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DATA EVALUATION RECORD

CHEM 108801 Metolachlor §162-1, -2

FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 41309801-A
Spare, W. 1989a. Identification of unknown metabolites of metolachlor in aerobic soil metabolism. Report No. ABR-89093. Unpublished study performed and submitted by Ciba-Geigy Corporation, Greensboro, NC.

STUDY ID 41309801-B
Spare, W.C. 1989b. Soil metabolism of metolachlor under aerobic, aerobic/anaerobic, and sterile conditions. Agrisearch Project No. 1258. Unpublished study performed by Agrisearch Incorporated, Frederick, MD, and submitted by Ciba-Geigy Corporation, Greensboro, NC.

DIRECT REVIEW TIME = 40

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CONCLUSIONS:

Metabolism - Aerobic Soil

1. This portion of the study is considered satisfactory and can be used to fulfill data requirements for an aerobic soil metabolism study.

2. In this study metolachlor was reported to degrade with a half-life of 67 days in sandy loam soil that was incubated in the dark at approximately 25^0 C and 75% of 0.33 bar moisture capacity. The major degradate was CGA-51202; other identified degradates were CGA-

50720, CGA-41638, and CGA-13656. Other possibly present degradates were CGA-40172, CGA-41507, CGA-40919, and CGA-37913.

4. The aerobic soil metabolism data requirement has been previously fulfilled with a satisfactory aerobic and anaerobic aquatic soil metabolism study (MRID No. 411857-01). In this referenced study the half-life of metolachlor under aerobic conditions was 47 days.

Metabolism - Anaerobic Soil

1. This portion of the study is satisfactory and can be used to fulfill data requirements for an anaerobic soil metabolism study.

2. Metolachlor degraded with a reported half-life of 81 days in a sandy loam soil that was incubated under anaerobic conditions (flooding plus nitrogen atmosphere) for 60 days at approximately 25° C following 30 days of aerobic incubation. The major degradate in both the soil and flood water was CGA-51202; other identified degradates were CGA-37735, CGA-41638, CGA-13656, and CGA-50720.

3. The anaerobic soil metabolism data requirement has been previously fulfilled with an anaerobic aquatic metabolism study (MRID No. 4118-57-01). In the referenced study the half-life of metolachlor under anaerobic conditions was 78 days.

METHODOLOGY:

Metabolism - Aerobic Soil

Two portions (320 g each for a total of 640 g dry weight) of sieved (2 mm) sandy loam soil (53.2% sand, 37.6% silt, 9.2% clay, pH 5.9, 5.0% organic matter, CEC 13.6 meq/100 g) were treated at 10.6 ppm with phenyl ring-labeled [^{14}C]metolachlor (average radiochemical purity 98.6%, specific activity 26.2 uCi/mg, Ciba-Geigy) dissolved in acetone. Each treated portion of soil was roller-milled for 1 hour, after which the portions were combined. Subsamples of the treated soil (25 g dry weight) were weighed into 20 Erlenmeyer flasks which were then wrapped in foil and stoppered with polyurethane foam plugs. The treated soil was incubated in the dark at 25 ± 1° C; soil moisture levels were maintained at 75% of 0.33 bar moisture. Two additional flasks of soil (designated as the 12-month samples) were sealed with Teflon-coated rubber stoppers and connected to the metabolism apparatus shown in Figure 2. To trap volatiles, humidified, CO_2-free compressed air was flushed through the flasks "at 40-60 mL/minute during working hours"; the exiting air was passed sequentially through tubes of ethylene glycol, 1 N sulfuric acid, and 1 N sodium hydroxide (two tubes). Soil samples for the time 0 interval were collected from the bulk treated soil; additional soil samples were collected in duplicate at 1, 3, 7, and 14 days, and at 1, 3, 6, 9, and 12 months posttreatment. The trapping solutions from the metabolism apparatus were replaced at each sampling interval.

In addition to the treated nonsterile soil, four samples (25 g) of sandy loam soil were autoclaved (1 hour at 121^0 C), then treated with [^{14}C]metolachlor at approximately 10 ppm. Two of the flasks were connected to the metabolism apparatus, the remainder were stoppered as previously described. The flasks were covered in foil and incubated aerobically at 25 $\pm$ 1^0 C and 75% of 0.33 bar moisture. The stoppered flasks of soil were sampled at 30 days posttreatment; the soil samples and trapping solutions in the metabolism apparatus were collected at 6 months.

Samples from time 0 through 90 days posttreatment (10-25 g dry weight) were extracted twice by sonication with methanol:acetone (1:1, v:v) for 30 minutes; the soil was removed by vacuum filtration. The extracted soil from time 0 through 90 days posttreatment and samples from 6 to 12 months posttreatment were refluxed with methanol:water (9:1, v:v) for 1 hour, and the solution removed by vacuum filtration. Aliquots of the extracts were analyzed for total radioactivity by LSC. Additional aliquots of the extracts were analyzed by one-dimensional TLC on silica gel plates developed in either methylene chloride:ethyl acetate (9:1, v:v, Solvent System 1) or chloroform:methanol:formic acid:water (75:20:4:2, v:v:v:v; Solvent System 2). The extracts were cochromatographed with unlabeled parent compound; additional unlabeled standards were chromatographed separately on the same TLC plate. Following development, the plates were air-dried and visualized under UV light (254 nm); radioactive areas on the plates were located using a TLC linear analyzer. The soil was analyzed for unextractable [^{14}C]residues by LSC following combustion. Duplicate aliquots of the trapping solutions from the 12-month nonsterile and 6-month sterile soil samples were analyzed for total radioactivity using LSC.

The methanol-extracted soil from samples taken at 3 days through 12 months posttreatment was further extracted by refluxing with methanol:water (50:50, v:v; duration not specified). The soil was then extracted twice by sonication with oxalic acid (concentration not specified) for 30 minutes, followed by refluxing for 2 hours; the oxalic acid solutions were combined. The methanol:water reflux extraction solution was processed as previously described. The oxalic acid solution was partitioned three times with ethyl acetate:chloroform (50:50, v:v) and the organic phases were combined; total radioactivity in each phase was quantified by LSC. The organic phase was concentrated under nitrogen and analyzed by one-dimensional TLC as previously described. The soil was analyzed for unextractable [^{14}C]residues by LSC following combustion.

"To confirm parent recoveries and to characterize remaining radioactive regions isolated from one-dimensional TLC analysis," aliquots of the methanolic extracts from the 6-month samples were analyzed by two-dimensional TLC on silica gel plates developed in Solvent System 1 in the first direction and chloroform:acetonitrile (8:2, v:v) in the second direction. Unlabeled standards were chromatographed in a separate column on the TLC plate (Figure 8). Following development,

the plates were air-dried and visualized under UV light (254 nm). Radioactive areas on the plates were located using autoradiography, removed from the plates by scraping, and analyzed for total radioactivity by LSC.

Additional aliquots of the extracts were evaporated to near dryness and methylated with diazomethane; unlabeled metolachlor and nine potential degradates were methylated also. All samples were analyzed for metolachlor and its degradates using GC with nitrogen phosphorus detection; the methylated standards were cochromatographed with the samples. The method (GC) detection limit was 0.4 ng.

Degradate identification

In order to determine the identities of unknown degradates, further characterization of the radioactivity in methanolic soil extracts from a 9-month soil sample was performed at Ciba-Geigy.

An aliquot of a 9-month soil extract was dried, the residue was dissolved in methanol, and diazomethane was added. After 3 hours, the solution was evaporated to dryness and the residue redissolved in methanol. The methylated soil extract material was analyzed by one-dimensional TLC on methanol-washed silica gel plates developed in Solvent System 2. Radioactive zones (Figure 4) were detected using a "Berta Spark Chamber"; zones were scraped and the silica gel extracted by stirring with methanol for approximately 30 minutes. Aliquots of the extracts were analyzed by two-dimensional TLC on silica gel plates developed in Solvent System 1 in the first direction and chloroform:methanol:ammonium hydroxide:water (80:30:4:2, v:v:v:v) in the second direction. The extracts were cochromatographed with unlabeled reference standards that were visualized with UV light; radioactive zones were detected with a Berta Spark Chamber. Additional aliquots of the extracts were analyzed using HPLC with UV light (254 nm) and radioactivity detection; the mobile phase was either a linear gradient from 5 to 100% acetonitrile, a linear gradient from 10 to 100% acetonitrile, or 17% acetonitrile (all eluting solutions were in 0.05 M ammonium acetate buffer). Aliquots of the fractions collected from the HPLC analyses were analyzed by two-dimensional TLC as previously described. The sequence of 2-D TLC/elution/HPLC/2-D TLC was repeated until unique peaks were obtained. The HPLC isolates for individual peaks were concentrated by rotary evaporation, and analyzed individually using GC/MS or EI/DIP/MS. 2-D TLC plates of isolated compounds were compared to 2-D plates from the original soil extract to confirm identification.

Metabolism - Anaerobic Soil

Following 30 days of aerobic incubation, 50 mL of distilled water was added to each of four flasks from the aerobic incubation study; the flasks were sealed with Teflon-coated rubber stoppers and flushed (approximately 60 mL/minute) for 60 minutes with compressed nitrogen gas. Following 2 weeks of incubation in the dark at 25 $\pm$ 1^0 C, the

soil was amended with 0.25 g of dextrose (1% of dry soil weight); the flasks were then flushed (60 mL/minute) with nitrogen for 30 minutes and returned to the incubator. After 29 days of anaerobic incubation, the flasks designated for 60-day sampling were again flushed with nitrogen. Samples were collected at 29 and 60 days of anaerobic incubation (59 and 90 days posttreatment).

The aqueous layer from each flask was decanted, centrifuged, and the supernatant removed; the remaining pellet was returned to the sediment in the flask. Aliquots of the aqueous layer were analyzed for total radioactivity by LSC; additional aliquots were analyzed using one-dimensional TLC as described for the aerobic metabolism study. The soil sediment was extracted and analyzed as previously described for the 3 to 90 days posttreatment samples in the aerobic soil metabolism portion of the study. Additional aliquots of extracts and water were methylated and analyzed by GC as described previously.

DATA SUMMARY:

Metabolism - Aerobic Soil

Phenyl ring-labeled [^{14}C]metolachlor (average radiochemical purity 98.6%), at 10.6 ppm, degraded with a registrant-calculated half-life of 67 days in aerobic nonsterile sandy loam soil that was incubated for 366 days at 25 $\pm$ 1^{0} C in the dark (Table XVII). [^{14}C]Metolachlor was an average of 87.94% of the applied immediately posttreatment, 64.48% at 14 days, 36.98% at 30 days, 11.57% at 90 days, and 3.35% at 366 days. The major degradate

CGA-51202

reached a maximum of 28.09% of the applied at 90 days posttreatment, then decreased to 17.44% at 366 days (Table XIX). Other identified degradates were:

CGA-50720, at a maximum of 14.85% of the applied at 272 days posttreatment (Table XIV);

CGA-41638, at a maximum of 2.06% at 90 days (Table XIX);

CGA-37735, at a maximum of 1.27% at 30 days (Table XIX); and

CGA-13656, at a maximum of 1.02% immediately posttreatment (Table IX).

Other degradates that were detected but not quantifiable were CGA-40172, CGA-41507, CGA-40919, and CGA-37913; unidentified extractable radioactivity was present at up to 3.91% of the applied at 181 days posttreatment (Table XIV). At 366 days posttreatment, unextractable [^{14}C]residues were 67.38% of the applied, and cumulative volatiles totaled 4.84% (Table IV). Additional two-dimensional TLC and GC/MS

analyses indicated the presence of the aldehyde and the keto-aldehyde of CGA-50720 (Table XX). Material balances ranged from 88.59 to 104.51% of the applied radioactivity (Table IV).

In autoclaved sandy loam soil, phenyl ring-labeled [^{14}C]metolachlor, at approximately 10 ppm, decreased from an average of 77.75% of the applied at 30 days posttreatment to 68.63% at 181 days (Tables IX and XI). After 181 days of incubation, the degradates CGA-13656 and CGA-50720 were 1.11 and 0.32% of the applied, respectively, unextractable [^{14}C]residues averaged 9.90%, and cumulative volatiles averaged 4.90% (Tables V, IX-XII). Material balances ranged from 85.18 to 95.56% of the applied (Table V).

Metabolism - Anaerobic Soil

Phenyl ring-labeled [^{14}C]metolachlor (radiochemical purity 97.3%), at 10.6 ppm, degraded with a registrant-calculated half-life of 81 days in sandy loam soil that was incubated anaerobically (flooding plus nitrogen atmosphere) at 25 $\pm$ 1^{0} C for 60 days following 30 days of aerobic incubation (Table XVIII). [^{14}C]Metolachlor decreased from an average 36.98% of the applied at the start of anaerobic incubation to 23.42% at 29 days (59 days posttreatment) and 22.07% at 60 days following the establishment of anaerobic conditions (90 days post-treatment). The major degradate in the soil and flood water was

CGA-51202

at a maximum of 23.33% of the applied at 29 days after anaerobic conditions were established (Table XIX). Other degradates isolated from the soil and water were:

CGA-41638, at a maximum of 8.30% of the applied at 60 days (Table XIX);

CGA-50720, at a maximum of 7.34% at 60 days (Table X);

CGA-13656, at a maximum of 1.46% at 29 days (Table IX); and,

CGA-37735, at a maximum of 1.25% at 29 days (Table XIX).

Unidentified compounds were present at a maximum of 1.61% of the applied at 29 days after anaerobic conditions were established (Table XV). Unextractable [^{14}C]residues decreased from 49.60% of the applied at the start of the anaerobic incubation period to 37.94% at 60 days after anaerobic conditions were established (Table IV). Material balances ranged from 106.01 to 94.80% of the applied (Table V).

COMMENTS:

General

1. It is preferable that [^{14}C]residues in samples be separated by chromatographic methods (such as TLC, HPLC, or GC) with at least three solvent systems of different polarity, 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. In both the aerobic and anaerobic soil metabolism portions of this study, the sample extracts were analyzed using one-dimensional TLC with only two solvent systems. Radioactive areas on the TLC plates were identified by comparison with the location of known reference standards chromatographed on the same plates. Additional two dimensional TLC and mass spectral analyses on extracts confirmed the identity of several TLC zones. GC was also used to provide confirmation of metabolites.

2. Not all degradates present at >0.01 ppm (approximately 0.1% of the applied) were identified. In the aerobic portion of the study, unidentified extractable radioactivity was present at up to 3.91% of the applied at 181 days posttreatment (Table XIV); in the anaerobic portion of the study, unidentified compounds were present at a maximum of 1.61% of the applied at 29 days after anaerobic conditions were established (Table XV). In addition, up to 17 metabolites were present in extracts from aerobic soil (Figure A); the identities of only four were confirmed by MS.

3. Recovery efficiencies from fortified samples were not provided. Method detection limits were provided for GC analysis but were not provided for the other analytical methods used (LSC, TLC, HPLC, and MS).

4. The study author mentioned that "a supplemental re-incubation of metolachlor was performed to generate additional fresh material for mass spectral analysis." He also stated that the sample was "a 6 month subsample from Agrisearch Project Number 12159 'Soil Metabolism of Metolachlor'"; this report was not provided for review. In addition, in ABR-89093, the author refers to Project No. 12159, indicating that some of the samples used for the identification of degradates originated from that study. The only sample used from the study under review was "a 9-month methanol extract".

6. The actual application rate may not have been confirmed. A known amount of radiolabel was added to a known amount of soil (104 uL at 30.8 mg/mL added to 320 g (dry weight) of soil), which would result in a concentration of 10.01 ppm; however, the stated concentration was 10.6 ppm. In the Protocol (p. 59 of report), it is stated that "soils were oxidized after extraction to determine percent of bound residues"; it was never explicitly stated that unextracted soil was

analyzed for total radioactivity by LSC following combustion, and no data were provided to indicate such.

Metabolism - Aerobic Soil

The concentrations reported for the degradate CGA-37735 in Table XIX of the original document are incorrect. Apparently, the study author simply used the data obtained from radioactive zones 5 or 6 for Solvent System 2 since CGA-37735 was detected in either of those zones, depending upon the extraction scheme used (Tables X, XII, XIV, and XVI). However, since radioactive zones 5 or 6 were composed of two degradates, CGA-37735 and CGA-37913, the reported values reflect the total percentage of applied radioactivity determined for these degradates, rather than for CGA-37735 alone.

Metabolism - Anaerobic Soil

The test water was not completely characterized; the pH was not reported.

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

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