conditions (30 days after aerobic incubation).

4. Soil samples were combusted and analyzed by LSC.

5. Not Applicable.

TABLE 6. SUMMARY OF ^{14}C-RESIDUES[1] (PERCENT[2] OF DAY 0 OF ANAEROBIC INCUBATION) AS DETERMINED BY HPLC ANALYSIS[3] OF EXTRACTS OF SANDY LOAM SOIL TREATED WITH ^{14}C-ALANAP AND INCUBATED UNDER ANAEROBIC CONDITIONS IN THE DARK AT 25.1±0.2°C.

	Incubation Time in Days		
Compound	0[4]	31	60
Alanap	75.3	71.9	63.3
alpha-naphthylamine (1-NA)	0.4	2.1	2.0
alanap imide	5.5	7.6	5.8

1. Average of two measurements at each sampling time.

2. Concentration of alanap residues remaining in the test soil at Day 0 of anaerobic incubation (30 days after aerobic aging) was 4.60×10^5 dpm/gram of test soil (5.50 ppm; see Table 2).

3. Detection limit was 0.005 ppm using a radioactive flow detector.

4. Samples were taken immediately prior to establishing anaerobic conditions (30 days after aerobic incubation).

TABLE 7. SUMMARY OF ^{14}C-RESIDUES[1] (PPM[2]) AS DETERMINED BY HPLC ANALYSIS[3] OF EXTRACTS OF SANDY LOAM SOIL TREATED WITH ^{14}C-ALANAP AND INCUBATED UNDER ANAEROBIC CONDITIONS IN THE DARK AT 25.1±0.1°C.

Compound	Incubation Time in Days		
	0[4]	31	60
Alanap	4.14	3.95	3.48
alpha-naphthylamine (1-NA)	0.02	0.12	0.11
alanap imide	0.30	0.42	0.32

1. Average of two measurements at each sampling time.

2. Concentration of alanap residues remaining in the test soil at Day 0 of anaerobic incubation (30 days after aerobic aging) was 4.60×10^5 dpm/gram of test soil (5.50 ppm; see Table 2).

3. Detection limit was 0.005 ppm using a radioactive flow detector.

4. Samples were taken immediately prior to establishing anaerobic conditions (30 days after aerobic incubation).

STUDY AUTHOR(S)'S RESULTS AND/OR CONCLUSIONS

5.0 RESULTS AND TABLES

The precise initial concentration of alanap immediately after application (before aerobic aging) in the test soil was determined to be 6.43 ppm. The concentration of alanap residues remaining in the soil after 30 days of aerobic aging (Day 0 of anaerobic incubation) was 5.50 ppm (alanap accounted for 64.4% of the initially-applied radioactivity). The extraction method of the aerobically aged soil provided recovery values ranging from 90.1 to 96.9% (extractable + unextractable + cumulative volatiles).

Alanap degraded during anaerobic incubation but at a much lower rate than observed under aerobic conditions. The metabolite alpha-naphthylamine (1-NA), present at 0.4% of the initial radioactivity at Day 0 of anaerobic incubation, increased to 2.1% by Day 31 then decreased to 2.0% by Day 60 of anaerobic incubation. The metabolite alanap imide, present at 5.5% of the initial radioactivity at Day 0 of anaerobic incubation, increased to 7.6% by Day 31 then decreased to 5.8% by Day 60 of anaerobic incubation (see Table 6).

CO_2 production reached 0.05 ppm (0.9% of Day 0 of anaerobic incubation) by the end of the 60-day anaerobic incubation period. No other volatile compounds were detected.

The material balance during the study ranged from 97.8 to 103.9% of the initial anaerobic Day 0 radioactivity (extractable + unextractable + volatiles).

The temperature of the test soil was $25.1 \pm 0.2^{o}C$ during the study period.

The level of dissolved oxygen in the reaction vessel was reduced from approximately 21% to less than 0.3% within 3 hours after humidified nitrogen was introduced. This level was maintained during the course of anaerobic incubation.

The soil used in this study was also tested for microbial activity and was found to contain viable populations of bacteria, actinomycetes and fungi (see Appendix 8).

The results of the aerobic aging of alanap in sandy loam soil are provided as 'percent of the initially-applied radioactivity' and 'ppm' in Tables 1-3. The results of the anaerobic soil metabolism of alanap in the sandy loam soil, reported as alanap residues, are presented in terms of "percent of Day 0 of anaerobic incubation" in Table 4 and in terms of "ppm" in Table 5. Tables 6-7 and Figure 6 show the metabolism of alanap in sandy loam soil incubated under anaerobic conditions in the dark. Copies of the individual chromatograms showing detection of alanap and its soil metabolites by HPLC equipped with a radioactive flow detector are in Appendix 9. Each replicate soil extract was analyzed by both UV detection at 280 nm and by radioactive flow detection. Concentration calculations of alanap and its soil metabolites were made based on results of the HPLC analysis with radioactive flow detection and LSC. Chromatograms of the developed TLC plates in Appendix 10 show alanap and its metabolites in the various soil extracts.

All calculations were done using a Lotus 1-2-3 spreadsheet and were validated using hand calculations.

6.0 CONCLUSIONS

Alanap was aged aerobically for 30 days since the half-life of alanap under aerobic soil conditions was greater than 30 days (approximately 41 days). After 30 days of aerobic aging, alanap accounted for 64.4% of the initially-applied radioactivity.

Analysis of anaerobically incubated soil samples showed that alanap degraded under anaerobic conditions, but at a much lower rate than under aerobic conditions. The half-life of alanap under anaerobic conditions was determined to be 245.8 days (R^2 = 0.922). The metabolite alpha-naphthylamine (1-NA), present at 0.4% of the initial radioactivity at Day 0 of anaerobic incubation, increased to 2.1% by Day 31 then decreased to 2.0% by Day 60 of anaerobic incubation. The metabolite alanap imide, present at 5.5% of the initial radioactivity at Day 0 of anaerobic incubation, increased to 7.6% by Day 31 then decreased to 5.8% by Day 60 of anaerobic incubation. No new metabolites were observed under anaerobic soil conditions.

The only volatile compound detected was CO_2 which totaled 0.9% of the Day 0 radioactivity by Day 60 of anaerobic incubation.

The material balance during the anaerobic soil metabolism study ranged from 97.8 to 103.9% of the initial anaerobic Day 0 radioactivity (extractable + unextractable + volatiles).