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CONCLUSIONS:

Metabolism - Aerobic Soil

1. This study is acceptable and fulfills EPA Data Requirements for Registering Pesticides by providing information on the aerobic metabolism of oxazolidine ring-labeled [4-^{14}C]MON 13900 in silt loam and sandy loam soils. No additional data on the aerobic soil metabolism of MON 13900 are required at this time.

2. MON 13900 degradation did not appear to follow first order kinetics. Initial half-lives, calculated as though first order kinetics had prevailed, were approximately 36 days in silt loam soil and approximately 48 days in sandy loam soil incubated in the dark at 25 C and 65-85% of 0.33 bar moisture. The major nonvolatile degradate was 2-[5-(2-furanyl)-2,2-dimethyl-3-oxazolidinyl]-2-oxoacetic acid (MON 13900 oxamic acid). Other nonvolatile degradates were 3-[(methylsulfinyl)acetyl]-2,2-dimethyl-5-(2-furanyl)oxazolidine (MON 13900 methyl sulfoxide); 5-(2-furanyl)-3-(hydroxyacetyl)-2,2-dimethyloxazolidine (MON 13900 alcohol).; and 5-(2-furanyl)-2,2-dimethyl-3-(methylthioacetyl)oxazolidine (MON 13900 methyl sulfide). At 12 months posttreatment, $^{14}CO_2$ was the major degradate and totaled 37.1-40.5% of the applied.

METHODOLOGY:

Samples (50 g, dry weight) of air-dried, sieved (2 mm) Sable silt loam and Sarpy sandy loam soils (Table 2) were placed in flasks and adjusted to 85% of 0.33 bar moisture. The flasks were stoppered with foam plugs, then incubated at 25 ± 1 C and 30% humidity in the dark for 13 days. Following the 13-day preincubation period, 28 flasks of each soil type were treated at 0.39 ppm with oxazolidine ring-labeled [4-$^{13}C/^{14}C$]MON 13900 (radiochemical purity 97.2-98.2%, specific activity 19.04 mCi/mMol, Monsanto), dissolved in acetonitrile. Following treatment, the soil moisture was readjusted to 85% of 0.33 bar moisture. Each flask was topped with a two-piece trapping tower (Figure 4) containing foam plugs to trap [^{14}C]volatiles and Ascarite II to trap evolved $^{14}CO_2$, then the samples were incubated at 25 ± 1 C in the dark. Duplicate flasks of each soil were collected at 0, 1, 3, 7, 14, 30, 62, 91, 122, 184, 273, and 365 days posttreatment. During the study, foam plugs in the trapping towers were replaced every 7-10 days, Ascarite was replaced every 2-3 weeks, the flasks were weighed at 7- to 10-day intervals, and water was added, as needed, to maintain the soil moisture at 65-85% of 0.33 bar.

Soil samples were extracted three times with 60% aqueous acetonitrile using a wrist-action shaker. Extracts were separated from the soil by centrifugation, then decanted, and aliquots of each extract were analyzed for total radioactivity using LSC. Selected soil samples were further extracted with various basic and/or acidic solvents, including 60% and 85% aqueous acetonitrile, 0.1 N ammonium hydroxide, acetonitrile:0.1 N ammonium hydroxide, acetonitrile:1 N HCl, 60% aqueous acetonitrile containing 1% EDTA, and 1% acetic acid (Summary Table). A pooled aqueous acetonitrile extract was prepared for each soil sample. The extract was concentrated by rotary evaporation, and aliquots were analyzed by LSC and by reverse phase HPLC using UV (254 nm) and radioactivity detection on a Brownlee RP-18 precolumn followed by either a Water u-Bondapak C-18 column (0- to 30-day soil extracts) or a Beckman Altex Ultrasphere-ODS C-18 column (62- to 365-day extracts) eluted with isocratic and linear gradients of acetonitrile and 0.001 M dibasic ammonium phosphate. Fractions were collected at 0.3-minute intervals and analyzed for radioactivity using LSC. To confirm degradate identifications, collected HPLC fractions were also analyzed using GC with flame ionization and radioactivity detection, GC/MS with chemical ionization, fast atom bombardment MS (FAB/MS), and NMR. Derivatives of degradates were produced, as needed, using diazomethane to form methyl esters of carboxylic acids and acetic anhydride plus pyridine for acetylations. Unextracted [^{14}C]residues remaining in the extracted soil were quantified using LSC following combustion.

To characterize unextracted [^{14}C]residues, the previously extracted 273-day soil samples were fractionated into humin, humic acid, and fulvic acid (Figure 26). A subsample of soil was extracted by shaking with 0.5 N NaOH for 60 hours. The extract was analyzed for total radioactivity using LSC, then acidified to pH 1 with concentrated HCl to precipitate the humic acid fraction. The supernatant containing the fulvic acid fraction was analyzed for total radioactivity using LSC, then partitioned with ethyl acetate; the resulting organic and aqueous phases were analyzed using LSC. The NaOH-extracted soil containing the humin fraction was lyophilized and analyzed for residual radioactivity by LSC following combustion.

Foam plugs from the trapping towers were placed in scintillation cocktail and analyzed for total radioactivity using LSC. Ascarite from the trapping towers was placed in a flask and dissolved in distilled water. The flask was immersed in ice; the adsorbed $^{14}CO_2$ was released from the Ascarite using concentrated sulfuric acid and trapped in phenethylamine solution. The trapping solution was analyzed for total radioactivity using LSC.

Extraction and analytical procedures were being developed during the main experiment, therefore, a supplemental experiment was conducted to obtain additional data during the first month after treatment. Eighteen flasks of each soil type were prepared, preincubated for 33 days, then treated at 0.42 ppm with oxazolidine ring-labeled [4-^{13}C/^{14}C]MON 13900 (radiochemical purity 98.8-99.5%, specific activity 18.13 mCi/mMol, Monsanto), and incubated as described above. Duplicate flasks of each soil were collected at 0, 1, 3, 7, 14, and 30 days posttreatment and analyzed as described above; a Brownlee RP-18 precolumn followed by a Beckman Altex Ultrasphere-ODS C-18 column were used for reverse phase HPLC analyses. Trapping towers were collected, replaced, and analyzed as described for the main experiment.

To produce additional material for degradate characterizations, a large scale experiment was performed. Twenty flasks of silt loam soil were prepared, preincubated for 34 days, then treated at 2.04 ppm with oxazolidine ring-labeled [4-^{13}C/^{14}C]MON 13900 (radiochemical purity 97.2-99.0%, specific activity 18.97 mCi/mMol, Monsanto), and incubated as described above. One to three flasks of soil were collected at 24, 56, 57, 127, and 274 days posttreatment and analyzed as described above. Two of the 274-days flasks were fitted with trapping towers that were collected, replaced, and analyzed as described above; the remaining flasks were fitted with modified trapping towers that were not analyzed. In an attempt to isolate and characterize polar degradates (HPLC Fraction A), the aqueous acetonitrile extracts from the 274-day soil samples were processed and analyzed by anion exchange chromatography (AG 1-X2 resin) according to the scheme presented in Figure 25. To further characterize unextracted [^{14}C]residues, the previously extracted 57- and 127-day soil samples were fractionated according to the schemes presented in Figures 27 and 28, respectively; extracts were analyzed using LSC and HPLC, and the extracted soil was lyophilized and analyzed for residual radioactivity by LSC following combustion.

DATA SUMMARY:

Oxazolidine ring-labeled [4-^{14}C]MON 13900 (radiochemical purity >97%), at 0.4 ppm, degraded with calculated initial half-lives of 32.8-40.1 days in silt loam soil and 45.0-52.5 days in sandy loam soil that were incubated in the dark at 25 ± 1 C and 65-85% of 0.33 bar moisture for up to 12 months (Figures 15-20); secondary half-lives were determined to be 263 days in silt loam soil and 156 days in sandy loam soil. In the silt loam soil, MON 13900 comprised an average 97.9% of the applied radioactivity at day 0, 78.5% at 7 days, 50.7% at 30 days, 27.9% at 184 days, and 16.7% at 365 days (Table 18). In the sandy loam soil, MON 13900 comprised an average 99.5% of the applied at day 0, 80.1% at 7 days, 66.1% at 30 days, 19.1% at 184 days, and 9.6% at 365 days (Table 21). The major nonvolatile degradate in the extracts of both soils was

> 2-[5-(2-furanyl)-2,2-dimethyl-3-oxazolidinyl]-2-oxoacetic acid (MON 13900 oxamic acid, Fraction B).

MON 13900 oxamic acid increased to maximum concentrations of 5.0% of the applied at 62 days posttreatment in the silt loam soil and 5.9% at 30 days in the sandy loam soil, then decreased to 1.4-1.6% of the applied by 365 days in extracts of both soils (Tables 17 and 20). Other nonvolatile degradates identified in the extracts of both soils were

> 3-[(methylsulfinyl)acetyl]-2,2-dimethyl-5-(2-furanyl)oxazolidine (MON 13900 methyl sulfoxide, Fraction C), present at a maximum concentration of 1.7-1.9% of the applied at 30 days posttreatment;

5-(2-furanyl)-3-(hydroxyacetyl)-2,2-dimethyloxazolidine (MON 13900 alcohol, Fraction D1), present at a maximum concentration of 0.9-1.7% at 62-91 days; and

5-(2-furanyl)-2,2-dimethyl-3-(methylthioacetyl)oxazolidine (MON 13900 methyl sulfide, Fraction D2), present at a maximum concentration of 0.5-0.9% at 62 days.

Unidentified polar [^{14}C]residues (Fraction A) consisting of two neutral [^{14}C]compounds and two acidic [^{14}C]compounds were present at total maximum concentrations of 4.7-6.8% (0.018-0.027 ppm) of the applied at 62 days posttreatment.

At 12 months posttreatment, $^{14}CO_2$ was the major degradate, totaling 37.1% of the applied in the silt loam soil and 40.5% in the sandy loam soil; organic volatiles totaled 0.3 and 0.9%, respectively (Tables 3-6). In the silt loam soil, unextracted [^{14}C]residues increased to an average 32.1% of the applied at 273 days posttreatment and were 28.6% at 365 days (Table 4); in the sandy loam soil, [^{14}C]residues gradually increased to an average 30.2% of the applied at 365 days (Table 6). Based on analysis of the unextracted [^{14}C]residues from the 273-day soil samples, 4.7-8.2% of the applied was in the fulvic acid fraction and 15.3-19.0% was in the humin fraction; residues in the humic acid fraction were not quantified (Figure 26). During the main and supplemental studies, material balances ranged from 87.3 to 102.3% of the applied (Tables 3, 5, 7, and 9).

COMMENTS:

1. The process did not appear to follow first order kinetics. However, using the 0- to 30-day sampling intervals from the main experiment and first order kinetics where x = time and y = ln(concentration of MON 13900), the study authors calculated initial half-lives of 34.5 days ($r^2 = 0.9181$) in the silt loam soil (Figure 15) and 45.0 days ($r^2 = 0.9210$) in the sandy loam soil (Figure 16). The study authors did not include the 62- to 365-day sampling intervals because by that time, degradation had slowed considerably. Using the 0- to 30-day sampling intervals from the supplemental experiment and first order kinetics, the study authors calculated half-lives of 32.8 days ($r^2 = 0.9487$) in the silt loam soil and 52.5 days ($r^2 = 0.7860$) in the sandy loam soil. Using the 62- to 365-day sampling intervals from the main experiment and first order kinetics, the Dynamac reviewer calculated second half-lives of 263 days ($r^2 = 0.9908$) in the silt loam soil and 156 days ($r^2 = 0.9905$) in the sandy loam soil.

   Since the degradation of MON 13900 did not appear to follow first order kinetics, the study authors proposed an alternative kinetic model (described in Appendix C). Using the 0- to 30-day sampling intervals from the supplemental experiment plus the 62- to 365-day sampling intervals from the main experiment, the study authors calculated initial half-lives of 40.1 ± 17.6 days in the silt loam soil (Figure 19) and 46.5 ± 10.7 days in the sandy loam soil (Figure 20).

2. The registrant reported that MON 13900 [3-(dichloroacetyl)-5-(2-furanyl)-2,2-dimethyloxazolidine] is a safener intended for use with chloroacetanilide and sulfonylurea herbicides in corn and sorghum. The maximum projected use rate for MON 13900 is 0.4 lb/A.

3. The registrant reported that for studies conducted using radiolabeled MON 13900, the compound was synthesized with the radiolabel in the carbon atom adjacent to the nitrogen in the oxazolidine ring portion of the molecule. Studies were not conducted with the compound labeled in the furan ring portion of the molecule because degradation of the radiolabeled furan ring would result in radiolabeled ring fragments that would be natural products composed of low numbers of carbon, hydrogen, and oxygen atoms.

Table 3: Total Accountability of Applied ^{14}C-Radioactivity in the MON 13900 Aerobic Soil Metabolism Study; Main Experiment; Sable Silt Loam Soil

Study Day	Replicate	% DPMA				Total Accountability (%)
		in Extracts	as Volatiles	as CO_2	Bound	
0	A	96.9	0.0	0.0	1.5	98.4
0	B	98.8	0.0	0.0	1.3	100.0
1	A	94.8	0.0	0.3	2.6	97.7
1	B	95.8	0.1	0.3	3.2	99.3
3	A	89.3	0.1	1.4	7.8	98.5
3	B	88.5	0.1	1.6	7.8	98.0
7	A	84.5	0.1	3.6	10.6	98.8
7	B	83.0	0.2	2.5	9.6	95.2
14	A	75.7	0.1	6.6	13.6	96.1
14	B	73.4	0.1	6.8	14.6	95.0
30	A	62.8	0.1	12.1	16.9	91.9
30	B	61.2	0.1	11.9	18.8	92.0
62	A	53.0	0.2	17.4	21.2	91.9
62	B	52.1	0.2	17.4	21.0	90.7
91	A	44.6	0.2	20.2	23.8	88.8
91	B	46.9	0.2	20.3	23.1	90.5
122	A	43.9	0.3	22.6	23.2	90.0
122	B	44.1	0.3	22.6	24.1	91.1
184	A	36.6	0.3	27.0	28.7	92.6
184	B	37.4	0.3	27.0	28.6	92.9
273	A	30.8	0.3	32.2	33.1	96.5
273	B	29.8	0.3	32.2	31.1	93.4
365	A	22.0	0.3	37.1	29.7	89.1
365	B	22.4	0.3	37.2	27.5	87.3
Mean						94.0
Std.Dev.						3.8

See Table 4 for averages of duplicate flasks.
DPMA = dpm applied

Table 4: Total Accountability of Applied ^{14}C-Radioactivity in the MON 13900 Aerobic Soil Metabolism Study; Main Experiment; Sable Silt Loam Soil; Averages of Duplicate Flasks (see Figure 11 for graph)

Study	% DPMA				Total
Day	in Extracts	as Volatiles	as CO_2	Bound	Accountability (%)
0	97.8	0.0	0.0	1.4	99.2
1	95.3	0.1	0.3	2.9	98.5
3	88.9	0.1	1.5	7.8	98.3
7	83.8	0.1	3.1	10.1	97.0
14	74.6	0.1	6.7	14.1	95.5
30	62.0	0.1	12.0	17.8	92.0
62	52.5	0.2	17.4	21.1	91.3
91	45.7	0.2	20.2	23.5	89.6
122	44.0	0.3	22.6	23.7	90.5
184	37.0	0.3	27.0	28.6	92.8
273	30.3	0.3	32.2	32.1	94.9
365	22.2	0.3	37.1	28.6	88.2
Mean					94.0
Std.Dev.					3.8

Data represents average of data from duplicate flasks.
See Table 3 for data from individual flasks.
DPMA = dpm applied.

Table 5: Total Accountability of Applied ^{14}C-Radioactivity in the MON 13900 Aerobic Soil Metabolism Study; Main Experiment; Sarpy Sandy Loam Soil

Study Day	Replicate	% DPMA in Extracts	% DPMA as Volatiles	% DPMA as CO_2	% DPMA Bound	Total Accountability (%)
0	A	100.7	0.0	0.0	0.5	101.2
0	B	100.8	0.0	0.0	0.5	101.3
1	A	93.9	0.1	0.4	4.7	100.0
1	B	93.4	0.1	0.4	6.7	100.5
3	A	93.9	0.0	1.7	3.5	99.1
3	B	89.9	0.1	1.2	6.7	97.9
7	A	85.0	0.1	2.4	9.3	96.8
7	B	87.5	0.2	2.3	9.0	99.0
14	A	75.9	0.4	7.1	14.0	97.4
14	B	74.8	0.4	7.1	17.2	99.4
30	A	71.2	0.4	8.9	14.4	95.0
30	B	70.2	0.4	9.0	13.1	92.7
62	A	54.7	0.6	16.8	23.9	96.0
62	B	53.7	0.6	16.4	23.9	94.6
91	A	46.1	0.7	21.6	25.6	94.0
91	B	46.5	0.7	21.7	23.9	92.7
122	A	40.3	0.8	25.2	32.9	99.3
122	B	39.1	0.8	25.2	26.0	91.1
184	A	31.4	0.9	30.1	28.1	90.5
184	B	32.1	0.9	30.1	28.1	91.1
273	A	23.5	0.9	35.8	30.3	90.5
273	B	23.2	0.9	35.8	29.3	89.2
365	A	18.7	0.9	40.5	29.7	89.7
365	B	17.9	0.9	40.5	30.8	90.0
Mean						95.4
Std.Dev.						4.1

See Table 6 for averages of duplicate flasks.
DPMA = dpm applied.

Table 6: Total Accountability of Applied ^{14}C-Radioactivity in the MON 13900 Aerobic Soil Metabolism Study; Main Experiment; Sarpy Sandy Loam Soil; Averages of Duplicate Flasks (see Figure 12 for graph)

Study Day	% DPMA				Total Accountability (%)
	in Extracts	as Volatiles	as CO_2	Bound	
0	100.8	0.0	0.0	0.5	101.3
1	93.6	0.1	0.4	5.7	100.3
3	91.9	0.1	1.4	5.1	98.5
7	86.3	0.2	2.3	9.1	97.9
14	75.3	0.4	7.1	15.6	98.4
30	70.7	0.4	9.0	13.8	93.9
62	54.2	0.6	16.6	23.9	95.3
91	46.3	0.7	21.7	24.7	93.4
122	39.7	0.8	25.2	29.5	95.2
184	31.8	0.9	30.1	28.1	90.8
273	23.4	0.9	35.8	29.8	89.8
365	18.3	0.9	40.5	30.2	89.8
Mean					95.4
Std.Dev.					4.1

Data represents average of data from duplicate flasks.
See Table 5 for data from individual flasks.
DPMA = dpm applied.

Table 7: Total Accountability of Applied ^{14}C-Radioactivity in the MON 13900 Aerobic Soil Metabolism Study; Supplemental Experiment; Sable Silt Loam Soil

Study Day	Replicate	% DPMA in Extracts	as Volatiles	as CO_2	Bound	Total Accountability (%)
0	A	100.7	0.0	0.0	0.9	101.5
0	B	100.9	0.0	0.0	1.1	102.0
1	A	96.1	0.0	0.2	2.5	98.9
1	B	94.8	0.0	0.1	2.2	97.1
3	A	88.3	0.0	0.9	7.0	96.2
3	B	91.4	0.0	1.0	5.6	98.1
7	A	83.1	0.1	1.6	10.8	95.6
7	B	82.8	0.1	2.4	9.5	94.7
14	A	67.8	0.1	6.6	15.5	90.1
14	B	69.3	0.1	6.7	16.3	91.1
30	A	59.8	0.2	8.4	21.2	89.6
30	B	58.7	0.2	9.4	20.7	88.9
Mean						95.3
Std.Dev.						4.5

See Table 8 for averages of duplicate flasks.
DPMA = dpm applied.

Table 8: Total Accountability of Applied ^{14}C-Radioactivity in the MON 13900 Aerobic Soil Metabolism Study; Supplemental Experiment; Sable Silt Loam Soil; Averages of Duplicate Flasks (see Figure 13 for graph)

Study Day	% DPMA in Extracts	as Volatiles	as CO_2	Bound	Total Accountability (%)
0	100.8	0.0	0.0	1.0	101.7
1	95.5	0.0	0.2	2.4	98.0
3	89.8	0.0	1.0	6.3	97.1
7	82.9	0.1	2.0	10.1	95.1
14	68.5	0.1	6.7	15.9	90.6
30	59.2	0.2	8.9	20.9	89.2
Mean					95.3
Std.Dev.					4.5

Data represents average of data from duplicate flasks.
See Table 7 for data from individual flasks.
DPMA = dpm applied.

Table 9: Total Accountability of Applied ^{14}C-Radioactivity in the MON 13900 Aerobic Soil Metabolism Study; Supplemental Experiment: Sarpy Sandy Loam Soil

Study Day	Replicate	% DPMA				Total Accountability (%)
		in Extracts	as Volatiles	as CO_2	Bound	
0	A	101.5	0.0	0.0	0.7	102.2
0	B	101.5	0.0	0.0	0.8	102.3
1	A	97.1	0.0	0.2	2.3	99.6
1	B	98.3	0.1	0.2	2.2	100.8
3	A	91.1	0.1	1.1	5.7	97.9
3	B	91.9	0.0	1.1	5.6	98.6
7	A	87.5	0.1	2.2	9.1	98.9
7	B	85.3	0.0	2.8	10.1	98.2
14	A	76.3	0.1	6.1	14.5	97.0
14	B	75.1	0.1	6.2	11.4	92.8
30	A	75.7	0.3	8.7	12.4	97.2
30	B	76.6	0.4	9.4	12.2	97.9
Mean						98.6
Std.Dev.						2.6

See Table 10 for averages of duplicate flasks.
DPMA = dpm applied.

Table 10: Total Accountability of Applied ^{14}C-Radioactivity in the MON 13900 Aerobic Soil Metabolism Study; Supplemental Experiment; Sarpy Sandy Loam Soil; Averages of Duplicate Flasks (see Figure 14 for graph)

Study Day	% DPMA				Total Accountability (%)
	in Extracts	as Volatiles	as CO_2	Bound	
0	101.5	0.0	0.0	0.7	102.2
1	97.7	0.1	0.2	2.2	100.2
3	91.5	0.0	1.1	5.7	98.2
7	86.4	0.1	2.5	9.6	98.5
14	75.7	0.1	6.1	13.0	94.9
30	76.2	0.4	9.1	12.3	97.9
Mean					98.6
Std.Dev.					2.6

Data represents average of data from duplicate flasks.
See Table 9 for data from individual flasks.
DPMA = dpm applied.

Table 15: Data Used for Half-Life Estimation for MON 13900 in the Aerobic Soil Metabolism Studies; Main and Supplemental Experiments

Study Day	Replicate	% DPMA		ln(% DPMA)	
		MON 13900 Sable	MON 13900 Sarpy	MON 13900 Sable	MON 13900 Sarpy
0	A	97.6	99.7	4.581	4.603
0	B	98.1	99.3	4.586	4.598
1	A	89.7	93.4	4.496	4.536
1	B	87.3	94.7	4.469	4.550
3	A	82.8	85.7	4.416	4.451
3	B	87.3	86.5	4.469	4.460
7	A	77.4	81.3	4.349	4.398
7	B	79.5	78.8	4.376	4.367
14	A	61.4	68.1	4.117	4.221
14	B	63.5	66.6	4.152	4.199
30	A	51.4	66.1	3.939	4.191
30	B	50.0	66.0	3.912	4.190
62	A	39.5	39.0	3.677	3.664
62	B	37.8	38.6	3.632	3.653
91	A	33.6	27.5	3.513	3.316
91	B	34.5	30.2	3.541	3.407
122	A	34.9	25.7	3.553	3.246
122	B	33.2	27.4	3.503	3.312
184	A	27.9	18.9	3.328	2.938
184	B	27.9	19.3	3.330	2.961
273	A	23.3	13.5	3.147	2.605
273	B	23.2	13.1	3.146	2.570
365	A	16.5	9.7	2.801	2.269
365	B	16.9	9.4	2.826	2.245

Data plotted in Figures 17 through 20 (pages 86 through 89).
Data for Days 0 through 30 are from the supplemental experiment.
Data for Days 62 through 365 are from the main experiment.
See Tables 13 and 14 for data from Days 0 through 30 from the main experiment.
DPMA = dpm applied.

Table 17: Quantification of MON 13900 and Soil Metabolites by HPLC/RAD; Percent of Applied ^{14}C-Radioactivity; Sable Silt Loam Soil

Study Day	Replicate	% DPMA Analyzed by HPLC/RAD	% DPMA as					
			Fraction A	Fraction B	Fraction C	Fraction D		MON 13900
						Peak 1	Peak 2	
0	A	100.7	0.1	1.0	0.6	0.3	0.3	97.6
0	B	100.9		1.1	0.7	0.4	0.3	98.1
1	A	96.1	0.4	3.5	1.5	0.5	0.4	89.7
1	B	94.2	0.3	3.2	0.9	0.3	0.4	87.3
3	A	88.3		3.5	1.5			82.8
3	B	91.4	0.8	2.1	0.5			87.3
7	A	83.4	1.1	3.5	0.9			77.4
7	B	82.6		2.8	0.3			79.5
14	A	67.8	0.8	2.6	1.1			61.4
14	B	69.3	0.9	2.5	1.0			63.5
30	A	59.8	1.3	3.4	1.9	0.5		51.4
30	B	58.7	0.7	3.1	1.8			50.0
62	A	51.9	4.7	2.8	1.0	1.7	0.9	39.5
62	B	51.1	4.2	5.0	1.5	1.4	0.3	37.8
91	A	39.7	1.3	2.8	0.9	0.6		33.6
91	B	41.9	1.1	3.1	1.2		0.8	34.5
122	A	41.1	1.5	2.6	0.9	0.4		34.9
122	B	40.9	1.4	3.1	1.2	0.8		33.2
184	A	34.6	1.2	3.0	1.3	0.4	0.1	27.9
184	B	35.2	1.5	2.7	1.3	0.4	0.2	27.9
273	A	28.6	0.5	2.1	1.3	0.6		23.3
273	B	27.8	0.6	2.0	0.9	0.4		23.2
365	A	21.1	0.8	1.5	0.8	0.4		16.5
365	B	21.1	0.9	1.4	0.7	0.4		16.9

DPMA = dpm applied.
No entry = not detected.
Fraction B = MON 13900 Oxamic Acid II; Fraction C = MON 13900 Methyl Sulfoxide III
Fraction D1 = MON 13900 Alcohol IV; Fraction D2 = MON 13900 Methyl Sulfide V
Data for Days 0 through 30 are from the supplemental experiment.
Data for Days 62 through 365 are from the main experiment.

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Table 18: Quantification of MON 13900 and Soil Metabolites by HPLC/RAD; Percent of Applied ^{14}C-Radioactivity; Sable Silt Loam Soil; Averages of Duplicate Flasks (see Figure 23 for graph)

Study Day	% DPMA Analysed by HPLC/RAD	% DPMA as					
		Fraction A	Fraction B	Fraction C	Fraction D		MON 13900
					Peak 1	Peak 2	
0	100.8	0.1	1.0	0.7	0.3	0.3	97.9
1	95.2	0.3	3.4	1.2	0.4	0.4	88.5
3	89.8	0.8	2.8	1.0			85.0
7	83.0	1.1	3.2	0.6			78.5
14	68.5	0.8	2.6	1.0			62.5
30	59.2	1.0	3.3	1.9	0.5		50.7
62	51.5	4.5	3.9	1.3	1.6	0.6	38.6
91	40.8	1.2	2.9	1.1	0.6	0.8	34.0
122	41.0	1.5	2.9	1.1	0.6		34.1
184	34.9	1.3	2.8	1.3	0.4	0.2	27.9
273	28.2	0.5	2.1	1.1	0.5		23.3
365	21.1	0.9	1.5	0.7	0.4		16.7

DPMA = dpm applied.
No entry = not detected.
See Figures 21 and 23 for graphs.
Fraction B = MON 13900 Oxamic Acid II; Fraction C = MON 13900 Methyl Sulfoxide III
Fraction D1 = MON 13900 Alcohol IV; Fraction D2 = MON 13900 Methyl Sulfide V
Data for Days 0 through 30 are from the supplemental experiment.
Data for Days 62 through 365 are from the main experiment.

-4.16-

Table 20: Quantification of MON 13900 and Soil Metabolites by HPLC/RAD; Percent of Applied ^{14}C-Radioactivity; Sarpy Sandy Loam Soil

Study Day	Replicate	% DPMA Analyzed by HPLC/RAD	% DPMA as Fraction A					
			Fraction A	Fraction B	Fraction C	Fraction D Peak 1	Fraction D Peak 2	MON 13900
0	A	101.5		0.7	0.4	0.3		99.7
0	B	101.5	0.2	0.7	0.4	0.4		99.3
1	A	97.1		2.5	0.3	0.3		93.4
1	B	98.3	0.4	2.3	0.5	0.2		94.7
3	A	91.1	0.4	3.3	0.7	0.1		85.7
3	B	91.9	0.5	3.8	0.6			86.5
7	A	87.5	1.3	3.6	0.7			81.3
7	B	85.3	1.7	3.3	0.9			78.8
14	A	76.3	1.3	5.2	1.3			68.1
14	B	75.1	1.2	4.4	1.2			66.6
30	A	75.7	0.8	5.6	1.5			66.1
30	B	76.6	0.8	5.9	1.7			66.0
62	A	54.7	6.0	5.5	1.3	0.7	0.5	39.0
62	B	53.7	6.8	5.2	1.4	0.8	0.5	38.6
91	A	40.3	4.9	3.8	1.4	0.9		27.5
91	B	41.0	4.7	3.6	1.1	0.6		30.2
122	A	37.7	4.5	3.9	1.4	0.5		25.7
122	B	36.7	3.9	3.4	1.2	0.5	0.5	27.4
184	A	27.6	4.4	2.9	0.8	0.4		18.9
184	D	28.4	4.3	2.8	0.8	0.7		19.3
273	A	20.4	3.6	2.0	0.6	0.5		13.5
273	B	19.8	3.9	1.6	0.7	0.5		13.1
365	A	16.3	3.2	1.6	0.5	0.5		9.7
365	B	15.3	3.2	1.5	0.5	0.5		9.4

DPMA = dpm applied.
No entry = not detected.
Fraction B = MON 13900 Oxamic Acid II; Fraction C = MON 13900 Methyl Sulfoxide III
Fraction D1 = MON 13900 Alcohol IV; Fraction D2 = MON 13900 Methyl Sulfide V
Data for Days 0 through 30 are from the supplemental experiment.
Data for Days 62 through 365 are from the main experiment.

Table 21: Quantification of MON 13900 and Soil Metabolites by HPLC/RAD; Percent of Applied ^{14}C-Radioactivity; Sarpy Sandy Loam Soil; Averages of Duplicate Flasks (see Figure 24 for graph)

Study Day	% DPMA Analyzed by HPLC/RAD	% DPMA as					
		Fraction A	Fraction B	Fraction C	Fraction D Peak 1	Fraction D (Peak 2)	MON 13900
0	101.5	0.2	0.7	0.4	0.3		99.5
1	97.7	0.4	2.4	0.4	0.3		94.0
3	91.5	0.5	3.5	0.7	0.1		86.1
7	86.4	1.5	3.4	0.8			80.1
14	75.7	1.2	4.8	1.2			67.4
30	76.2	0.8	5.8	1.6			66.1
62	54.2	6.4	5.3	1.4	0.7	0.5	38.8
91	40.6	4.8	3.7	1.3	0.7		28.9
122	37.2	4.2	3.7	1.3	0.5	0.5	26.6
184	28.0	4.3	2.8	0.8	0.5		19.1
273	20.1	3.8	1.8	0.7	0.5		13.3
365	15.8	3.2	1.6	0.5	0.5		9.6

DPMA = dpm applied.

No entry = not detected.

See Figures 22 and 24 for graphs.

Fraction B = MON 13900 Oxamic Acid II; Fraction C = MON 13900 Methyl Sulfoxide III

Fraction D1 = MON 13900 Alcohol IV; Fraction D2 = MON 13900 Methyl Sulfide V

Data for Days 0 through 30 are from the supplemental experiment.

Data for Days 62 through 365 are from the main experiment.

-4.18-

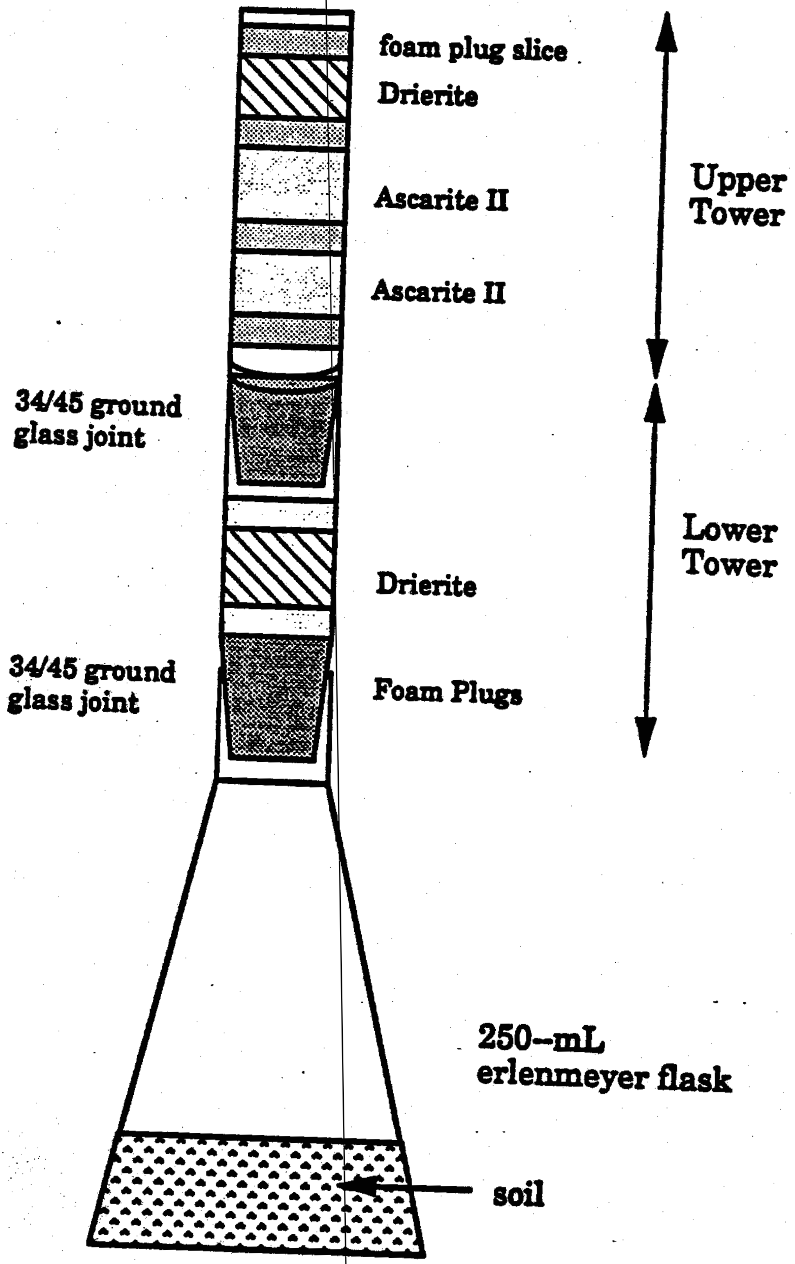
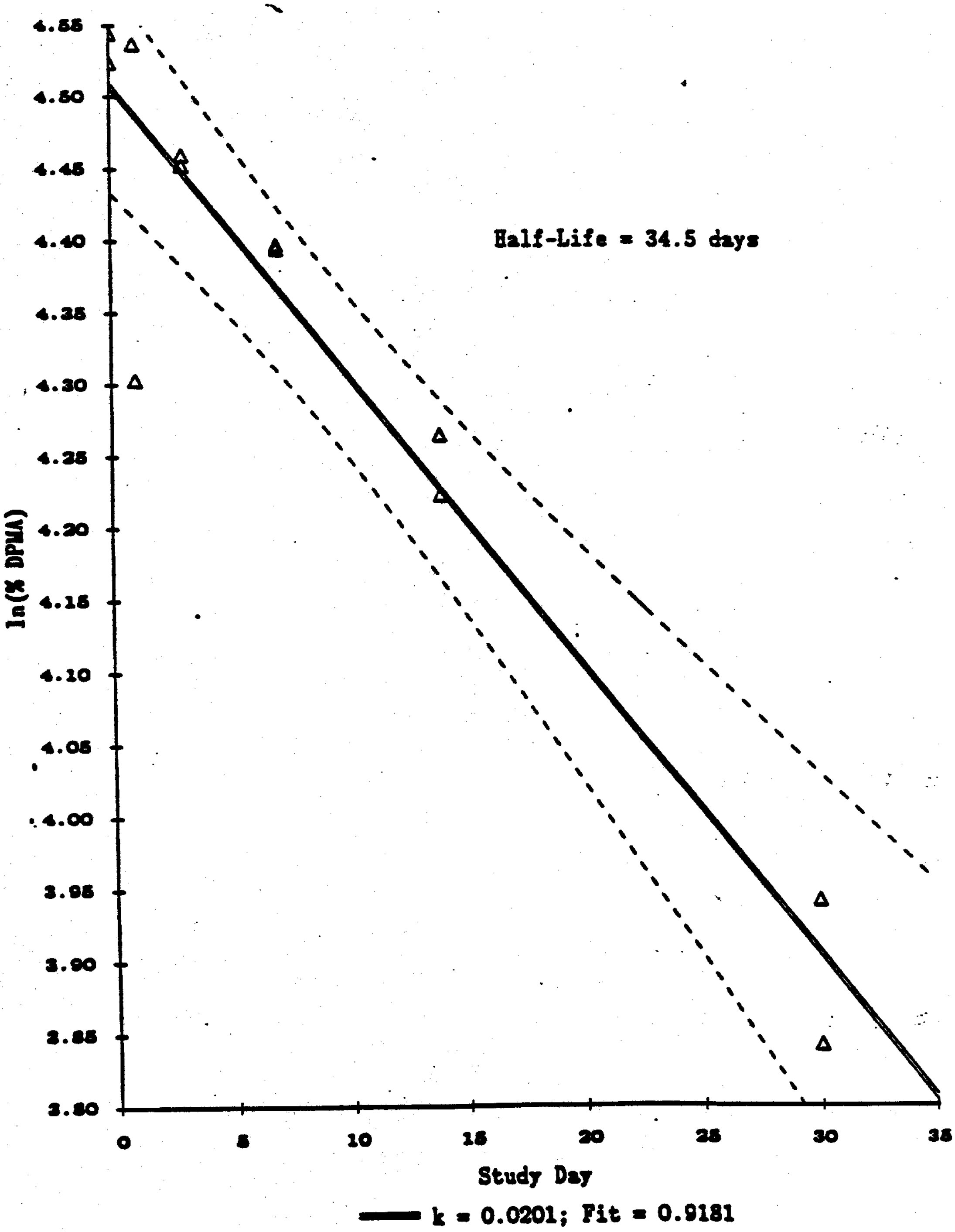


Figure 4: Apparatus Used in the MON 13900 Aerobic Soil Metabolism Studies

Figure 15: Half-Life Calculation for MON 13900 in Sable Silt Loam Soil under Aerobic Conditions; Main Experiment; First-Order Model (see Table 13 for data)



Dashed lines indicate 95% confidence intervals.

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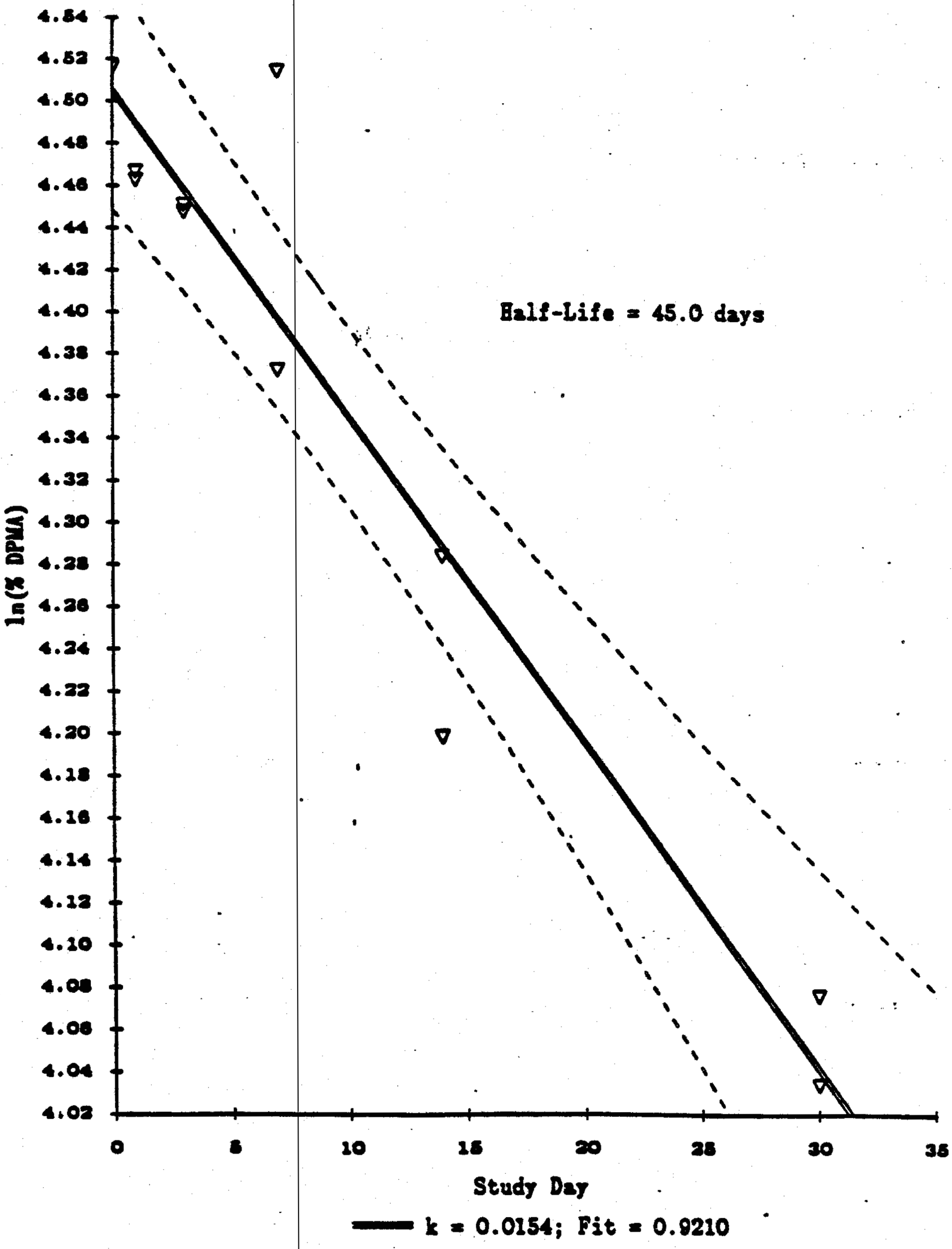
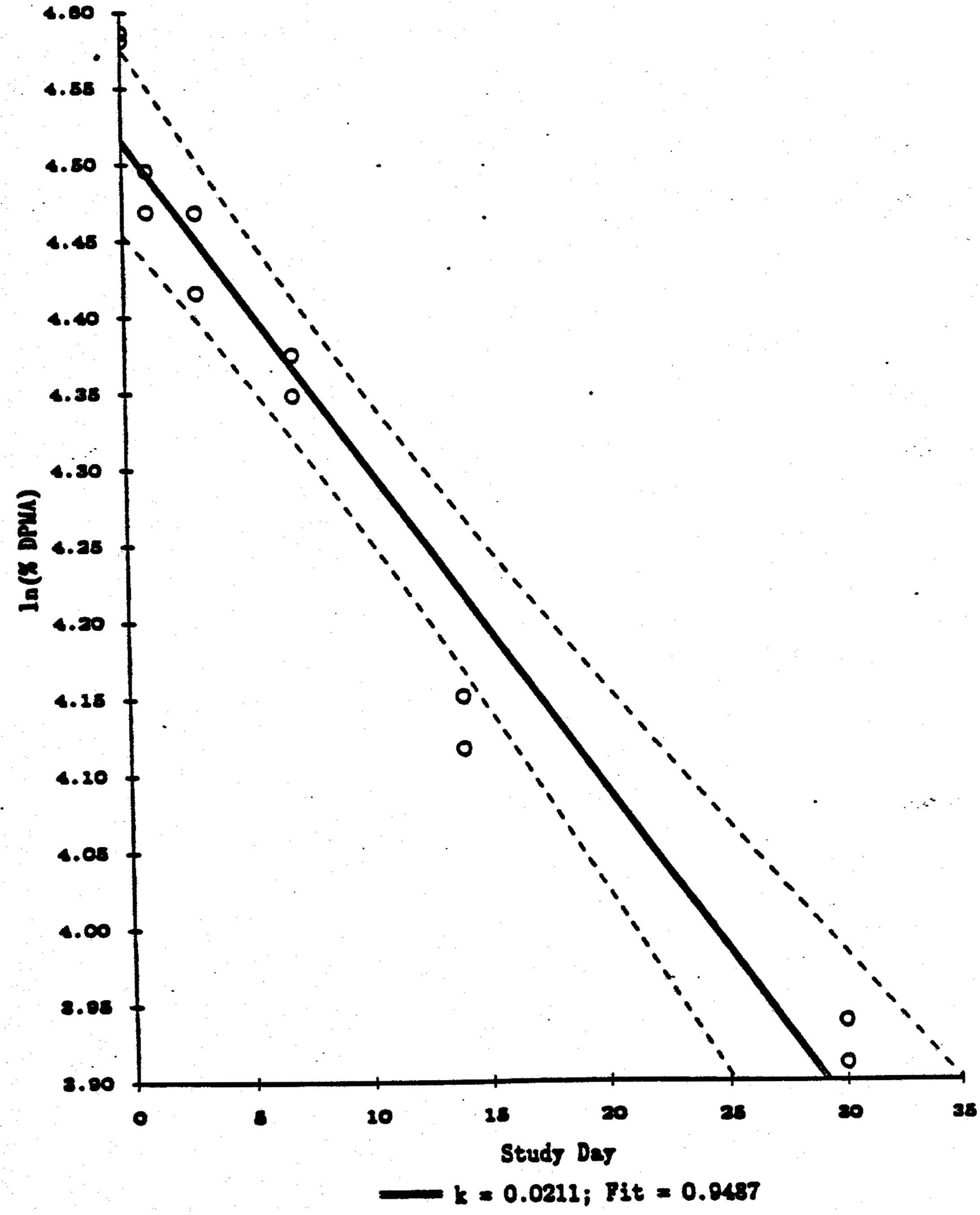
Figure 16: Half-Life Calculation for MON 13900 in Sarpy Sandy Loam Soil under Aerobic Conditions; Main Experiment; First-Order Model (see Table 14 for data)



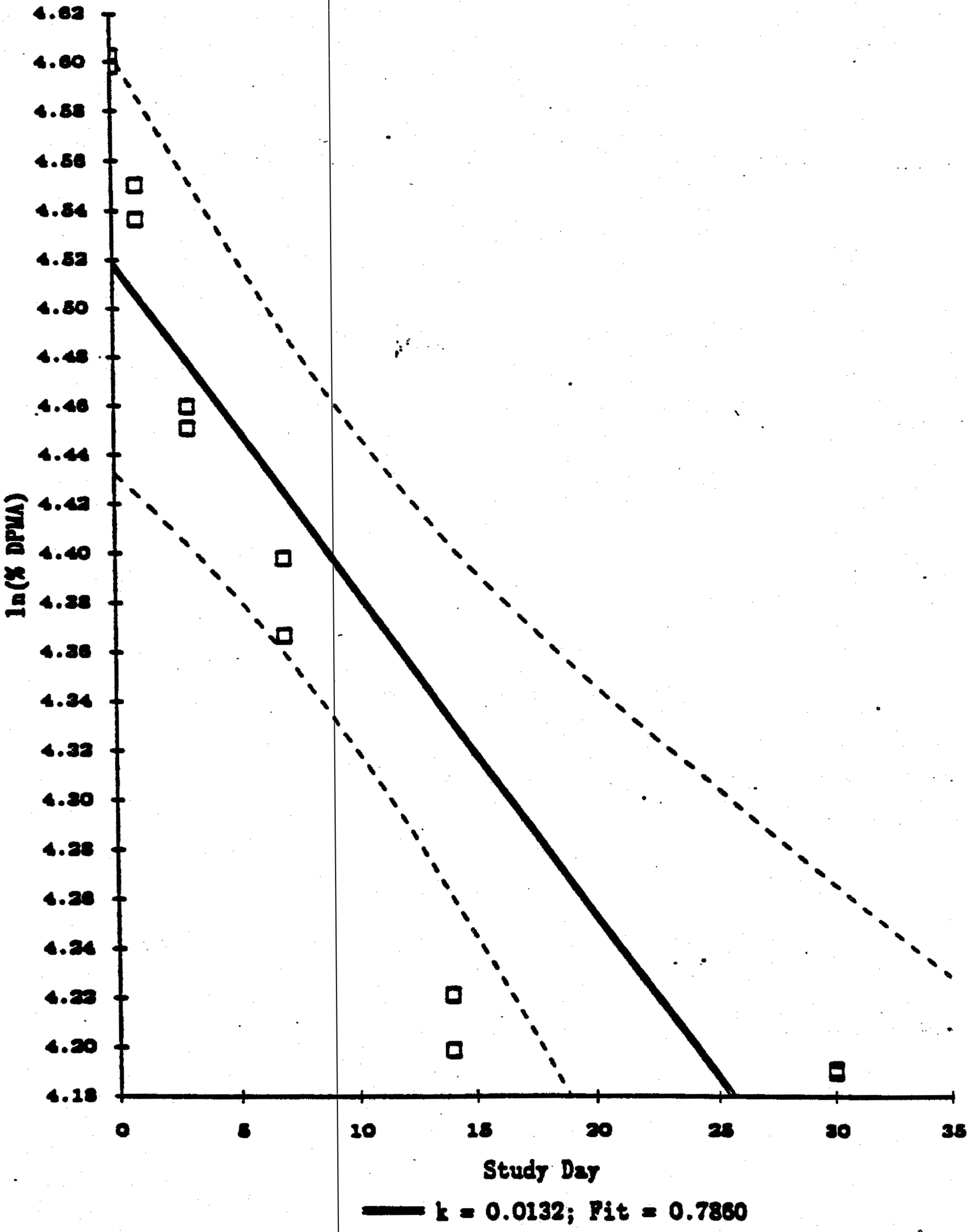
Dashed lines indicate 95% confidence intervals.

Figure 17: Half-Life Calculation for MON 13900 in Sable Silt Loam Soil under Aerobic Conditions; Supplemental Experiment; First-Order Model (see Table 15 for data)



Dashed lines indicate 95% confidence intervals.

Figure 18: Half-Life Calculation for MON 13900 in Sarpy Sandy Loam Soil under Aerobic Conditions: Supplemental Experiment: First-Order Model (see Table 15 for data)



Dashed lines indicate 95% confidence intervals.

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Figure 19: Half-Life Calculation for MON 13900 in Sable Silt Loam Soil under Aerobic Conditions; Alternative Kinetic Model (see Table 15 for data)

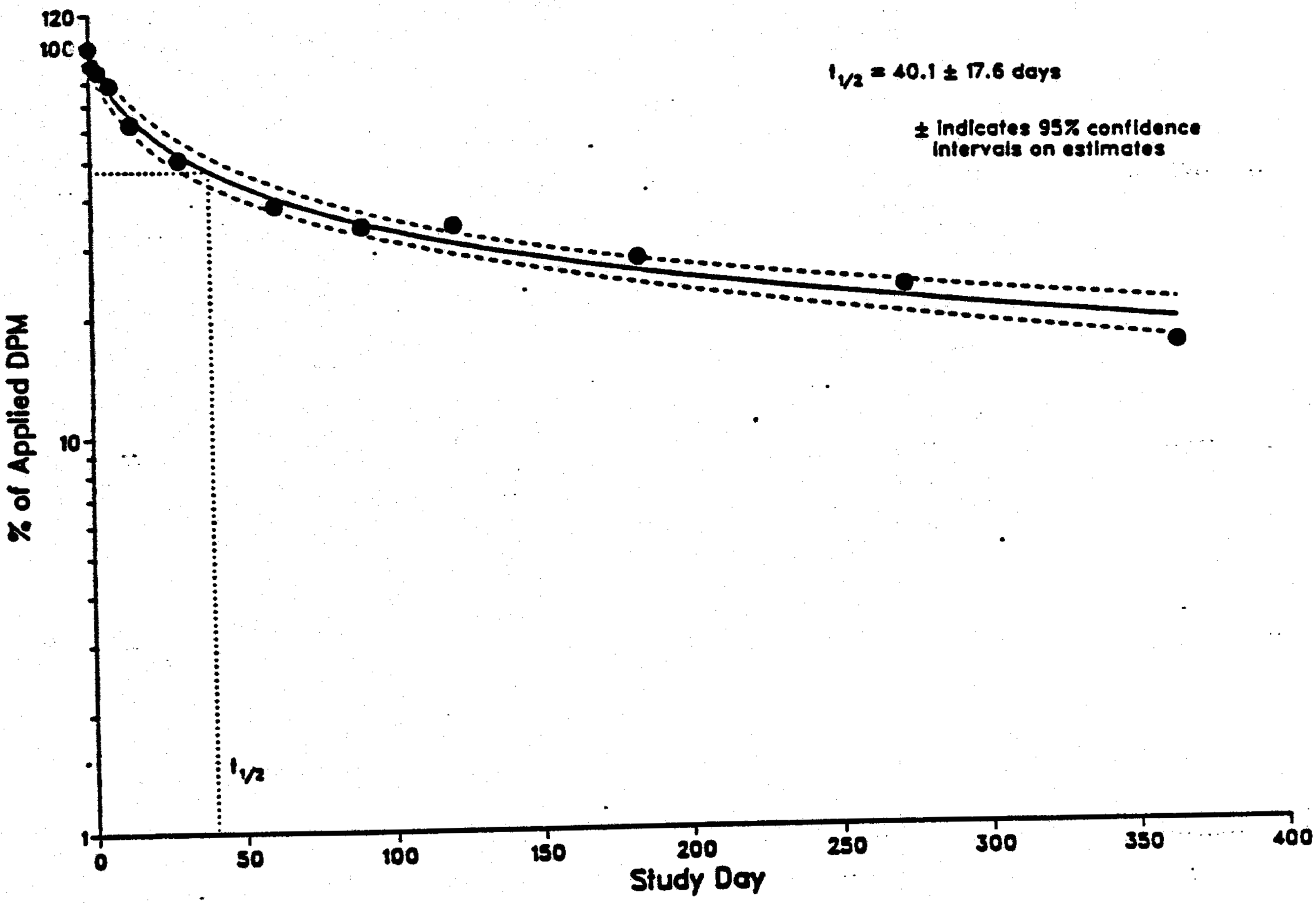

Figure 20: Half-Life Calculation for MON 13900 in Sarpy Sandy Loam Soil under Aerobic Conditions; Alternative Kinetic Model (see Table 15 for data)

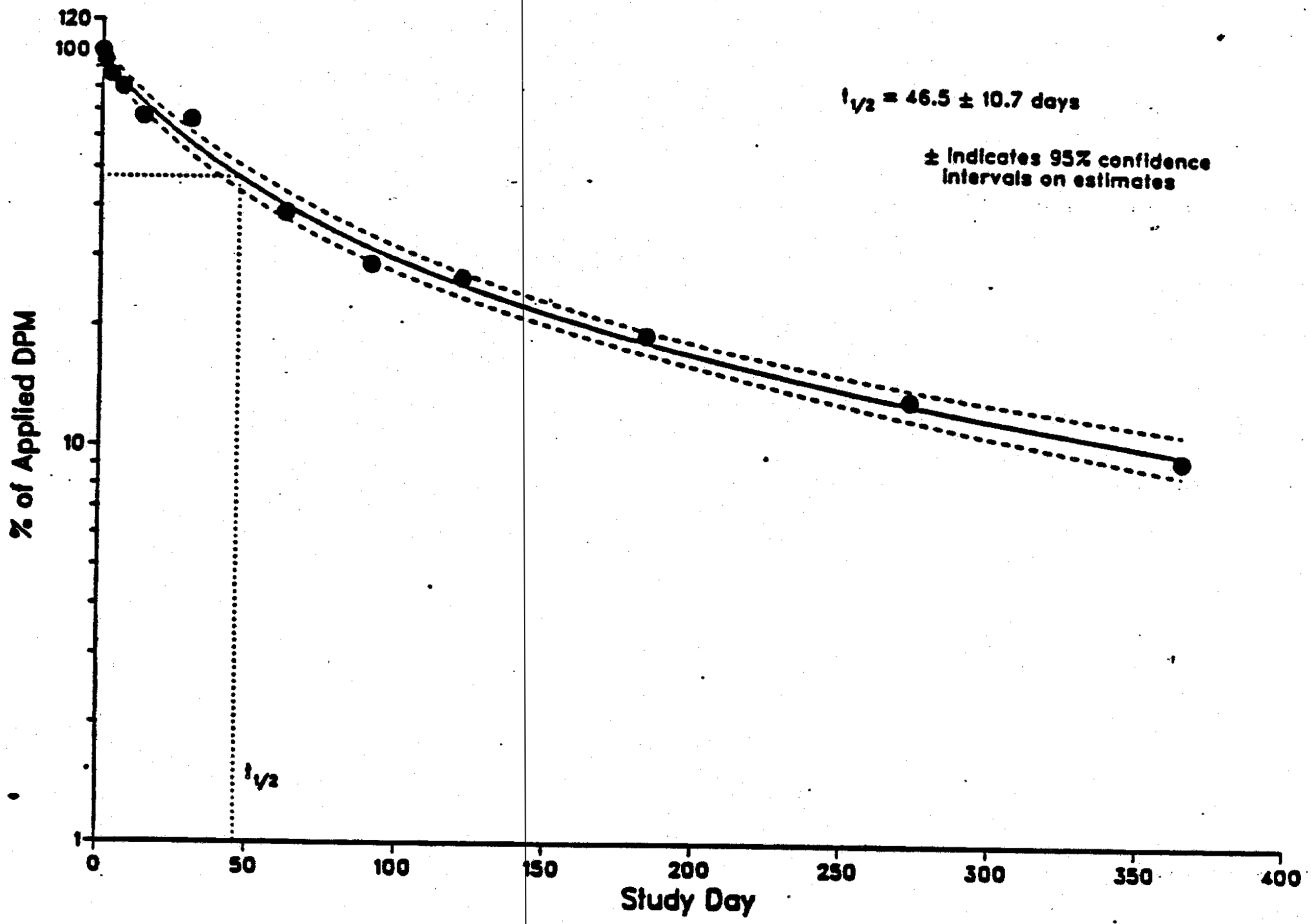

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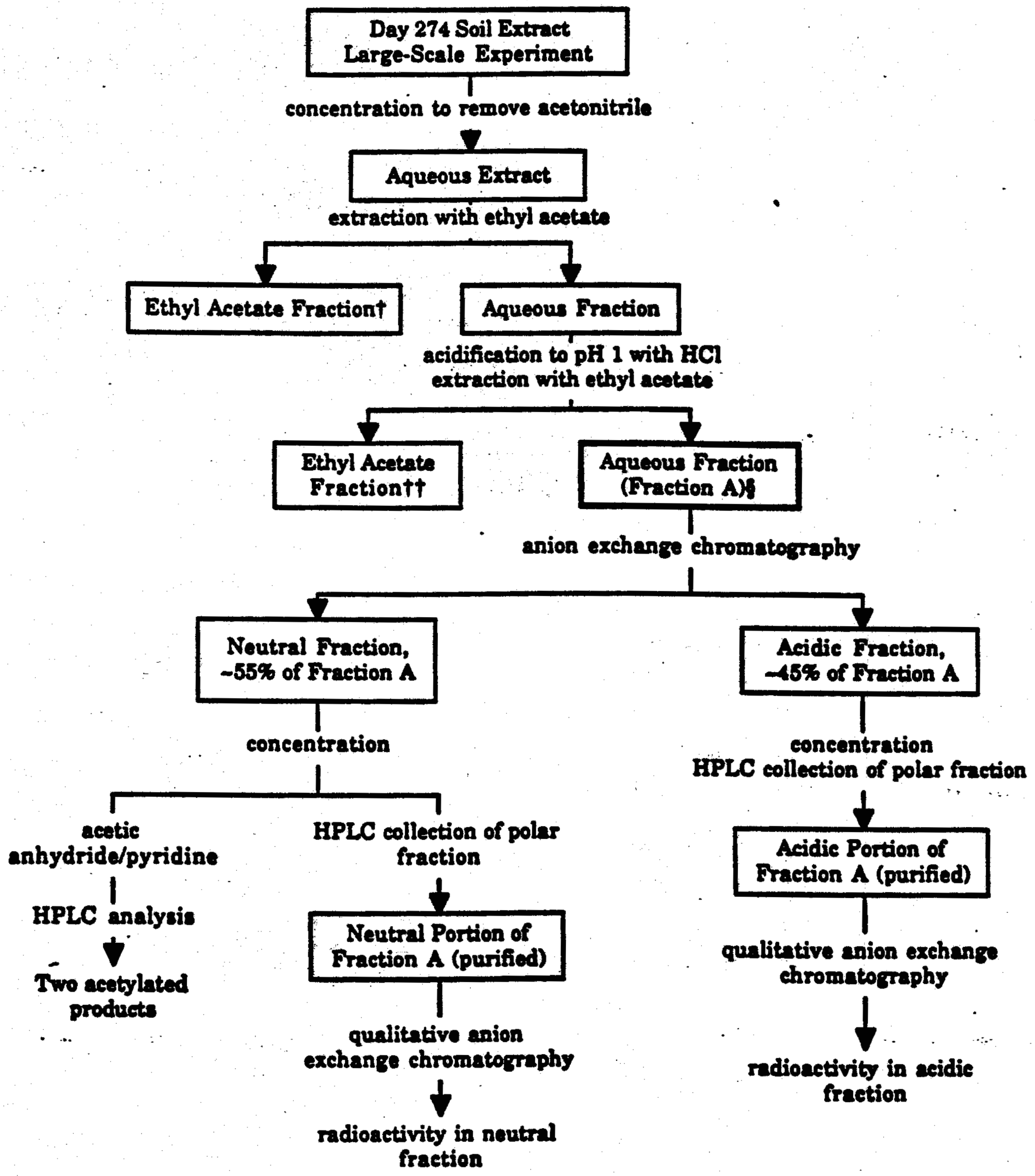
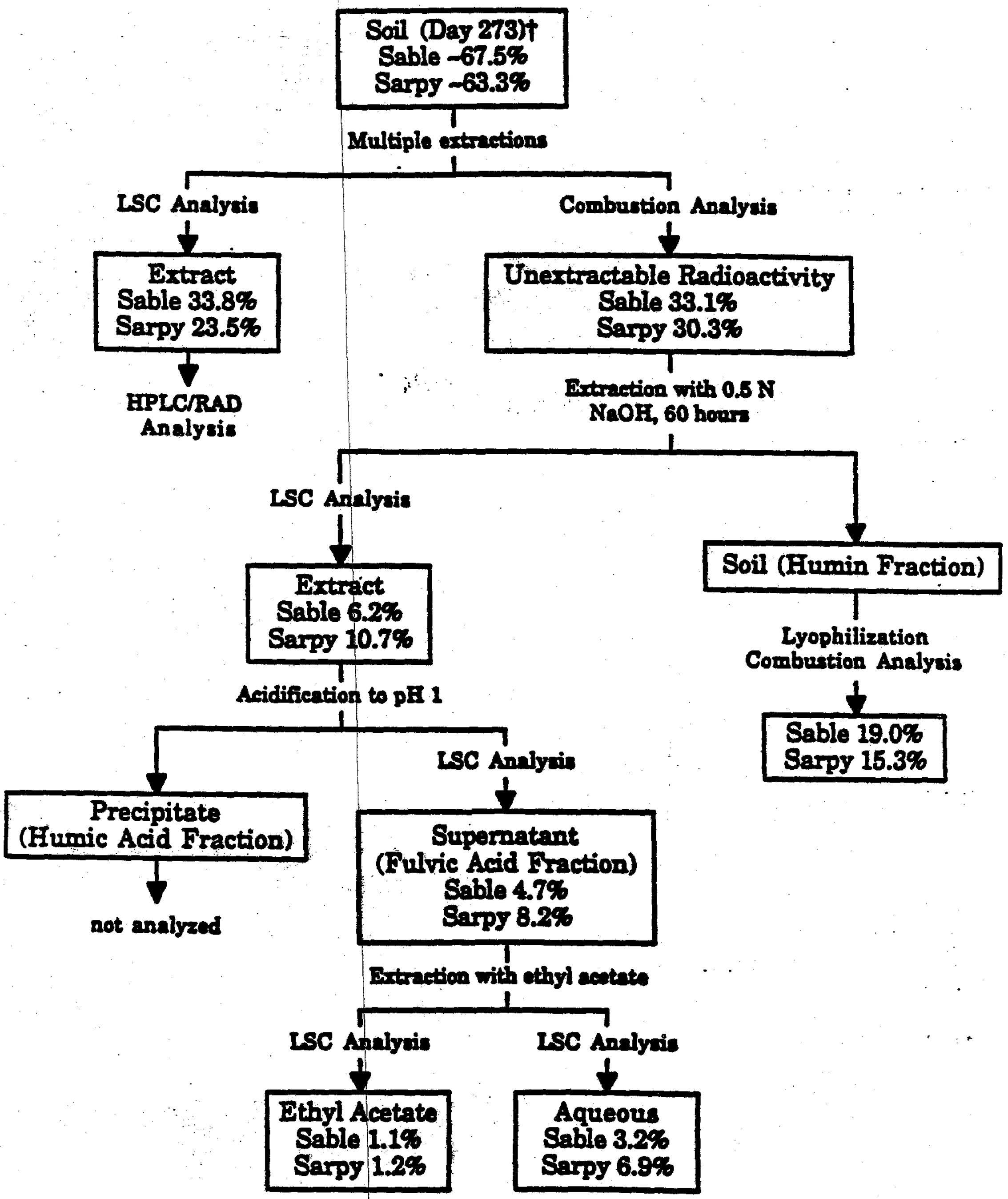
Figure 25: Procedure for Characterization of Metabolite Fraction A from Day 274 Soil Extract, Large-Scale Experiment



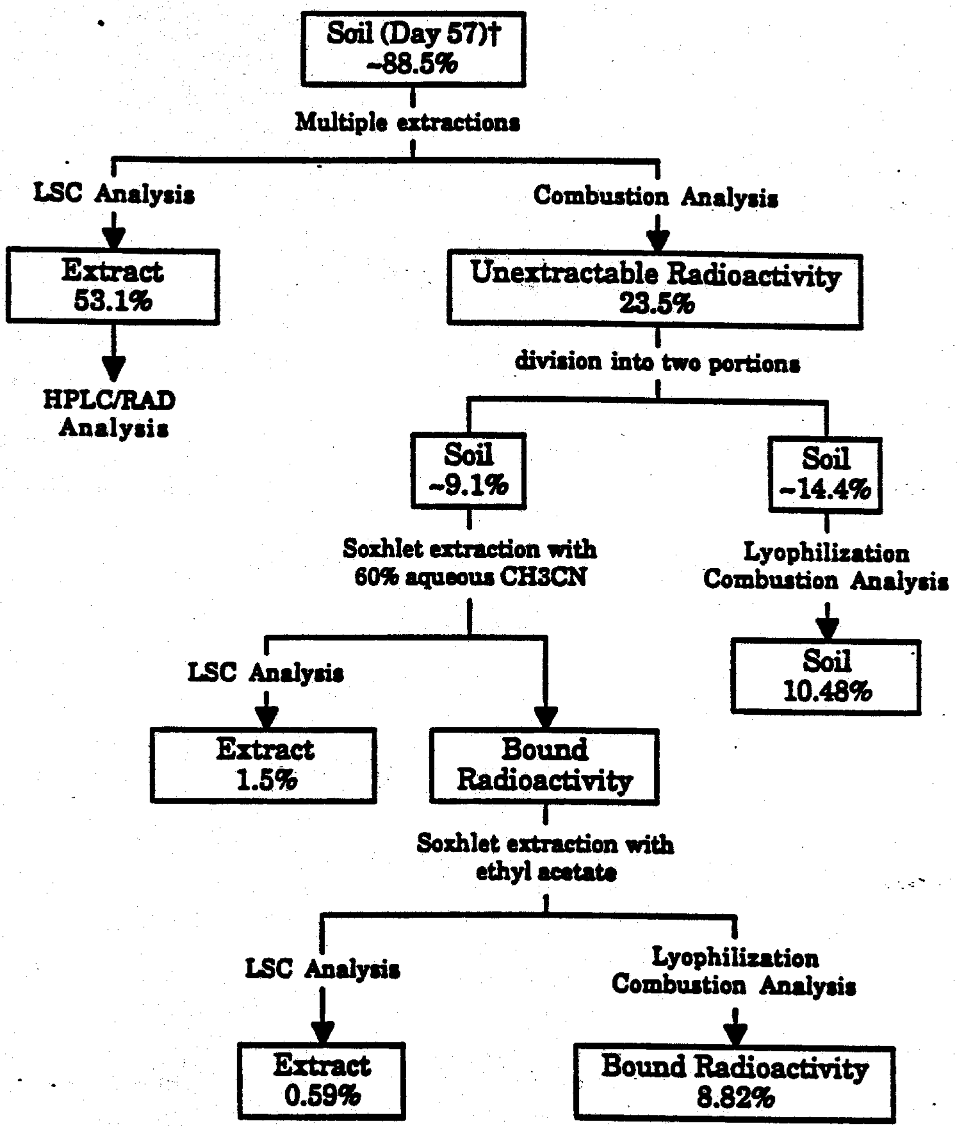
†This fraction contained MON 13900 I and the alcohol IV.
††This fraction contained MON 13900 oxamic acid II.
§The polar metabolites of interest represented 80% of this fraction.

Figure 26: Procedure for Characterization of Bound Residues in Day 273 Soil from the MON 13900 Main Experiment



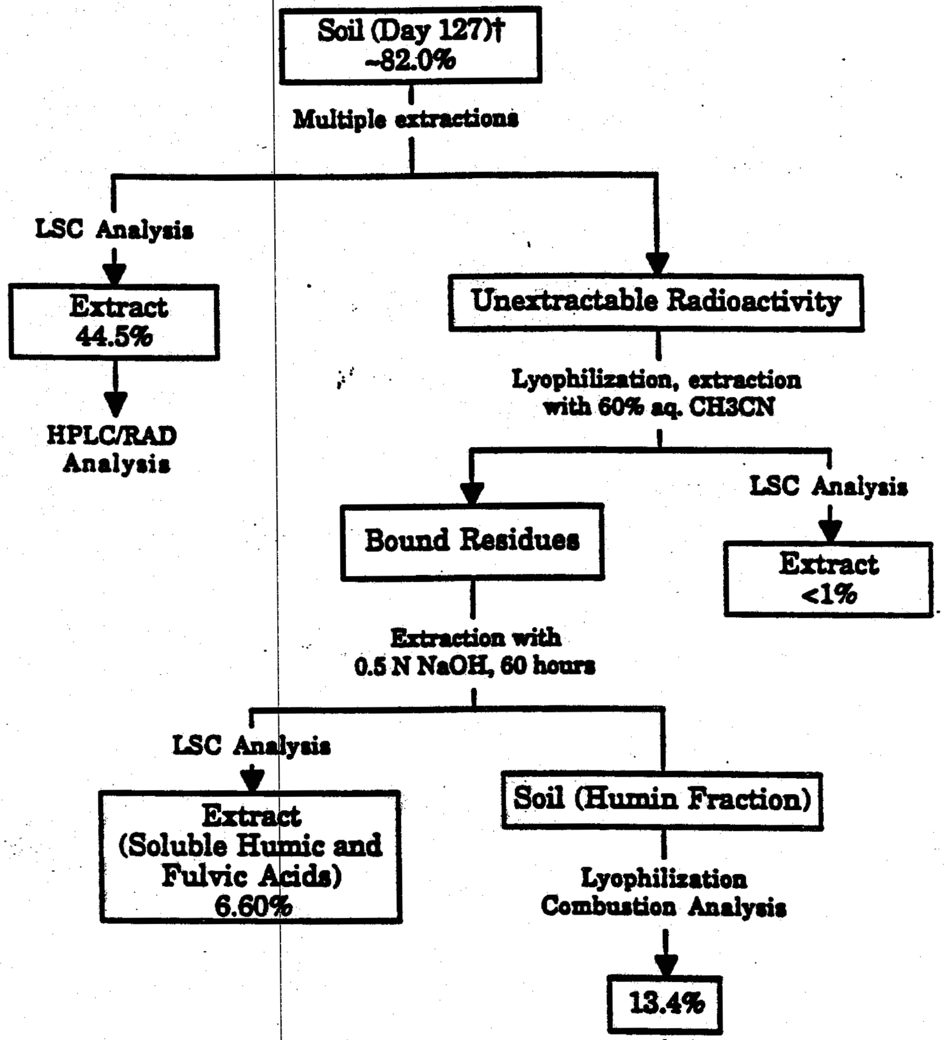
†All values are expressed as percent of applied dpm. The soil was not analyzed by combustion analysis prior to extraction. Initial percent dpm values were estimated based on radiolabeled CO2 and volatile production.

Figure 27: Procedure for Characterization of Bound Residues in Day 57 Soil from the MON 13900 Large Scale Experiment



†All values are expressed as percent of applied dpm. The soil was not analyzed by combustion analysis prior to extraction. The initial percent dpm value was estimated based on the CO2 and volatile production.

Figure 28: Procedure for Characterization of Bound Residues in Day 127 Soil from the MON 13900 Large Scale Experiment



†All values are expressed as percent of applied dpm. The soil was not analyzed by combustion analysis prior to extraction. Initial percent dpm values were estimated based on CO2 and volatile production.

SUMMARY TABLE.

Summary of Extraction Solvents and Shaking Times for Soil Extractions

Study	Study Day	First Extract	Second Extract	Third Extract	Fourth Extract
Main Experiment	0	1-hour	1-hour	1-hour	
	1	1-hour	1-hour	1-hour	
	3	0.5-hour	0.5-hour	0.5-hour	3-hour[a]
	7	1-hour	1-hour	1-hour	
	14	1-hour	1-hour	1-hour	18-hour[b]
	30	1-hour	1-hour	1-hour	18-hour[c]
	62	1-hour	1-hour	1-hour	4-hour[d]
	91	1-hour	1-hour	18-hour	2-hour[e,f]
	122	1-hour	1-hour	1-hour	1-hour[e,g]
	184	4-hour	2-hour	2-hour	18-hour[e]
	273	4-hour	4-hour	18-hour	18-hour[e]
	365	2.5-hour	1.2-hour	18-hour	4.5-hour[e]
Supplemental Experiment	0	3-hour	2-hour	overnight	
	1	3-hour	2-hour	overnight	
	3	3-hour	2-hour	overnight	
	7	6-hour	18-hour	4-hour	6-hour
	14	6-hour	18-hour	4-hour	
	30	6-hour	18-hour	5-hour	
Large Scale Experiment	24	1-hour	1-hour	1-hour	
	56[h]	1-hour	1-hour	1-hour	
	56[i]	18-hour	1.5-hour	1.5-hour	
	127	4-hour	2-hour	18-hour	60-hour[e]
	274	18-hour	4-hour	2-hour	

Unless otherwise specified, solvent is 60% aqueous CH_3CN.

[a] Fourth extraction: 50:50 CH_3CN:0.1 N NH_4OH.
Fifth extraction: 95:5 CH_3CN:1 N HCl, 1-hour.

[b] For Sable soil only: Fifth extraction with 0.1 N NH_4OH, 2-hour.
Sixth extraction with 95:5 CH_3CN:1 N HCl, 2-hour.

[c] Fifth extraction with 0.1 N NH_4OH, 1-hour.

[d] Fifth extraction: 60% CH_3CN, 4-hour.
For Sable soil only: sixth extraction with 60% CH_3CN with 1% EDTA.

[e] Solvent for fourth extraction: 0.1 N NH_4OH.

[f] Fifth extraction with 1% acetic acid, 2-hour.

[g] Fifth extraction: 95:5 CH_3CN:1 N HCl, 1-hour.
Sixth extraction: 60% CH_3CN, 18-hour.

[h] Replicate A: 85% aqueous CH_3CN.

[i] Replicate B: 60% aqueous CH_3CN.

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Table 2: Physical Properties of Soils Used in the MON 13900 Aerobic Soil Metabolism Study[1]

	Sable	Sarpy
Textural Classification	silt loam	sandy loam
Order [2]	*	Entisol
USDA Classification [2]	*	mixed,mesic, Typic Udipsamment
Location	Monmouth, IL	New Bloomfield, MO
% Sand	19.00	59.00
% Silt	59.00	31.00
% Clay	22.00	10.00
pH (1:1 soil:H_2O)	6.70	8.00
Cation Exchange Capacity (meq/100g)	55.80	10.30
% Moisture at 1/3 atm (Field Capacity)	29.08	12.99
% Moisture at 15 atm (Wilting Point)	18.08	6.60
Bulk Density (g/mL)	1.10	1.11
% Organic Matter [3]	4.9	0.80
% Organic Carbon	*	0.58
% $CaCO_3$	1.10	4.58
Extractable Cations		
Ca	3210.00	1200.00
Mg	703.00	225.00
Na	42.00	120.00
K	242.00	131.00
H[4]	*	*

* Information not available.

[1] All data was generated (except where indicated) by A & L Agricultural Laboratories, 411 N. Third St., Memphis, TN; 1986 (Sable), 1987 (Sarpy).

[2] Information was obtained from Dr. Robert Held, soil scientist with the USDA Soil Conservation Service, Franklin, MO via phone communication, 1985.

[3] Determined colormetrically.

[4] Lab was unable to analyze for Hydrogen.

13 APPENDIX C: Alternate Kinetic Model for Half-Life Calculations

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Monsanto Agricultural Company

Background

Aerobic soil metabolism studies are required in order to register a compound for its planned use as a pesticide. As part of the report on dissipation kinetics, regulatory agencies generally request that the registrant provide an estimate of the half-life of the compound from the dissipation data. This requirement carries with it the implicit assumption that the dissipation curve may be approximated using a simple first-order kinetic model. During the aerobic soil metabolism studies of MON 13900, it was found that the dissipation of MON 13900, as with many other compounds, was not adequately described using the traditional first-order model. Thus, a new methodology had to be developed in order to estimate the half-lives requested by the regulatory agencies. The new technique is shown below to give an excellent fit to the data, and it allowed precise estimates to be made of the time required for residues in soil to reach 50% of their initial levels. This time, usually denoted DT_{50}, may be thought of as the *first* half-life of dissipation, but it should not be used in a simple first-order model to estimate concentrations later in the experiment. If such estimates are required, then the new methodology described in this document, which includes the non-linear effects, should be used.

Methodology

A first-order reaction model is commonly used to describe dissipation kinetics.[*] The model is based on a solution to the following differential equation:

$$\frac{dC}{dt} = -kC \tag{1}$$

in which the time derivative of the concentration, C, is directly proportional to the concentration. The proportionality constant, k, is usually denoted the first-order reaction rate constant. This rate equation is first-order because the reaction rate dC/dt is proportional to the first power of C. Reaction models of different order (e.g. second- or third-order) result then C is raised to a power of 2 or 3 on the right-hand-side of equation (1).

---

[*] Lyman, W.J.; Reehl, W.F.; Rosenblatt, D.H.; "Handbook of Chemical and Property Estimation Methods"; McGraw Hill: New York; 1982.

Equation (1) may be integrated directly with the initial condition that the concentration is C_o:

$$C = C_o e^{-kt} \tag{2}$$

Estimation of the two parameters, C_o and k, from a set of data is usually accomplished by regressing the natural logarithm of the concentration onto time, as seen by taking the logarithm of both sides of equation (2):

$$ln(C) = ln(C_o) - kt \tag{3}$$

According to equation (3),the logarithm of the concentration should be linear when plotted as a function of time, with slope $= -k$ and intercept $= ln(C_o)$.

An interesting and useful feature of the kinetic model described by equations (1) to (3) is that the time necessary for the concentration to decline by 50% is a constant, determined entirely by the first-order rate constant. This time is usually called the half-life, $t_{1/2}$, and is related to k as follows:

$$t_{1/2} = \frac{ln(2)}{k} \tag{4}$$

Unfortunately, the dissipation data for MON 13900 are non-linear when plotted as $ln(C)$ versus time. This is illustrated in Figures 19 and 20 (pages 89 and 90). This curvature immediately suggests that the dissipation of MON 13900 is not adequately described by the traditional first-order model presented above, as often reported by other authors for other compounds.[†],[‡],[§] However, a generalization of the first-order kinetic model was found that gives an excellent fit to the data, as shown by the smooth curve in Figures 19 and 20.

Equation (1) was generalized by assuming that k, the first-order rate constant, is spatially variable. If we assume the soil to be an infinite collection of segregated compartments, each with its own first-order rate constant, then we can replace the single exponential solution by an integral over all possible exponential decay curves:

$$C = \int_0^\infty C_o e^{-kt} f(k) dk \tag{5}$$

in which the probability density function of the spatially variable rate constant, k, is represented by $f(k)$. This equation has a simple solution when we assume that the rate constants vary spatially according to the standard Gamma Distribution:

$$f(k) = \frac{k^{\alpha-1} e^{-k/\beta}}{\beta^\alpha \Gamma(\alpha)} = GammaDistribution : \alpha > 0, \beta > 0, k > 0 \tag{6}$$

[†] Lyman, et al.; 1982.

[‡] Hance, R.J.; McKone, C.E.; in "Herbicides"; L.J.Audus Ed.; Academic: New York; 1976; p. 393.

[§] Graham-Bryce, I.J.; in "The Chemistry of Soil Processes"; D.J. Greenland and M.H.B. Hayes, Eds.; Wiley: New York; p. 621.

Substitution of (6) into (5), followed by integration, yields the following expression for the concentration, C , as a function of time:

$$C = C_o(1+\beta t)^{-\alpha} \tag{7}$$

Taking the logarithm of both sides results in the following expression:

$$ln(C) = ln(C_o) - \alpha ln(1+\beta t) \tag{8}$$

Equation (9) results from the substitution, $\mu = \alpha\beta$, where μ is the mean of the Gamma Distribution:

$$ln(C) = ln(C_o) - \mu\frac{ln(1+\beta t)}{\beta} \tag{9}$$

Equation (9) is given in order to demonstrate that as β vanishes, this model reduces to the linear, first-order kinetic model described by equations (1) through (3) with $\mu = k$. By direct analogy with the methods commonly employed to estimate the first-order reaction rate constant (regressing $ln(C)$ onto t), a non-linear regression model can be applied directly to equation (8) in order to fit the dissipation function. The SAS procedure NLIN was used to estimate values for the parameters appearing in equations (8) and (9). NLIN fits non-linear regression models by least squares.[¶] The iterative method GAUSS-NEWTON was used within NLIN, which is the default method in cases where derivatives are provided (see Section 13 for a sample program). Because the Gamma Distribution is defined only for α and β greater than zero, the two parameters were restricted to have values greater than zero (see the bounds statement in Section 13).

The dissipation parameter of most interest is DT_{50} , which is found by solving equation (7) for the time at which $C = C_o/2$. This yields:

$$DT_{50} = \frac{2^{1/\alpha}-1}{\beta} \tag{10}$$

The estimated value for DT_{50} was found by substituting the estimates for α and β from the NLIN-generated fit into equation (10). The estimated variance of DT_{50} can be related to the variances and covariances of α and β coming from the statistics of the fit to the data reported by NLIN:

$$\widehat{var}(DT_{50}) = \left(\frac{\partial DT_{50}}{\partial\alpha}\right)\Big|^2_{\hat{\alpha},\hat{\beta}}\widehat{var}(\hat{\alpha}) + \left(\frac{\partial DT_{50}}{\partial\alpha}\right)\Big|^2_{\hat{\alpha},\hat{\beta}}\widehat{var}(\hat{\beta}) + \tag{11}$$

$$2\left(\frac{\partial DT_{50}}{\partial\alpha}\right)\Big|_{\hat{\alpha},\hat{\beta}}\left(\frac{\partial DT_{50}}{\partial\beta}\right)\Big|_{\hat{\alpha},\hat{\beta}}\widehat{cov}(\hat{\alpha},\hat{\beta}) \tag{12}$$

in which the partial derivatives are:

$$\frac{\partial DT_{50}}{\partial\alpha} = \frac{-2^{1/\alpha}ln(2)}{\beta\alpha^2} \tag{13}$$

[¶] SAS® Users Guide: Statistics, Version 5 Edition; SAS Institute: CARY, NC; 1985; p. 575.

$$\frac{\partial DT_{50}}{\partial \beta} = \frac{1 - 2^{1/\alpha}}{\beta^2} \tag{14}$$

Estimates for DT_{50} and its standard errors were derived using this methodology for MON 13900 and are reported in Section 4.3.2 (page 41). Approximate 95% confidence intervals for any parameter or functions of parameters, $\omega = \omega(\alpha\beta)$, can be computed as:

$$\hat{\omega} \pm t^{*}_{n-3}\hat{\sigma}_{\hat{\omega}} \tag{15}$$

where $\hat{\omega}$ is the estimate of ω, $\hat{\sigma}$ is its estimated standard error (the square root of the estimated variance). The coefficient t^* is the 5% two-tailed percentile from the Student's t-distribution with $n-3$ degrees of freedom. These intervals were calculated and are reported in Section 4.3.2 (page 41).

Sample SAS Program

```
options ls=80;
data raw;
input dat ppm;
logppm = log(ppm);
cards;
0 97.9
1 88.5
3 85.0
7 78.5
14 62.5
30 50.7
62 38.6
91 34.0
122 34.1
184 27.9
273 23.3
365 16.7
;
Proc nlin outest=parest;
  parms logc0=4.5 alpha=1.0 beta=0.1;
  bounds alpha>0, beta>0;
  model logppm = logc0 - (alpha*log(1.0+(beta*dat)));
  der.logc0=1;
  der.alpha=-log(1+(beta*dat));
  der.beta=-alpha*dat/(1+(beta*dat));
Proc reg data=raw outest=regest covout;
  model logppm = dat;
```

```
 output out=confidnt l95=l95 u95=u95;
Proc print data=confidnt;
 var u95 l95;
```