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DATA EVALUATION RECORD

STUDY 4a

CHEM 030703 Naptalam sodium salt §162-1

FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 41427201
Cohen, S.P., and A.M. Levine. 1990a. Aerobic soil metabolism of alanap. Uniroyal Project No. 8966. CHMR Project No. 008/005/004/89. Unpublished study performed by Center for Hazardous Materials Research, Pittsburg, PA, and submitted by Uniroyal Chemical Company, Inc., Middlebury, CT.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 - Aerobic Soil

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

2. Naptalam degraded with a half-life of 36.7 days in sandy loam soil that was incubated in the dark at 25 C and 75% of 0.33 bar moisture capacity. 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 at 0, 2, 13, 20, 30, 41, 50, 75, and 135 days posttreatment. The trapping solutions were replaced with fresh trapping solution at various intervals.

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 6.43 ppm, degraded with a registrant-calculated half-life of 36.7 days in nonsterile sandy loam soil that was incubated in the dark at 25 C and 75% of 0.33 bar moisture. [^{14}C]Naptalam decreased from 93.0% of the applied (5.98 ppm) immediately posttreatment to 39.4% (2.53 ppm) at 41 days and 3.6% (0.23 ppm) at 135 days (Tables 3 and 4). Two nonvolatile degradates were identified:

N-1-naphthylphthalimide (alanap imide),

which was a maximum of 6.1% of the applied (0.39 ppm) at 41 days posttreatment and decreased to 3.6% (0.17 ppm) by 135 days, and

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

which was a maximum of 2.2% (0.14 ppm) immediately posttreatment, decreased to 0.4% (0.03 ppm) by 30 days, and was not detected (<0.005 ppm) by 41 days.

At 135 days posttreatment, $^{14}CO_2$ totaled 39.5% of the applied and unextracted [^{14}C]residues were 12.3% of the applied (Table 1). During the study, material balances ranged from 87.1 to 96.9% of the applied with no discernible pattern (Table 1).

COMMENTS:

1. The study is acceptable and fulfills the 162-1 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 to the location of known reference standards on the same plates.

   EFGWB prefers that [^{14}C]residues in samples be analyzed by 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 the Rf of reference standards.

TABLE 1. DISTRIBUTION OF RADIOACTIVITY (PERCENT OF INITIALLY-APPLIED RADIOACTIVITY[1]) AS DETERMINED BY LSC ANALYSIS IN SANDY LOAM SOIL TREATED WITH ^{14}C-ALANAP AND INCUBATED UNDER AEROBIC CONDITIONS IN THE DARK AT 25.1±0.1°C.

Recovery of Radioactivity[2]	Incubation Time in Days								
	0	2	13	20	30	41	50	75	135
Extractable Radioactivity									
Acetonitrile Extract	45.1	44.7	30.1	31.1	27.2	14.7	13.1	8.9	5.2
Propanol/Water Extract	43.0	33.3	30.5	25.1	13.7	4.3	3.1	2.8	1.8
Sodium Hydroxide Extract	7.5	12.5	22.6	24.7	28.8	35.4	39.5	32.0	32.0
Total Extractable Radioactivity	95.6	90.5	83.2	85.0	69.7	54.4	55.7	43.7	39.0
Unextractable Radioactivity	1.3	1.5	5.6	7.3	13.9	21.3	9.5	13.9	12.3
Cumulative Volatiles									
5% NaOH ($^{14}CO_2$) Trap	NA[3]	0.2	2.7	3.8	6.5	14.8	22.0	29.5	39.5
Ethylene Glycol Trap	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
H_2SO_4 Trap	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total Cumulative Volatiles	NA	0.2	2.7	3.8	6.5	14.8	22.0	29.5	39.5
Total Radioactivity Recovered (Extractable + Bound + Cumulative Volatiles)	96.9	92.2	91.5	96.1	90.1	90.5	87.2	87.1	90.8

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

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TABLE 2. DISTRIBUTION OF RADIOACTIVITY (PPM[1]) AS DETERMINED BY LSC ANALYSIS IN SANDY LOAM SOIL TREATED WITH ^{14}C-ALANAP AND INCUBATED UNDER AEROBIC CONDITIONS IN THE DARK AT 25.1±0.1°C.

Recovery of Radioactivity[2]	Incubation Time in Days								
	0	2	13	20	30	41	50	75	135
Extractable Radioactivity									
Acetonitrile Extract	2.90	2.87	1.94	2.00	1.75	0.95	0.84	0.57	0.33
Propanol/Water Extract	2.76	2.14	1.96	1.61	0.88	0.28	0.20	0.18	0.12
Sodium Hydroxide Extract	0.48	0.80	1.45	1.59	1.85	2.28	2.54	2.06	2.06
Total Extractable Radioactivity	6.14	5.81	5.35	5.20	4.48	3.51	3.58	2.81	2.51
Unextractable Radioactivity	0.08	0.10	0.36	0.47	0.89	1.37	0.61	0.89	0.79
Cumulative Volatiles									
5% NaOH ($^{14}CO_2$) Trap	NA[3]	0.01	0.17	0.24	0.42	0.95	1.41	1.90	2.54
Ethylene Glycol Trap	NA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
H_2SO_4 Trap	NA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total Cumulative Volatiles	NA	0.01	0.17	0.24	0.42	0.95	1.41	1.90	2.54
Total Radioactivity Recovered (Extractable + Bound + Cumulative Volatiles)	6.22	5.92	5.88	5.91	5.79	5.83	5.60	5.60	5.84

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

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

	Incubation Time in Days								
	0	2	13	20	30	41	50	75	135
Compound	Percent of Initially-Applied Radioactivity[2]								
Alanap	93.0	85.7	76.6	73.1	56.2	39.4	43.3	25.1	3.6
alpha-naphthylamine (1-NA)	2.2	1.7	0.4	0.8	0.4	ND[3]	ND	ND	ND
alanap imide	0.2	1.1	3.4	1.9	4.7	6.1	5.1	2.4	2.7

1. Initial Alanap concentration immediately post-treatment was 5.38 x 10^5 dpm/gram of test soil (6.43 ppm).

2. Average of two measurements at each sampling time.

3. Not detected; detection limit 0.005 ppm.

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TABLE 4. SUMMARY OF ^{14}C-RESIDUES (PPM[1]) AS DETERMINED BY HPLC ANALYSIS OF EXTRACTS OF SANDY LOAM SOIL TREATED WITH ^{14}C-ALANAP AND INCUBATED UNDER AEROBIC CONDITIONS IN THE DARK AT 25.1±0.1°C.

	Incubation Time in Days								
	0	2	13	20	30	41	50	75	135
Compound	PPM[2]								
Alanap	5.98	5.51	4.93	4.70	3.62	2.53	2.78	1.61	0.23
alpha-naphthylamine (1-NA)	0.14	0.11	0.03	0.05	0.03	ND[3]	ND	ND	ND
alanap imide	0.01	0.07	0.22	0.12	0.30	0.39	0.33	0.15	0.17

1. Initial Alanap concentration immediately post-treatment was 6.43 ppm.

2. Average of two measurements at each sampling time.

3. Not detected; detection limit 0.005 ppm.

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

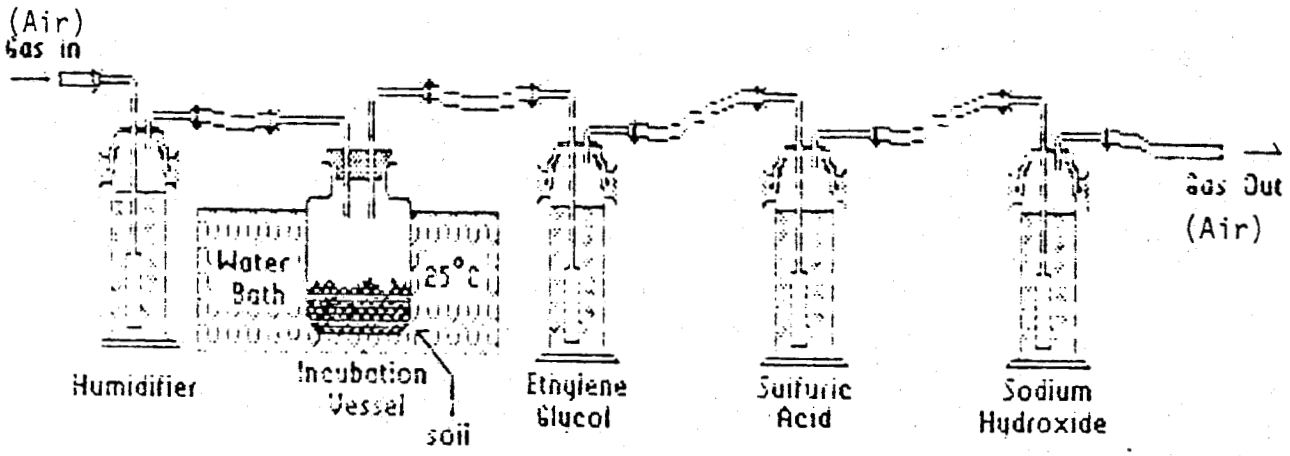
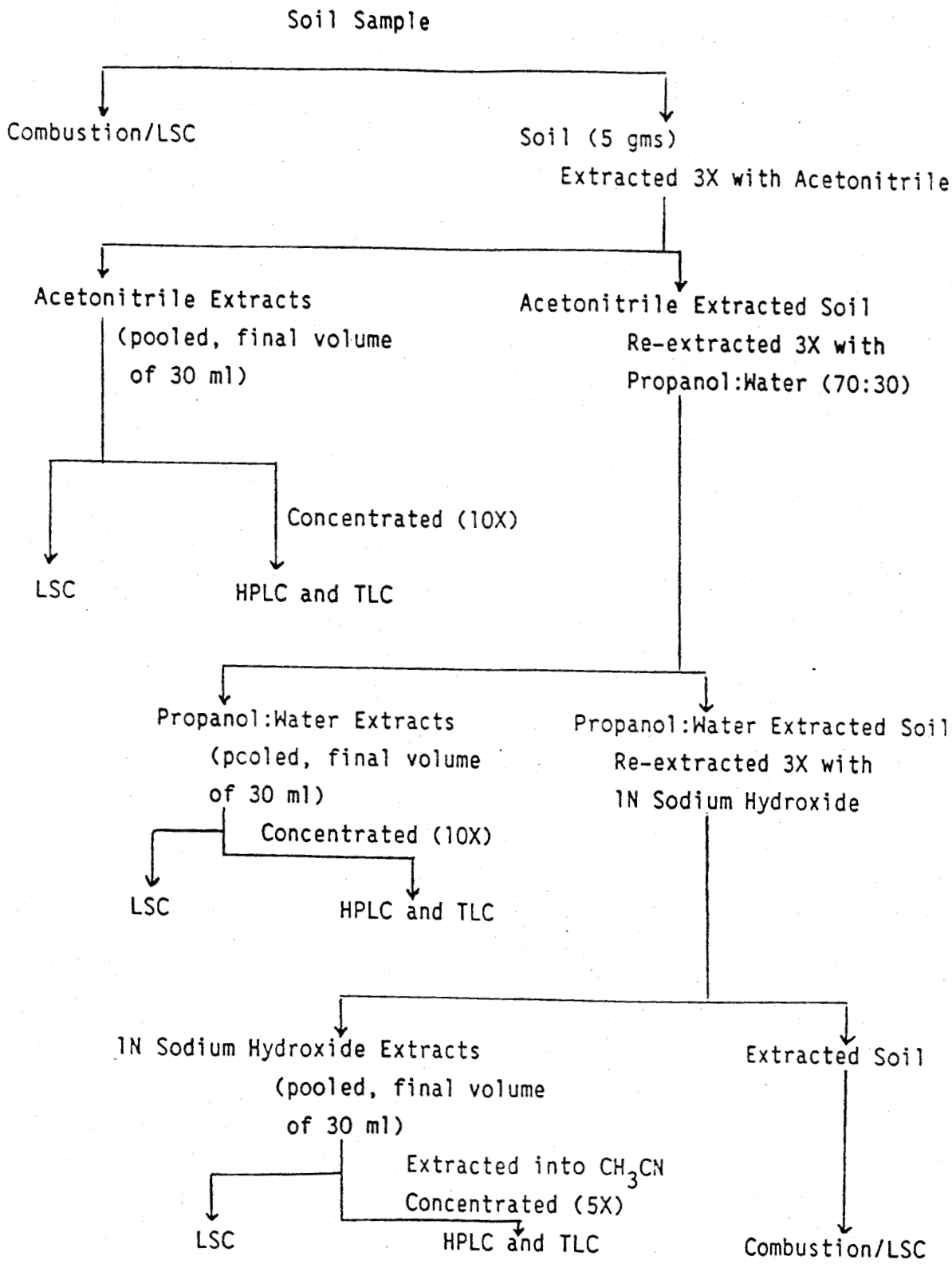


FIGURE 5. EXTRACTION PROCEDURE FOR SOIL SAMPLES.



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

5.0 RESULTS AND TABLES

The precise initial concentration of alanap, determined by combustion immediately after application to the test soil (prior to extraction), was 6.43 ppm. The efficiency of the soil extraction methodology, based on the recovery of radiolabeled material (determined by combustion prior to extraction at each sampling interval), ranged from 92.9 to 104.9 %. The material balance (extractable + unextractable + cumulative volatiles), ranged from 87.2 to 96.9% of the initially-applied radioactivity (Table 1).

Alanap degraded with a half-life of 36.7 days (R^2 = 0.9627) based on linear regression analysis (see Tables 3 and 4 and Figure 6).

The metabolite alanap imide (AC-974-61) increased from 0.2% to 6.1% of the initially-applied radioactivity (0.01 to 0.39 ppm) by Day 41 then decreased to 2.7% (0.17 ppm) of the initially-applied radioactivity by Day 135. Alpha-naphthylamine (AC-974-60), present at 2.2% (0.14 ppm) of the initially-applied radioactivity on Day 0, decreased to 0.4% by Day 30 and was not detected thereafter.

CO_2 production (trapped in 5% sodium hydroxide solution) reached 39.5% of initially-applied radioactivity (2.54 ppm) by Day 135. No other volatile metabolites were observed in either the ethylene glycol or 1M sulfuric acid trap.

The temperature of the test soil was 25.1±0.1°C during the study period.

Determination of the microbial activity of the soil used in this study yielded the following results: Total bacteria, 4.58 x 10^6 colony forming units (C.F.U.)/gram of soil; actinomycetes, 7.56 x 10^5 C.F.U./gm; fungi, 1.69 x 10^4 C.F.U./gm (Appendix 8).

The results of the aerobic soil metabolism of alanap in sandy loam soil are provided as 'percent of the initially-applied radioactivity' and 'ppm' in Tables 1-4. Copies of the individual chromatograms showing detection of alanap and its soil metabolites by HPLC 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.