

## DATA EVALUATION RECORD

## STUDY 1

CHEM 041402

## Molinate

§162-3, §162-4

CAS No. 2212-67-1

## FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 44956603

Thomas, V. M., J. E. Dennison, and D. G. Takahashi. 1981. Behavior of Ordram in the environment, Report No. 2, anaerobic and aerobic aquatic metabolism. Report No. PMS-112/MRC-B-112. Unpublished study performed by Stauffer Chemical Co., Mountain View Research Center, Mountain View, CA; and submitted by Zeneca Ag Products, Wilmington, DE.

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1. As a result of the company responses for MRID 41421801, the additional anaerobic aquatic metabolism study (MRID 44956603), the simulated rice field study (MRID 44956602), and consistent results in the aquatic field dissipation studies below, EFED now considers the anaerobic aquatic metabolism guideline (162-3) to be satisfied for molinate and metabolites. When applied to standing water in the field, molinate rapidly volatilizes and reversibly sorbs to sediment with some degradation. The difference in half-lives for this guideline (6.7-130 days) may be attributed to the fact that the longest half-life (130 days) was in the laboratory study and the other half-lives (6.7-11 days) were associated with studies conducted in the greenhouse and in open air, allowing more volatility.

2. The aerobic aquatic soil metabolism data requirement is satisfied with the combination of MRID's 41421802 and 44956603. In the 1999 RED, EFED concluded that a half-life for molinate could not be determined because less than 50% of the applied material had degraded by the time the laboratory study was terminated (MRID 41421802). As a result, the long-term behavior of molinate in natural aerobic aquatic systems could not be predicted with certainty from the results of this study. However, the registrant submitted an aerobic aquatic metabolism study conducted outdoors (MRID 44956603) that showed consistent results with field dissipation studies and laboratory greenhouse studies conducted to simulate field studies.
3. This study does not provide acceptable information for the 162-3 and 162-4 data requirements by itself, but when taken with the weight of evidence, provides supplemental information. The primary reasons include:
  - Poor material balance and no volatility trapping (Comment 1)
  - Study conducted in the presence of light and at excessive temperatures (Comment 2)
  - No redox, pH, or dissolved oxygen measurements (Comment 3)
  - No storage stability data (Comment 4)

## ABSTRACT

### Metabolism - Anaerobic Aquatic

Under anaerobic aquatic conditions, radiolabeled [ $^{14}\text{C}$ ]molinate, at a nominal concentration of 3.5 ppm, dissipated with a total system half-life of 11 days ( $r^2 = 0.89$ ,  $F=16.2$ ,  $P=5.7 \times 10^{-2}$ ) in flooded Biggs clay loam sediment that was incubated outdoors at  $37 \pm 3^\circ\text{C}$  (99 oF) for up to 56 days (MRID 44956603). All reported data are the means of duplicates. The minor degradates, 2-keto Ordram, 2-OH Ordram, 3-OH Ordram, 4-OH Ordram, 4-keto Ordram, N-formyl HMI, Ordram sulfoxide, HMI, Carboxy-Ordram, and Caprolactam were each present at  $\leq 5.6\%$  of the applied radioactivity during the incubation period. Total degradates (polar and non-polar) were 17.9 % of applied at 7 days, 12.5 % 21 days, and 5.6 % at 56 days posttreatment. In the sediment phase, the parent compound was 19.1% of the applied radioactivity at 7 days posttreatment, the first sampling interval for which separate sediment phase residues were characterized, decreased to 5.5% by 21 days, and was 1.9% at 56 days. In the sediment phase, the minor degradates, 2-keto Ordram, 3-OH Ordram, 4-OH Ordram, 4-keto Ordram, N-formyl HMI, and Ordram sulfoxide each accounted for  $\leq 1.0\%$  of the applied radioactivity throughout the incubation period. Nonextractable [ $^{14}\text{C}$ ]residues accounted for 7.9% of the applied radioactivity at 7 days posttreatment, were a maximum of 34.6% at 21 days, and were 28.3% at 56 days. The distribution ratio (reviewer-calculated) of [ $^{14}\text{C}$ ]residues between the sediment and water phases was 1.05:1 at 7 days posttreatment, 5.2:1 at 21 days posttreatment, and 8.4:1 at 56 days posttreatment. Volatile [ $^{14}\text{C}$ ]residues were not determined.

## Aerobic Aquatic Metabolism

Under aerobic aquatic conditions, radiolabeled [ $^{14}\text{C}$ ]molinate, at a nominal concentration of 3.5 ppm, dissipated in water with a reviewer-calculated half-life of 5.6 days ( $r^2 = 0.99$ ,  $F=410$ ,  $P=3.1 \times 10^{-2}$ ) in flooded Biggs clay loam sediment that was incubated **outdoors** at  $35 \pm 2^\circ\text{C}$  ( $95^\circ\text{F}$ ) for up to 56 days (MRID 44956603). All reported data are the means of duplicates. All data represent water phase samples (no sediment phase was prepared for aerobic aquatic samples). The minor degradates, 2-keto Ordram, 2-OH Ordram, 3-OH Ordram, 4-OH Ordram, 4-keto Ordram, N-formyl HMI, Ordram sulfoxide, HMI, Carboxy-Ordram, and Caprolactam were each  $\leq 3.8\%$  of the applied radioactivity during the incubation period. Total degradates (polar and non-polar) were 19.7 % of applied at 7 days, 15.8 % 21 days, and 11 % at 56 days posttreatment. Volatile [ $^{14}\text{C}$ ]residues were not determined.

## MATERIALS AND METHODS

Soil samples (600 g) of Biggs clay loam (collected near a rice paddy in California; 36% sand, 30% silt, 34% clay, 2.2% organic matter, pH 6.0, CEC 25.3 meq/100 g; Table 1, p. 13), were weighed into six glass jars and flooded with creek water (pH 8.3; 1600 mL) collected from Santa Clara County, California (p. 12); the final sediment:water ratio was 1:2.6 (w:v; reviewer-calculated; p. 18). The flasks were maintained outdoors for 30 days; the volume of creek water was maintained daily. Following pre-incubation, the aqueous phase was removed and discarded. Creek water (1600 mL) treated with [ $^{14}\text{C}$ ]molinate {Ordram; S-ethyl hexahydro-1H-azepine-1-carbothioate; specific activity 21.25 mCi/mM; radiochemical purity 98%; Stauffer Chemical Co., Richmond, CA; p. 12}, dissolved in acetone, at a nominal concentration of 3.5 ppm, was added to the pre-incubated sediment (anaerobic study) or six glass tanks without sediment (aerobic study). The test systems were maintained outdoors for up to 56 days; light intensity and air and water temperature were measured daily. Sterile control samples were not prepared. Sediment/water systems and water systems were removed for analysis at 2 hours and 1, 3, 7, 10, 14, 17, 21, 24, 28, 31, 35, 42, 49, and 56 days posttreatment (Table IV, p. 21).

At each sampling interval, triplicate aliquots of the water phase were analyzed for total radioactivity by LSC (p. 18); the limit of quantitation was not reported. Selected samples (days 7, 21, 56; both studies) of the water phase were extracted with methylene chloride. Sediment samples (anaerobic study only) were extracted with chloroform:methanol (3:1, v:v) by Soxhlet extraction; the organic phase extract (from sediment and water samples) was filtered through sodium sulfate and concentrated by a Kuderna-Danish apparatus, while the aqueous phase extract was evaporated to dryness by lyophilization and reconstituted with methanol. For both studies, replicate organic phase extracts from the water phase were combined, concentrated, and analyzed by one-dimensional TLC using silica gel plates developed with 2,2,4-trimethylpentane:p-dioxane (2:1; Table III, p. 16) with radioimage analysis or x-ray film (p. 37). Samples were co-chromatographed with

nonradiolabeled reference standards of the parent and potential degradates (Appendix B, pp. 56-57), which were visualized with UV (wavelength not specified) or iodine vapor detection (pp. 15, 24). Radiolabeled residues were scraped from TLC plates and analyzed by LSC (p. 15). Aqueous phase extracts from the water phase (both studies) were analyzed by two-dimensional TLC using silica gel plates developed with butanol:acetic acid:water (4:1:1; v:v:v; Table III, p. 16; p. 44). [ $^{14}\text{C}$ ]Compounds with similar  $R_f$  values were combined (method not specified) and reanalyzed by two-dimensional TLC as previously described, except plates were developed with propanol:ammonia:water (8:1:1, v:v:v). [ $^{14}\text{C}$ ]Compounds were reanalyzed by thin-layer electrophoresis on silica gel plates saturated with 0.05 M  $\text{NH}_4\text{HCO}_3$  electrolyte and maintained at 400 V (pp. 16, 44). The organic phase extracts from sediment samples (anaerobic study only) were analyzed by two-dimensional TLC (solvent system not specified; p. 28). Samples were co-chromatographed with nonradiolabeled reference standards of the parent and potential degradates 2-keto Ordram, 2-OH Ordram, 3-OH Ordram, 4-OH Ordram, 4-keto Ordram, N-formyl HMI, and Ordram sulfoxide (Table VIII, p. 31).

To confirm identities of the parent compound and potential degradates in the organic phase extracts from the water phase (both studies), selected samples (days 1, 21, 56) containing degradates at >1% of the applied radioactivity were analyzed by GC (Gas Chrom Q column, 1.8 m; 100-290°C, ramped at 12°C/min) with flame ionization detection, radioactivity monitoring, and mass spectral detection (pp. 17, 24). Organo-soluble extracts from the water phase were also analyzed by two-dimensional TLC using silica gel plates developed with toluene:ether (2:3, v:v) followed by 2,2,4-trimethylpentane:p-dioxane (2:1, v:v; Table III, p. 16; Figures 2-4; pp. 26, 27, 29) with radioimage analysis or x-ray film. Samples were co-chromatographed with nonradiolabeled reference standards of the parent and potential degradates (not specified), which were visualized by UV (wavelength not specified) or iodine vapor detection. To confirm the identities of the parent compound and potential degradates in the aqueous phase extracts from the water phase (both studies), selected samples (days 1, 21, 56) were co-chromatographed with nonradiolabeled reference standards of the parent and potential degradates by GC with radioactive monitoring and/or two-dimensional TLC using silica gel plates developed with butanol:acetic acid:water (4:1:1; v:v:v) followed by propanol:ammonia:water (8:1:1, v:v:v; Table III, p. 16; Figures 5-7, pp. 33-35).

Post-extracted sediment samples were analyzed by LSC following combustion (p. 20); combustion efficiency was not reported.

To confirm the presence of anaerobic conditions, the redox potential of the sediment was measured using a platinum electrode (anaerobic study only; p. 17).

## RESULTS/DISCUSSION

### Anaerobic Aquatic Metabolism

Radiolabeled [ $^{14}\text{C}$ ]molinate (radiochemical purity 98.0%), at a nominal concentration of 3.5 ppm, dissipated with a reviewer-calculated half-life of 11 days ( $r^2 = 0.89$ ,  $F=16.2$ ,  $P=5.7 \times 10^{-2}$ ) in flooded Biggs clay loam sediment that was incubated outdoors at  $37 \pm 3^\circ\text{C}$  ( $99^\circ\text{F}$ ) for up to 56 days (p. 20; Tables IV, VII, pp. 21, 30). All reported data are the means of duplicates. In the total sediment/water system, the parent compound was initially present at 79.3% of the applied radioactivity at 2 hours posttreatment, decreased to 31.5% by 7 days, was 6.0% at 21 days, and was 1.9% at 56 days (Tables IV, VII, VIII, pp. 21, 30, 31). In the water phase, the parent compound was initially present at 12.4% of the applied radioactivity at 7 days posttreatment, the first sampling interval for which separate water phase residues were characterized, decreased to 0.5% by 21 days, and was not detected at 56 days (Table VII, p. 30). The minor degradates,

2-keto Ordram [S-ethyl-1-azacycloheptan-2-one-1-carbothioate],

2-OH Ordram [S-ethyl-1-azacycloheptan-2-ol-1-carbothioate],

3-OH Ordram [S-ethyl-1-azacycloheptan-3-ol-1-carbothioate],

4-OH Ordram [S-ethyl-1-azacycloheptan-4-ol-1-carbothioate],

4-keto Ordram [S-ethyl-1-azacycloheptan-4-one-1-carbothioate],

N-formyl HMI [N-formyl hexamethyleneimine]

Ordram sulfoxide [1-[(ethylsulfinyl)carbonyl]hexahydro-1H-azepine],

HMI [hexamethyleneimine],

Carboxy-Ordram [S-carboxymethylhexahydro-1H-azepine-1-carbothioate], and

Caprolactam [2-oxohexamethyleneimine]

were each present at  $\leq 5.6\%$  of the applied radioactivity during the incubation period (Tables VII, IX, pp. 30, 36). Uncharacterized [ $^{14}\text{C}$ ]residues (designated "organo-soluble unknowns I, IV, V, VI," "polar unknowns I, II, IV, VIII, XIV, XV" and "other unknowns") accounted for  $\leq 1.3\%$  of the applied radioactivity during the incubation period.

In the sediment phase, the parent compound was present at 19.1% of the applied

radioactivity at 7 days posttreatment, the first sampling interval for which separate sediment phase residues were characterized, decreased to 5.5% by 21 days, and was 1.9% at 56 days (Table VIII, p. 31). The minor degradates, 2-keto Ordram, 3-OH Ordram, 4-OH Ordram, 4-keto Ordram, N-formyl HMI, Ordram sulfoxide each accounted for  $\leq 1.0\%$  of the applied radioactivity throughout the incubation period (Table VIII, p. 31). Nonextractable [ $^{14}\text{C}$ ]residues accounted for 7.9% of the applied radioactivity at 7 days posttreatment, were a maximum of 34.6% at 21 days, and were 28.3% at 56 days (Table V, p. 23). The distribution ratio (reviewer-calculated from data in Table V, p. 23) of [ $^{14}\text{C}$ ]residues between the sediment and water phases was 1.05:1 at 7 days posttreatment, 5.2:1 at 21 days posttreatment, and 8.4:1 at 56 days posttreatment. Volatile [ $^{14}\text{C}$ ]residues were not determined.

Material balances (based on LSC analysis) were 79.3% at 2 hours posttreatment, decreased to 57.3% of the applied by 7 days posttreatment, were 53.1% at 21 days, and were 35.8% at 56 days (Tables IV, V, pp. 21, 23); a pattern of loss of [ $^{14}\text{C}$ ]residues from the water phase was observed over time (Figure 1, p. 22).

### Aerobic Aquatic Metabolism

Radiolabeled [ $^{14}\text{C}$ ]molinate (radiochemical purity 98.0%), at a nominal concentration of 3.5 ppm, dissipated with a reviewer-calculated half-life of 5.6 days ( $r^2 = 0.99$ ,  $F=410$ ,  $P=3.1 \times 10^{-2}$ ) in flooded Biggs clay loam sediment that was incubated outdoors at  $35 \pm 2^\circ\text{C}$  ( $95^\circ\text{F}$ ) for up to 56 days (p. 20; Tables IV, VII, pp. 21, 30). All reported data are the means of duplicates. All data represent water phase samples (no sediment phase was prepared for aerobic aquatic samples). The parent compound was present at 98.2% of the applied radioactivity at 2 hours posttreatment, decreased to 36.7% by 7 days, was 7.1% of the applied at 21 days posttreatment, and was not detected at 56 days (Table IV, VII, pp. 21, 30). The minor degradates, 2-keto Ordram, 2-OH Ordram, 3-OH Ordram, 4-OH Ordram, 4-keto Ordram, N-formyl HMI, Ordram sulfoxide, HMI, Carboxy-Ordram, and Caprolactam were each present at  $\leq 3.8\%$  of the applied radioactivity during the incubation period (Tables VII, IX, pp. 30, 36). Uncharacterized [ $^{14}\text{C}$ ]residues (designated "organo-soluble unknowns I, II, III, IV, V, VI," "polar unknowns I, III, IV, VI-XIV, XVI" and "other unknowns") accounted for  $\leq 3.4\%$  of the applied radioactivity during the incubation period. Volatile [ $^{14}\text{C}$ ]residues were not determined.

Material balances (based on LSC analysis) were 98.2% at 2 hours posttreatment, decreased to 56.3% of the applied by 7 days posttreatment, was 22.9% at 21 days, and was 11.0% at 56 days (Tables IV, V, pp. 21, 23); a pattern of loss of was observed over time (Figure 1, p. 22).

### Comments (Deficiencies/Deviations)

1. The material balances (both studies) were incomplete (Tables IV, V, pp. 21, 23). Subdivision N Guidelines require that the material balance be 90-110% of the applied radioactivity. The maximum material balance reported in the study was 57 % of applied (Table 5, p. 23). This could have been a result of the fact that volatiles were not trapped. Volatility is a major route of dissipation of molinate, and any molinate studies must quantify this route of dissipation.
2. Metabolism studies are required to be conducted in darkness because light can lead to degradation of compounds. Both of these studies were conducted in light, and therefore the identity and proportion of degradates cannot be attributed to either metabolism or photodegradation. Also, the incubation temperature was not maintained between 18-30°C and at  $\pm 1^\circ\text{C}$  as required by Subdivision N Guidelines. The incubation temperature was  $36.6 \pm 2.7^\circ\text{C}$  (anaerobic study) and  $34.7 \pm 2.2^\circ\text{C}$  (aerobic study; Appendix C, p. 58); the maximum water phase temperature was  $40.0^\circ\text{C}$  (anaerobic study) and  $43.0^\circ\text{C}$  (aerobic study). Conducting a study in excessive temperatures can create artifacts in the data and lead to erroneous conclusions about the route(s) of dissipation. In this case, elevated water and air temperatures may have increased the loss of the parent compound and degradates due to volatilization.
3. The reviewer could not determine whether anaerobic conditions were achieved and maintained in the anaerobic study; similarly, it could not be determined whether aerobic conditions existed in the aerobic study. In the sediment (anaerobic study only), redox potential was -0.1 mV at 56 days posttreatment (p. 20; tabular data not provided); however, dissolved oxygen and pH were not measured for either study. Measurements at each sampling point for pH, Eh, and dissolved oxygen must be presented to determine if anaerobic or aerobic conditions are present in a study.
4. Nonextractable residues accounted for 34.6% and 28.3% of the applied radioactivity at 21 and 56 days posttreatment, respectively (Table V, p. 23). Subdivision N Guidelines require that [ $^{14}\text{C}$ ]residues present at greater than 10% of the applied should be identified.
5. Storage stability data were not reported. The reviewer could not confirm whether samples were analyzed immediately following collection. Storage stability data should be collected if samples are stored.
6. Method detection limits were not reported for LSC and TLC analyses. It is necessary that both limits of detection and quantitation be reported to allow the reviewer to evaluate the adequacy of the method for the determination of the test compound and its degradates.
7. The water solubility of the test compound was not reported.

8. The study authors presented data as percentages of the nominal application; concentration data were not provided. In future studies submitted to the EPA, it is necessary that data be reported as both percentages of the applied and in units of concentration (e.g., ppm).
9. Data reporting was insufficient. In both studies, separate data for the parent compound and degradates were not reported at each sampling interval; data were reported at only 7, 21, and 56 days posttreatment (Tables VII, VIII, pp. 30, 31).



ATTACHMENT 1  
Tables cited in DER

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