

UNDATED

DATA EVALUATION RECORD

DER 3

SHAUGHNESSY No. 041402
COMMON NAME: Molinate
CHEMICAL NAME: S-Ethyl hexahydro-1H-azepine-1-carbothioate
FORMULATION: Not formulated, 99.5% pure unlabeled active ingredient, ring-2-¹⁴C-labeled (radiochemical purity 97.67%).
DATA REQUIREMENT: Anaerobic Aquatic Metabolism (162-3)

MRID No: 41421801

Tarr, J. B. 1990. Anaerobic aquatic metabolism of [ring-2-¹⁴C]molinate. Report No. PMS-268/WRC-90-075. Unpublished study performed and submitted by ICI Americas, Inc., Mountain View, CA.

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CONCLUSIONS:

1. This study is scientifically invalid and cannot be used to fulfill the anaerobic aquatic metabolism data requirement for molinate.
2. These data are considered to be of uncertain value and should not be used to predict the environmental behavior of molinate and its degradates.
3. This study is unacceptable because it is not certain that conditions were anaerobic during the study (¹⁴CO₂ was the major degradate, formed at >40% of the applied).
4. Since it is not certain that conditions were anaerobic during the study, the problems with this study cannot be resolved with the submission of additional data. A new study is required.

METHODOLOGY:

Portions (250 g) of air-dried, sieved (2 mm) Stockton adobe clay soil (20.2% sand, 27.8% silt, 52.0% clay, 1.60% organic matter, pH 6.0, CEC 30.2 meq/100 g) were weighed into side-arm biometer flasks; an additional three flasks containing sterilized (autoclaved) soil and filter-sterilized water were prepared. The side-arms contained 1 N potassium hydroxide trapping solutions; a polyurethane plug was fitted between the flask and the side-arm to trap organic volatiles (Figure 1). Two flasks containing only KOH solution and foam plugs were maintained as blanks for CO₂ and volatiles. The soil flasks were flooded (300 mL) and connected to a humidified nitrogen source maintained at a positive pressure of 1 psi; the flasks were vented through the traps. The flooded flasks were incubated in the dark at 30°C for 31 days. After the 31 day incubation period, 20 soil flasks plus the three sterile controls were treated with 1 mL of a mixture of unlabeled molinate (purity 99.5%, ICI) and ring-2-labeled [¹⁴C]molinate (average radiochemical purity 97.67%, specific activity 27.3 uCi/mmol, Wizard Laboratories) dissolved in acetone at a nominal rate of 5.1 µg/g soil. All flasks were reconnected to the nitrogen source and incubated in the dark at 30°C. Duplicate flasks were removed for analysis at 0, 3, 9, 17, 23, 56, 95, 196, 273, and 365 days posttreatment; the flasks containing sterilized soil were removed at 95 and 196 days posttreatment. At each sampling interval, foam plugs and potassium hydroxide trapping solutions were replaced. The flasks were either processed to separate the phases immediately or stored at -20°C (length of storage not specified).

The contents of the flask were thoroughly shaken and then the soil and floodwater were separated by centrifugation. Aliquots of the supernatant floodwaters were analyzed by LSC. The soil pellets were stored at -20°C "for later analysis" (length of storage not specified). Floodwater samples were acidified with hydrochloric acid and partitioned five times with methylene chloride. The methylene chloride extracts were dried over sodium sulfate and concentrated to a small volume by rotary evaporation under vacuum at 8-10°C. The concentrated extracts were diluted with acetone and analyzed by one- or two-dimensional TLC on silica gel plates using the solvent systems listed in Table II. Radioactivity was quantified by LSC of radioactive zones or by beta scanning. There were no losses of volatile radioactivity during LSC (p.18 of study). Extracts were cochromatographed with unlabeled standards which were visualized by uv light (254 nm) or exposure to iodine vapor. The aqueous fractions remaining after extraction from single flasks from the 23, 95, 196, and 365 day sampling intervals were concentrated by rotary evaporation at 25-40°C; acetonitrile was added to form a low boiling temperature azeotrope. The concentrated aqueous fractions were then diluted with water and methanol and aliquots were analyzed by TLC as previously described.

Soil samples were extracted using one of two methods. In Scheme I (Figure 3), the soil was mixed with water (50 mL:250 g). The moist soil was extracted three times with acetone:methanol (22:3, v:v) by shaking for 30 minutes. The slurries were centrifuged, the supernatants were combined and concentrated to "an aqueous residue" under vacuum at 8-10°C. The concentrate was partitioned three times with methylene chloride; the methylene chloride extracts were combined, dried over sodium sulfate and concentrated by rotary evaporation under vacuum at 8-10°C. Aliquots of the concentrated extract were analyzed by TLC as previously described.

In the second method (Scheme II, Figure 6), the soil sample was extracted three times with methylene chloride:methanol:1 N HCl (9:20:9, v:v:v) by shaking for 60 minutes at room

temperature. The extracts separated into a clear upper phase and a dark brown lower phase. Aliquots of the lower phase were analyzed by LSC and TLC as described above. The clear upper phase was partitioned with methylene chloride, the methylene chloride phase was concentrated by rotary evaporation under vacuum at 8-10°C, and aliquots were analyzed by LSC and TLC as previously described. The aqueous phase was not further analyzed since most of the radioactivity was recovered in the methylene chloride fraction. Unextracted radioactivity in the soil was quantified by LSC following combustion of the soil.

Aliquots of the potassium hydroxide trapping solutions were analyzed by LSC. The CO₂ was precipitated from the trapping solutions with barium chloride; the supernatant was titrated with hydrochloric acid and compared with the trapping solutions from the empty flasks to calculate the amount of CO₂ evolved from biological activity. The foam plugs were extracted three times with acetone:methanol (22:3, v:v); aliquots of the acetone extracts were analyzed by LSC and by one- or two-dimensional TLC as previously described.

DATA SUMMARY:

Ring-2-¹⁴C -labeled molinate (radiochemical purity 98%), at 5.1 µg/g soil, degraded with a registrant-calculated half-life of 129 days in flooded clay loam soil that was incubated in the dark at 30°C for 365 days under a nitrogen atmosphere. However, it is not certain that conditions were anaerobic during the study, since the major degradate was ¹⁴CO₂ (40.9-45.4% of the applied at 365 days posttreatment; Table V and Figure 8). In addition, volatilization appeared to be a major route of dissipation; molinate and methyl molinate trapped in the foam plugs increased to 21.7-25.7% of the applied after 365 days (Table V and Figure 10). Molinate in the soil:water systems was 83.1-85.3% of the applied radioactivity immediately posttreatment, 55.7-57.9% at 23 days, 40.8-42.1% at 56 days, and 10.4-10.9% at 365 days (Tables X-XII). No non-volatile degradate was present at > 2.6% of the applied in either the floodwater or the soil. The degradates isolated from both the floodwater and the soil were:

4-hydroxymolinate, at maximum concentrations of 1.5% of the applied radioactivity in the floodwater and 2.6% in the soil (both at 95 days posttreatment);

molinate acid, at maximum concentrations of 2.2% in the floodwater (at 95 days) and 0.6% in the soil (at 23 days);

4-oxomolinate, at maximum concentrations of 1.0% in the floodwater (at 95 days) and 1.2% in the soil (at 23 days);

3-hydroxymolinate, at maximum concentrations of 0.9% in the floodwater and 0.5% in the soil (both at 9 days);

N-formyl-hexamethyleneimine (N-formyl-HMI), at maximum concentrations of 0.5% in the floodwater and 0.3% in the soil (both at 273 days);

molinate alcohol, at maximum concentrations of 0.1% in the floodwater (at 23 days) and 0.3% in the soil (at 9 days);

methyl molinate, at maximum concentrations of 0.2% in the floodwater (at 273 days) and 0.9% in the soil (at 196 days);

2-oxomolinate, at maximum concentrations of 0.1% in the floodwater (at 23 days) and 0.1% in the soil (at 3 days); and,

3-oxomolinate, at maximum concentrations of 0.4% in the floodwater and 0.3% in the soil (both at 9 days; Tables XI and XII).

Uncharacterized [^{14}C]residues (up to 17) in the floodwater each comprised up to 0.026 ppm (Results). Unextracted residues ranged from 2.0 to 14.3% of the applied during the study, and material balances ranged from 85.3-97.4% (Table V).

COMMENTS:

1. The study may not have been conducted under anaerobic conditions; large amounts of CO_2 were produced during the flooded incubation period. By the end of the study, nearly half of the applied radioactivity had been evolved as $^{14}\text{CO}_2$. Normally, under anaerobic conditions, the carbon-containing volatile degradate would be methane. In addition, no redox, pH, or dissolved oxygen measurements were taken to confirm that anaerobic conditions were present.
2. It is unclear which extraction scheme was used for which soil samples. In the text, the study author states that "the second method was adopted because it was more efficient at extracting [^{14}C]molinate from freshly treated soil samples" (p.22). However, in Table V, footnote (d) indicates that samples that were incubated for 0 and 3 days before analysis were extracted using Scheme I, and all other were extracted using Scheme II. In addition, in Tables VII and VIII, samples numbered 22 (designated a 56-days sample in Table IV) and 25 (designated a 196-day sample in Table IV) are noted as having been extracted using Scheme I.
3. The data from three flasks with total material balances of 58.0, 77.8, and 82.6% were excluded from the half-life calculations. The causes of the poor material balances were identified in footnote (e) of Table V.
4. Additional extractions were performed on: one extracted 9-day soil sample (containing 5.5% of the applied radioactivity); both extracted 196-day soil samples (5.9 and 14.3%); and the aqueous fraction remaining after methylene chloride partitioning of the "upper phase" of a 365-day soil extract (not specified). The extracted 196-day soil sample containing 14.3% of the applied radioactivity was extracted using the scheme in Figure 5; the maximum radioactivity found in any fraction was 5.1%, and TLC analysis indicated that the radioactivity that particular fraction was primarily molinate. In all other cases, the total radioactivity that could be extracted from these samples was $< 5\%$ of the applied and did not isolate any degradates that were not extracted by Schemes I and II. The study author stated that the variability of the unextracted molinate residues was probably due to the difficulties in extracting this soil, which was resistant to dispersal due to the high clay content.
5. In floodwater from flasks incubated 95 and 365 days, up to 17 radiolabeled compounds were detected that did not cochromatograph with any of the reference standards (Figures 17-20). Quantitative data were not provided for review, the study author stated that "the most abundant component" was present at a maximum of 0.026 ppm at

196 days posttreatment and declined to 0.01 ppm at 365 days. He also stated that "certain components were present in all of the samples". These compounds were determined not to be carboxylic acids.

6. The study author stated that molinate comprised all of the radioactivity in foam plugs until day 273, when 3% of the radioactivity recovered in the plug was methyl molinate (97% was molinate). The foam plugs at 196, 273 and 365 days contain 6% of the total radioactivity so the author assumes that the amount of volatile methyl molinate contained in those plugs was "less than 0.2% of the total radioactive material applied...". No data were provided to confirm this assumption.
7. A parallel experiment was conducted using an treatment rate of 255 ug molinate/g soil to three flasks. One flask was sacrificed at 95, 273, and 365 days posttreatment. Only the floodwater was analyzed and one uncharacterized degradate not found at the lower treatment rate was isolated. Since no additional data were presented from this experiment, the experiment was not described in this report.
8. The same degradates were identified in the flasks that were sterilized before incubation. At 196 days posttreatment, CO₂ was 5.1-5.6% of the applied; volatile degradates ranged from 22.5 to 36.0% (Table V). Parent molinate was 9.5% and 26.2% of the applied in the floodwater and soil, respectively; molinate acid was 6.2 and 0.7%; 4-hydroxymolinate was 1.0 and 1.4%; and 4-oxomolinate was 1.1% and 0.7% at 196 days posttreatment. All other degradates in the sterilized flasks were ≤0.1% of the applied (Tables XI and XII).
9. Refluxing the soil with sodium hydroxide yielded more total radioactivity than did combustion. The study author hypothesized that the radioassay following combustion was inefficient due to evaporation of molinate during heating for combustion. Most of radioactivity was accounted for in the extracts and by combustion; the amount of additional radioactivity lost by combustion did not seriously affect the material balance of the study. The study author also stated that in order to prevent loss of molinate and volatile degradates the temperature must kept low and evaporation to dryness avoided while concentrating the organic extracts.
10. The soil had been stored at 0-5°C before use.