DATA EVALUATION REPORT

AC 303,630 (PIRATE): Anaerobic Soil Metabolism (162-2)
MRID 434928-47

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EFGWB/EFED/OPP/OPPTS/USEPA

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9/12/96

CONCLUSIONS. *This study is acceptable and satisfies the data requirement. No additional information on the anaerobic metabolism of AC-303630 in soil is required at this time. However, the registrant should regard the comment section of this report before submitting future studies.*

This study provided information on the anaerobic metabolism of phenyl ring- and pyrrole-labeled [^{14}C]AC-303,630 [4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-pyrrole-3-carbonitrile]. This compound degraded only slightly ($\leq$10% degradation) in treated sandy loam soil that was incubated for 8 weeks under anaerobic (nitrogen atmosphere plus flooding) conditions following 30 days of incubation under aerobic conditions; aerobic incubation was conducted at 75% of field moisture capacity at 0.33 bar, and both anaerobic and aerobic incubations were conducted in the dark at 25°C. The registrant roughly estimates a first-order half-life to be about two years. The only degradate identified in the soil was CL-312,094 [2-(p-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-pyrrole-3-carbonitrile].

METHODOLOGY

Sieved (2 mm), air-dried sandy loam soil (55% sand, 29% silt, 16% clay, 0.6% organic carbon, pH 5.4, CEC 5.1 meq/100 g) was weighed (100 g dry weight) into 16-ounce wide mouth jars and treated at approximately 1 lb ai/A (equivalent to 1 ppm based on a 3-inch soil depth) with either phenyl ring-labeled [U-^{14}C]AC-303630 or pyrrole-labeled [2-^{14}C]AC-303630 [4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-pyrrole-3-carbonitrile; radiochemical purities $\geq$97%; specific activities 53.39 and 56.53 uCi/mg, respectively; Moravek Biochemicals], dissolved in water:acetone (6:4, v:v). The treated soil was mixed by manually shaking. The final moisture content of the treated soil was 75% of field capacity at 0.33 bar. The sample jars were "covered" and connected in series per radiolabel; the samples were placed in a dark environmental chamber. The treated soils were maintained in the dark at 25 $\pm$ 1 C for 30 days; during incubation, humidified air was drawn (rate unspecified) through the sample jars and sequentially through ethylene glycol and 0.1 N NaOH trapping solutions. At weekly intervals during aerobic incubation, the soils were remoistened to 75% of field capacity. At 30 days posttreatment, the samples were flooded with aliquots (250 mL) of deoxygenated purified water, then returned to the environmental

chamber and maintained in the dark at 25 $\pm$ 1 C for an additional 60 days; during incubation, nitrogen was drawn through the sample jars and sequentially through the trapping solutions. Duplicate jars of soil per label treatment were collected for analysis at 0 and 30 days posttreatment during aerobic incubation, and at 2, 4, 6, and 8 weeks after the initiation of anaerobic conditions. Aliquots of the ethylene glycol and NaOH trapping solutions were collected for analysis at weekly intervals, and the trapping solutions were replaced at 30 days posttreatment and after 4 weeks of anaerobic incubation.

The pH and redox potential of the samples were measured at each sampling interval during anaerobic incubation. The anaerobic samples collected were then centrifuged, and the water layer was removed; aliquots were analyzed by LSC. The floodwater was partitioned one or two times with ethyl acetate, and the ethyl acetate extracts were removed. The water samples were then acidified to pH 2.3 and again partitioned with ethyl acetate. Aliquots of the extracts and extracted water were analyzed by LSC. Aliquots of all of the extracts from both replicates of the samples collected after 8 weeks of anaerobic incubation were combined per radiolabel and concentrated under a stream of nitrogen. Aliquots were analyzed by LSC, normal-phase TLC on silica gel plates developed in hexane:ethyl acetate (8:2, v:v), normal-phase TLC on silica gel plates developed in hexane:ethyl acetate:acetic acid (100:50:0.75, v:v:v), reverse-phase TLC on C-18 plates developed in acetonitrile:water:acetic acid (160:40:1, v:v:v), and normal-phase TLC on silica gel plates developed in toluene:hexane (2:1, v:v). [^{14}C]Compounds on the plates were located using autoradiography, and were identified by comparison to unlabeled reference standards of AC-303630 and CL-312094 that had been cochromatographed with the samples and visualized using UV light. The TLC plates were scored into zones, and the gel within the zones was scraped from the glass; the radioactivity was eluted from the gel with water, and the water extracts were analyzed using LSC.

The soil samples collected during aerobic incubation were extracted once with water by shaking on a reciprocal shaker for 1 hour; after the extraction, the extracts were removed, and aliquots were analyzed by LSC. These extracted samples as well as the soil samples collected during anaerobic incubation were extracted four times with methanol by shaking as described; aliquots of the extracts were analyzed by LSC, and the remaining extracts were combined. The 30-day aerobic samples and all of the anaerobic samples were further extracted with methanol:HCl (98:2, v:v) by shaking on a reciprocal shaker for 2 hours; the extracts were combined and analyzed by LSC. The samples collected after 8 weeks of anaerobic incubation were then extracted sequentially with 0.24 N NaOH in methanol:water (84:16, v:v), aqueous 0.1 N NaOH, and 1 N NaOH; aliquots of the extracts were analyzed by LSC. The extracted soil samples were air-dried, and subsamples were analyzed by LSC following combustion.

The methanol extracts were concentrated to dryness by rotary evaporation and under a stream of nitrogen; the residues were dissolved in acetone. Aliquots were analyzed by LSC, normal-phase TLC on silica gel plates developed in hexane:ethyl acetate (8:2, v:v), normal-phase TLC on silica gel plates developed in hexane:ethyl acetate:acetic acid (100:50:0.75, v:v:v), and reverse-phase TLC on C-18 plates developed in acetonitrile:water:acetic acid (160:40:1, v:v:v). Extracts of one of the 8-week samples from each label treatment were also analyzed by normal-phase TLC on silica

gel plates developed in toluene:hexane (2:1, v:v).

The methanol:HCl extracts of the samples collected after 8 weeks of anaerobic incubation were diluted with water, then partitioned with ethyl acetate; the ethyl acetate extracts were concentrated by rotary evaporation, and the water layer was concentrated under a stream of nitrogen. Aliquots of the concentrated extracts and water layers were analyzed by LSC; additional aliquots of the extracts were analyzed by TLC as previously described.

Aliquots of the ethylene glycol and NaOH trapping solutions were analyzed for total radioactivity using LSC.

DATA SUMMARY

[^{14}C]AC-303630 [4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-pyrrole-3-carbonitrile] degraded only slightly ($\leq$10% degradation) in sandy loam soil that was treated at approximately 1 lb ai/A (equivalent to 1 ppm based on a 3-inch soil depth) with either phenyl ring-labeled [U-^{14}C]AC-303630 or pyrrole-labeled [2-^{14}C]AC-303630 (radiochemical purities $\geq$97%), then incubated for 8 weeks under anaerobic (nitrogen atmosphere plus flooding) conditions following 30 days of incubation under aerobic conditions. Aerobic incubation was conducted at 75% of field moisture capacity at 0.33 bar, and both anaerobic and aerobic incubations were conducted in the dark at 25 C $\pm$ 1 C. The only degradate identified in the soil was

CL-312,094 [2-(p-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-pyrrole-3-carbonitrile].

In the methanol extracts of samples treated with phenyl ring-labeled [U-^{14}C]AC-303630, total [^{14}C]residues decreased from 98.1-99.2% of the radioactivity in samples at time 0 (% of time 0) immediately posttreatment to 92.1-93.4% after 8 weeks of anaerobic incubation (Table IV). AC-303630 averaged 95.8-96.7% of time 0 (97.5-97.6% of the radioactivity recovered during TLC analysis) immediately posttreatment, 91.8-92.5% at 30 days posttreatment (immediately prior to initiation of anaerobic conditions), 88.9-89.0% after 4 weeks of anaerobic incubation (58 days posttreatment), and 86.0-87.4% after 8 weeks of anaerobic incubation (86 days posttreatment; Table VI). CL-312094 averaged 1.1-1.4% of time 0 immediately posttreatment (reviewer-calculated; 1.1-1.4% of the recovered), 2.3-2.8% at 30 days, 3.7-3.8% after 4 weeks of anaerobic incubation, and 4.9-5.0% after 8 weeks of anaerobic incubation (Tables IV and B through M). In the methanol:HCl extracts, total [^{14}C]residues increased from 0.9-1.7% of time 0 at 30 days posttreatment to 2.5% after 8 weeks of anaerobic incubations (Table IV); in the extracts of samples collected after 8 weeks of anaerobic incubation, AC-303630 was 1.9% of time 0 (reviewer-calculated; 77.7-77.9% of the recovered), and CL-312094 was 0.1% (5.6-5.7% of the recovered; Tables IV and N). In the water extracts of the aerobic samples or the floodwater of the anaerobic samples, total [^{14}C]residues were 0.4-0.6% of time 0 during aerobic incubation and 1.1-1.2% during the anaerobic phase (Table IV); after 8 weeks of anaerobic incubation, AC-303630 was 45.1-45.3% of the recovered radioactivity in the water layer, and CL-312094 was 20.2% (first two TLC systems in Table O). After 8 weeks of anaerobic incubation, NaOH-extractable soil residues totaled 1.0% of time 0, and $^{14}CO_2$ totaled 0.1% (Table IV). Throughout the study, unextracted soil

residues ranged from 0.3 to 1.6%, and material balances ranged from 97.3 to 100.4% of time 0.

In the methanol extracts of samples treated with pyrrole-labeled [2-^{14}C]AC-303630, total [^{14}C]residues decreased from 98.2-98.5% of the radioactivity in samples at time 0 (% of time 0) immediately posttreatment to 91.9-93.1% after 8 weeks of anaerobic incubation (Table V). AC-303630 averaged 94.7-94.9% of time 0 (96.3-96.5% of the radioactivity recovered during TLC analysis) immediately posttreatment, 90.3-90.9% at 30 days posttreatment (immediately prior to initiation of anaerobic conditions), 85.2-85.5% after 4 weeks of anaerobic incubation (58 days posttreatment), and 84.3-86.1% after 8 weeks of anaerobic incubation (86 days posttreatment; Table VI). CL-312094 averaged 1.9-2.1% of time 0 immediately posttreatment (reviewer-calculated; 1.95-2.1% of the recovered), 3.1-3.6% at 30 days, 4.8-5.1% after 4 weeks of anaerobic incubation, and 5.6-6.2% after 8 weeks of anaerobic incubation (Tables V and B through M). In the methanol:HCl extracts, total [^{14}C]residues increased from 1.8% of time 0 at 30 days posttreatment to 2.6-2.7% after 8 weeks of anaerobic incubation (Table V); in the extracts of samples collected after 8 weeks of anaerobic incubation, AC-303630 was 2.0% of time 0 (reviewer-calculated; 75.1-75.2% of the recovered), and CL-312094 was 0.2% (7.4-7.8% of the recovered; Tables V and N). In the water extracts of the aerobic samples or the floodwater of the anaerobic samples, total [^{14}C]residues were 0.5-0.7% of time 0 and 1.1-1.4% during the anaerobic phase (Table V); after 8 weeks of anaerobic incubation, AC-303630 was 41.6-42.3% of the recovered radioactivity in the water layer, and CL-312094 was 21.4-21.6% (first two TLC systems in Table O). After 8 weeks of anaerobic incubation, NaOH-extractable soil residues totaled 1.0% of time 0, and $^{14}CO_2$ totaled 0.1% (Table V). Throughout the study, unextracted soil residues ranged from 0.5 to 2.3%, and material balances ranged from 96.0 to 100.4% of time 0.

COMMENTS

1. Dynamac Corporation of Rockville, MD, provided the technical bulk of this report.

2. According to the study author, samples were analyzed by four different TLC systems. However, data from only two of these methods were generally provided within the study. For the methanol extracts, results reported for AC-303630 as "% of time 0" in Table VI were calculated from the average of results from the two TLC systems using hexane:ethyl acetate and acetonitrile:water:acetic acid as the solvent systems. Results from the TLC systems using hexane:ethyl acetate:acetic acid and toluene:hexane were not reported in the raw data for these extracts.

3. The Dynamac reviewer employed the same technique as described in Comment #1 for calculation of results as "% of time 0" for the degradate CL-312094 in the methanol extracts, and for calculation of results for AC-303630 and CL-312094 in the methanol:HCl extracts. Because replicates A and B were combined for TLC analysis of the water layers, the Dynamac reviewer did not calculate these results in terms of "% of time 0".

4. Unidentified [^{14}C]residues isolated in individual TLC regions during analysis of the various

extracts and water layers were $\leq$0.7% of time 0 ($\leq$0.007 ppm).

5. The redox potentials of the samples collected during the anaerobic phase of the study ranged from -107 to -458 mV.

TABLES, FIGURES, AND OTHER PERTINENT INFORMATION

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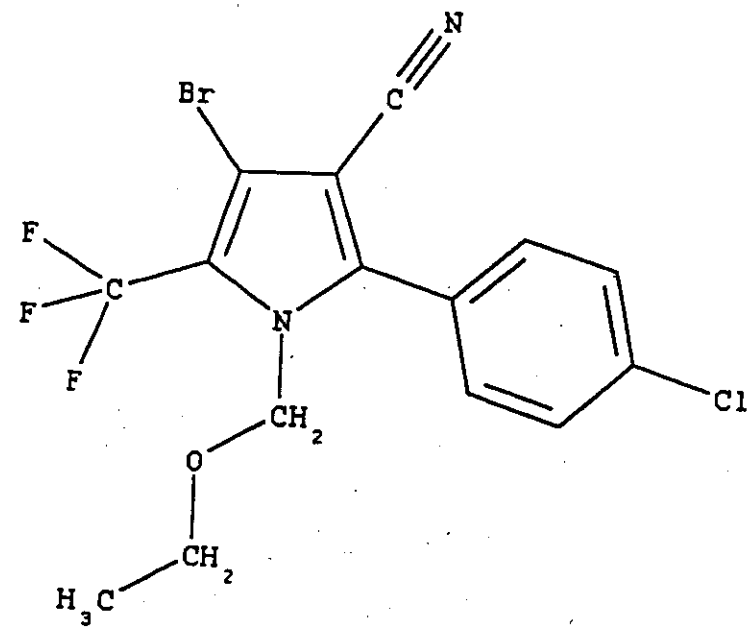
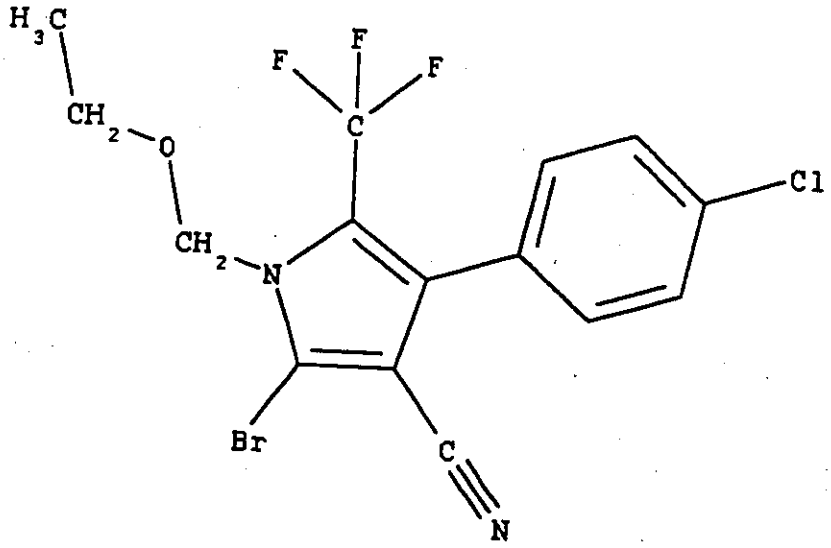
APPENDIX

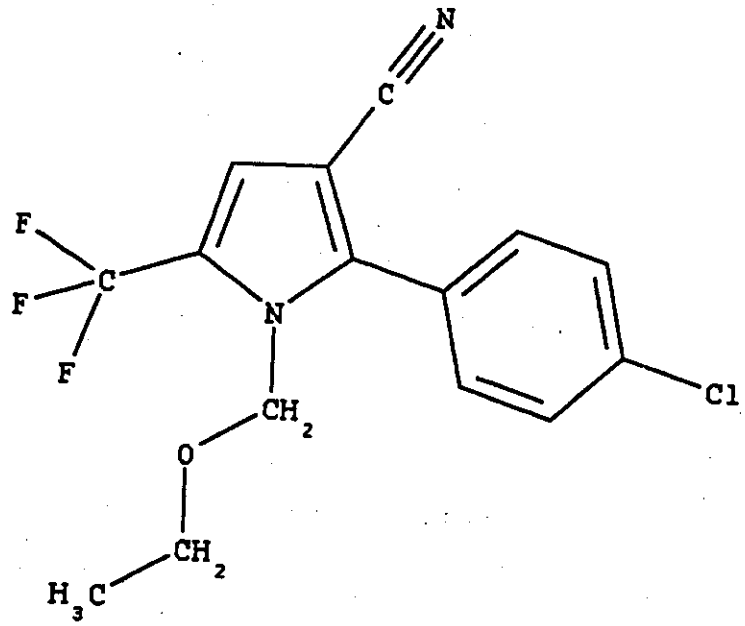
AC-303630 AND ITS DEGRADATES



4-Bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-pyrrole-3-carbonitrile
(AC-303630)



2-Bromo-4-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-pyrrole-4-carbonitrile
(CL-357806)



2-(p-Chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-pyrrole-3-carbonitrile
(CL-312094)