STUDY SC900021

DATA EVALUATION RECORD

STUDY 2

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CONCLUSIONS:

Metabolism - Aerobic Soil

1. This study is acceptable and satisfies data requirements for aerobic soil metabolism of ancymidol.

2. Ancymidol degraded with a half-life of 14.9 days in sandy loam soil that was incubated in the dark at 25±1° C and 75% of 0.33 bar moisture for up to 61 days. Six unidentified degradates were detected at ≤0.01 ppm.

METHODOLOGY:

Portions (50 g dry weight) of sieved (2 mm) sandy loam soil (60.8% sand, 29.9% silt, 9.2% clay, 1.22% organic matter, pH 6.9, CEC 11.2 meq/100 g) were weighed into glass dishes and treated with [^{14}C]ancymidol (labeled at the alpha carbon; radiochemical purity >99%, 10.6 uCi/mg specific activity, DowElanco) at a nominal concentration of 0.5 ppm. Additional deionized water was added to each aliquot to adjust the soil moisture to 75% of 0.33 bar. Soil moisture was adjusted periodically throughout the study by the addition of deionized water. The dishes containing the soil were placed in an incubation chamber (Figure 3) and incubated in the dark at 25$\pm$1^{0} C for 61 days. Humidified, carbon dioxide-free air was drawn through the chamber at 10-20 mL/min and vented through ethylene glycol (two tubes) and NaOH (2-3 tubes) trapping solutions. Duplicate soil samples were removed for analysis at 0, 1, 3, 7, 14, 30, and 61 days posttreatment. The trapping solutions were replaced at each sampling interval, and at 20, 37, 48, and 56 days posttreatment.

The soil was extracted three times by sonicating with acetonitrile; the slurries were centrifuged, and extracts were combined. Triplicate aliquots of each combined extract were counted by LSC. Additional aliquots (100 mL) of the extracts were concentrated by rotary evaporation, and the residues were redissolved in acetonitrile. Aliquots of the concentrated extracts were analyzed by two-dimensional TLC on silica gel plates developed in methylene chloride:ethyl acetate (25:75, v:v) in the first direction, and methylene chloride:tetrahydrofuran (75:25, v:v) in the second direction. An unlabeled reference standard of ancymidol was cochromatographed with the soil extracts, then visualized under UV light. Radioactive areas on the plates were located and quantified by radioscanning. The presence of [^{14}C]ancymidol was confirmed in the extracts from the 14-, 30-, and 61-day sampling intervals by HPLC. HPLC analyses were conducted using a YMC ODS column eluted with a 0.03% aqueous H_3PO_4 and acetonitrile gradient with absorbance detection (wavelength not reported). Extracted soils were air-dried, and subsamples were analyzed by LSC following combustion.

DATA SUMMARY:

[^{14}C]Ancymidol (labeled at the alpha carbon; radiochemical purity >99%), at 0.511 ppm, degraded with a half-life of 14.9 days in sandy loam soil that was incubated in the dark at 25 $\pm$ 1^{0} C and 75% of 0.33 bar moisture for 61 days. [^{14}C]Ancymidol was 97.5-98.7% of the applied radioactivity at day 0, 71.0-71.4% at 7 days posttreatment, 45.8-47.3% at 14 days, 21.8-23.4% at 30 days, and 5.7% at 61 days (Table V). Six unidentified, nonvolatile degradates were detected; each was $\leq$0.01 ppm (Table VI). Carbon dioxide increased to a maximum of 44.89% of the applied by 61 days posttreatment, and unextracted radioactivity increased from 0.7% of the applied at day 0 to 42.9-

43.7% at 61 days posttreatment (Table II). Material balances ranged from 94.0 to 99.4%.

COMMENTS:

1. The study authors stated that the radioactivity in the volatile traps was primarily located in the NaOH traps; however, no supporting data were provided.

2. Subsamples of the extracted soil from the 30- and 61-day posttreatment sampling intervals were further extracted to isolate the soluble and insoluble humin, fulvic acid, alpha and beta humus, and hymatomelanic acid fractions. The soluble humin contained 2.2-2.6% of the applied radioactivity, the insoluble humin contained 6.1-7.0%, the fulvic acid contained 7.9-11.1%, the hymatomelanic acid contained 4.0-4.2%; and the alpha and beta humus contained 8.3-9.9% and 2.5-2.8%, respectively (Table IX).

3. The study authors stated that the soil samples were either extracted immediately following sampling or were stored at -20^{0} C prior to extraction; the length of frozen storage was not reported.

TABLE II. Distribution of Radioactivity and Mass Balance (Individual Values).

Sample	Length of Incubation	Percent of Radioactivity Applied to Sample			
		Extractable	Soil-Bound	Traps	Total
1	0 Days	98.7	0.7	0.00	99.4
2	0 Days	97.9	0.7		98.6
3	1 Day	91.1	7.0	0.03	98.1
4	1 Day	91.1	6.8		97.9
5	3 Days	83.7	12.6	0.64	96.9
6	3 Days	84.6	13.3		98.5
7	7 Days	74.4	21.2	3.52	99.1
8	7 Days	74.8	20.4		98.7
9	14 Days	50.1	35.5	11.09	96.7
10	14 Days	50.8	33.7		95.6
11	30 Days	25.4	44.5	28.36	98.3
12	30 Days	23.8	44.3		96.5
13	61 Days	6.3	43.7	44.89	94.9
14	61 Days	6.2	42.9		94.0

TABLE V. Percent Distribution of Applied Radioactivity in ^{14}C-Ancymidol and Metabolites.

Sample	Length of Incubation	Percent of Radioactivity Applied to Sample[a]					
		^{14}C-Ancymidol	A	B	C	D[b]	E
1	0 Days	98.7	0.0	0.0	0.0	0.0	0.0
2		97.5	0.1	0.2	0.0	0.0	0.0
3	1 Day	89.8	0.2	0.2	0.1	0.5	0.3
4		90.1	0.3	0.0	0.0	0.5	0.4
5	3 Days	81.3	0.2	0.1	0.1	1.2	0.8
6		82.3	0.3	0.1	0.1	1.1	0.7
7	7 Days	71.0	0.4	0.1	0.0	1.6	1.2
8		71.4	0.3	0.1	0.2	1.6	1.1
9	14 Days	45.8	0.4	0.4	0.5	2.1	1.1
10		47.3	0.4	0.2	0.2	1.8	1.1
11	30 Days	23.4	0.2	0.1	0.1	0.8	0.8
12		21.8	0.2	0.4	0.0	0.7	0.7
13	61 Days	5.7	0.1	0.1	0.0	0.2	0.2
14		5.7	0.1	0.0	0.0	0.2	0.2

[a] A, B, C, D, E are the observed metabolites.
[b] Metabolite D was a mixture of two components.

TABLE VI. Concentration of Extractable ^{14}C-Ancymidol and Metabolites.

Length of Incubation	^{14}C-Ancymidol	A[a]	B	C	D[b]	E
0 Days	98.1[c] 0.511[d]	0.0 0.000	0.1 <0.001	0.0 0.000	0.0 0.000	0.0 0.000
1 Day	89.9 0.469	0.2 0.001	0.1 <0.001	0.0 0.000	0.5 0.003	0.3 0.002
3 Days	81.8 0.426	0.2 0.001	0.1 <0.001	0.1 <0.001	1.1 0.006	0.8 0.004
7 Days	71.2 0.371	0.4 0.002	0.1 <0.001	0.1 <0.001	1.6 0.008	1.2 0.006
14 Days	46.5 0.242	0.4 0.002	0.3 0.001	0.3 0.001	1.9 0.010	1.1 0.006
30 Days	22.6 0.118	0.2 0.001	0.3 0.001	0.0 0.000	0.7 0.003	0.8 0.004
61 Days	5.7 0.030	0.1 <0.001	0.0 0.000	0.0 0.000	0.2 0.001	0.2 0.001

[a] A, B, C, D, E are the observed metabolites.
[b] Metabolite D was a mixture of two components.
[c] Mean values of values in Table V expressed as percent of radioactivity applied to the sample.
[d] Mean values expressed as ppm.

TABLE IX. Fractionation of Soil-Bound Residues: Distribution of Radioactivity in Components of Soil Organic Matter.

Sample	Length of Incubation	Component (Percent of Radioactivity Applied to Sample)						Total[a]
		Soluble Humin	Insoluble Humin	Fulvic Acid	β-Humus	Hymatomelanic Acid	α-Humus	
11	30 Days	2.2	6.1	11.1	2.5	4.0	8.3	34.1
13	61 Days	2.6	7.0	7.9	2.8	4.2	9.9	34.4

[a] Before organic matter fractionation, the extracted soil samples contained 44.5% (Day 30) and 43.7% (Day 61) of ^{14}C labeled material bound to the soil, thus the recoveries were 76.6% and 78.7%, respectively.

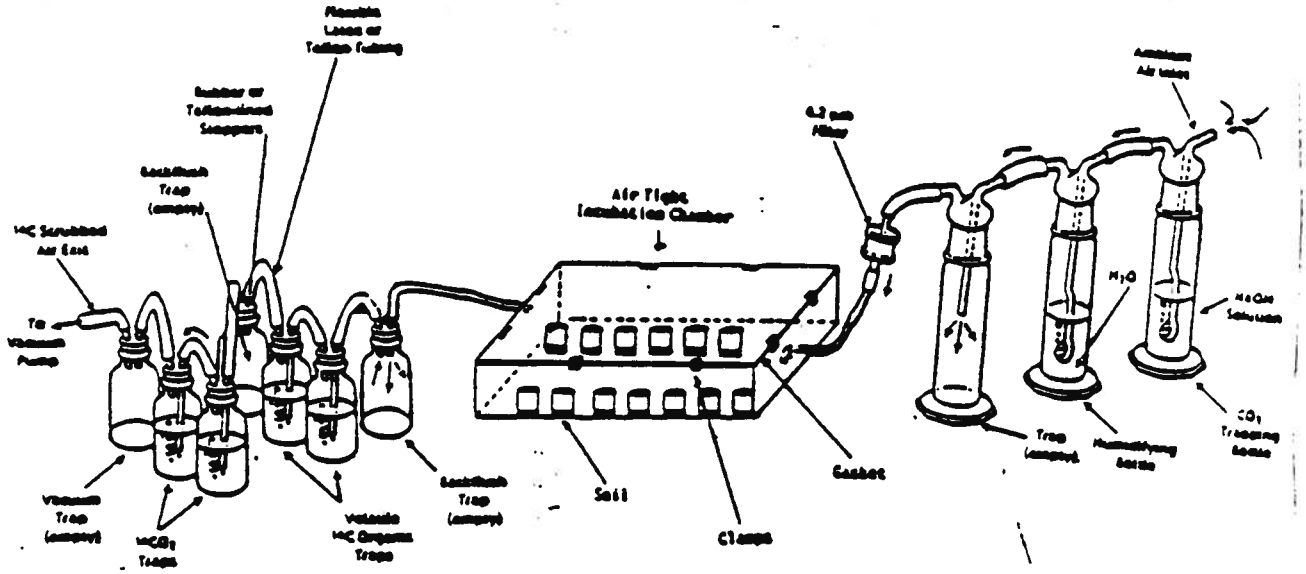

FIGURE 3. Schematic of Incubation Chamber.

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

RESULTS

Distribution of Radioactivity and Mass Balance.

The percent of applied radioactivity recovered in the various components of the samples is in Table II (individual values) and Table III (mean values). Plotted data are in Figure 6. The radioactivity in the acetonitrile soil extracts declined from 98.3% 0 Days, to 6.3% 61 Days, Table III. The soil-bound (non-extractable) radioactivity and radioactivity in the traps for volatile components increased with length of incubation. The soil-bound radioactivity increased from 0.7%, 0 Days to a maximum of 44.4%, 30 Days and was 43.3% on 61 Days (Table III). The radioactivity in the volatile traps was primarily located in the NaOH traps indicating the formation of $^{14}CO_2$. A maximum of 44.89% of the applied radioactivity was recovered as $^{14}CO_2$. The second NaOH trap at any interval contained a maximum 0.07% (436 dpm) of the applied radioactivity. Treatment of the first NaOH trap with saturated barium chloride resulted in a decline of soluble radioactivity by 99.0% indicating that trapped $^{14}CO_2$ was precipitated as $BaCO_3$. The high level of $^{14}CO_2$ indicated a rapid metabolism of ^{14}C ancymidol by soil bacteria. The high level of bound residues indicate incorporation of the radioactivity into microbial biomass and/or humus components. Further characterization of the soil-bound radioactivity is addressed in the section "Fractionation of Soil-Bound Residues". The total mass balance of radioactivity applied to the soils ranged from 99.0% (0 Days) to 94.4% (61 Days) (Table III).

Thin Layer Chromatography of Soil Extracts.

The acetonitrile soil extracts were analyzed by 2D TLC to determine the percent of ^{14}C-ancymidol remaining and the metabolite profile. The distribution of radioactivity in ^{14}C-ancymidol and metabolites expressed as a percent of radioactivity detected by the scanner is in Table IV. ^{14}C-Ancymidol and six other unknown metabolites were detected by TLC. Metabolite D was actually a mixture of two components that were not completely resolved as observed from the TLC of samples 5 (Figure 10), 6 and 7. Since the separation of the two components was observed to be inconsistent they were

quantified as a single component and are represented by a single spot in Figures 10, 11, and 12. Data for the percent of applied radioactivity in ^{14}C-ancymidol and metabolites is in Table V. Mean values for each sampling interval, expressed as percent of applied radioactivity and as ppm are reported in Table VI. Plotted data are in Figures 7 and 8. A representative TLC scan for the 0 Days Sample 1 is in Figure 9; 14 Days, Sample 9 in Figure 11, and for 61 Days Sample 14 is in Figure 12. The percent of ^{14}C-ancymidol declined from 98.1% (0 Days, Table VI) to 22.6% over a 30 day period and declined further to 5.7% or 0.03 ppm (61 Days). All metabolites that were observed did not individually exceed 2% of the applied radioactivity or 0.01 ppm at any sampling interval. Metabolites A, D, and E appeared to peak in the 7 Days - 14 Days period and were subsequently metabolized to levels of 0.2% or less of the applied radioactivity by 61 Days. The level of metabolite B was a maximum at 30 Days and was not detected at 61 Days. Metabolite C was observed at 0.1% at a maximum. Since all of the metabolites were present at levels less than 0.01 ppm, they were not identified further.

High Performance Liquid Scintillation Analysis of Selected Soil Extracts.

Selected acetonitrile soil extracts were analyzed by a second method using HPLC and fraction collection to confirm the presence of ^{14}C-ancymidol. Data are summarized in Table VII for column recoveries and ^{14}C-ancymidol. The radioactivity in the fractions for Sample 9 (14 Days) is in Figure 11. ^{14}C-Ancymidol was observed in fractions 21 to 29 and contained 91.3% of the injected radioactivity. The five peaks corresponding to the metabolites appeared to peak at fractions 5, 10, 15, 17, and 19. The most polar metabolite (fractions 3-6) contained 2.4% of the injected radioactivity. The other metabolite that was prominently observed, fractions 13-16, contained 3.0% of the injected radioactivity. The total column recovery was 100.4%.

Half-Life of ^{14}C-Ancymidol.

Data used to calculate the half-life of ^{14}C-ancymidol when incubated under aerobic conditions in soil are in Table VIII and the least squares linear regression plot is in Figure 14. The $t_{½}$ for ^{14}C-anycimidol was 14.9

days (y-intercept = 4.55; correlation coefficient = 0.998; k or slope of the regression line =
-0.0465).

Fractionation of Soil-Bound Residues.

Data for the distribution of non-extractable, soil-bound radioactivity in the various fractions of soil organic matter are in Table IX. Fractionation of soil organic matter was conducted according to the scheme in Figure 5. The samples 11 and 13 had 44.5% and 43.7%, respectively of the applied radioactivity that was not extracted with acetonitrile. 34.1% and 34.4% of the applied radioactivity was recovered in the various fractions for samples 11 and 13, respectively or 76.6% and 78.7% of the available radioactivity. These recoveries were influenced by the fact that the coloidal materials encountered may be difficult to transfer quantitatively due to adsorption to glass, small sample volumes and low levels of radioactivity. For sample 11, the low molecular weight, acid-soluble fraction, containing fulvic acid, contained the greatest percentage of the applied radioactivity, 11.1%. The α-humus and insoluble humin fraction contained 8.7% and 6.1%, respectively. For sample 13, the α-humus fraction contained 10.8% of the applied radioactivity. The fulvic acid and insoluble humin fraction contained 7.9% and 7.0% applied radioactivity, respectively. For both samples the radioactivity in the remaining fractions, soluble humin, β-humus and hymatomelanic acid, ranged from approximately 2-4% of the applied radioactivity. Since the soil organic matter is a continuum and the cut-off between these various fractions is ill-defined, the exact significance of these results is of less importance. It is interesting to note that the highest amounts of radioactivity were distributed in fulvic acid and insoluble humin, both of which lie at either end of the soil organic matter spectrum. Since extensive mineralization of ^{14}C-ancymidol to $^{14}CO_2$ occurred via bacterial metabolism, some of the radiolabel was presumably incorporated into biomass and subsequently into soil organic matter.

CONCLUSION

^{14}C-Ancymidol was metabolized in soil under aerobic conditions with a half-life of 14.9 days. The major product observed, $^{14}CO_2$, accounted for 44.9% of the applied radioactivity. Soil-bound residues accounted for 43.4% of the applied radioactivity at the end of 61 days of incubation. Six minor unknown products were observed by TLC analysis. None of the metabolites individually exceeded 2.0% of the applied radioactivity or the 0.01 ppm parent equivalent. ^{14}C-Ancymidol and metabolites were also confirmed by HPLC analysis. The results of this study indicated that ^{14}C-ancymidol was mineralized in soil with incorporation of radiocarbon into microbial biomass and/or soil organic matter components.