DATA EVALUATION RECORD

STUDY 9

CHEM 122990 Mesotrione §162-3
CAS No. 104206-82-8
FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 44505132
Vispetto, A. R. and M. Tovshteyn. 1996. [Cyclohexane-2-^{14}C]ZA1296 anaerobic aquatic soil metabolism. Laboratory Study No.: PMS 405. Report No.: RR 95 048B. Unpublished study performed by ZENECA Inc., Richmond, CA; and submitted by ZENECA Inc., Wilmington, DE.

DIRECT REVIEW TIME = 55 Hours

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CONCLUSIONS

Metabolism - Anaerobic Aquatic

1. This study provides useful information and ***partially satisfies data requirements*** for anaerobic aquatic (flooded soil) metabolism. Without supporting data available from other fate studies, the deficiencies in this study and the associated degree of uncertainty would prohibit its acceptability.

   There were numerous deficiencies in this study, as noted in the Comments section of this report, and similar deficiencies in an anaerobic aquatic soil study conducted using [phenyl-U-^{14}C]labeled mesotrione (MRID 44505131). However, the present study, in conjunction with the aforementioned study and other fate studies, is part of a consistent picture of the environmental behavior of mesotrione. ***Therefore, in combination with results from this and other fate studies, data requirements for anaerobic aquatic (flooded soil) metabolism are satisfied.***

2. The registrant should carefully consider the critical elements in the Comments section (and elsewhere), and, where possible, reply with plausible explanations, calculations or recalculations, or additional data. The many study deficiencies would, in most cases, vitiate future study results. For example, there are questions about the adequacy of extraction (Comments 1 and 2) and identification of degradates (Comment 5); concentration data reported for the aqueous phase do not accurately depict actual aqueous concentrations since they were reported on a soil-weight basis (Comment 3); tables were apparently mislabeled, key duplicate values were not tabulated, and day 30 values were not included in the analysis for parent (Comment 4). There were also irregularities in the measurement and maintenance of anaerobic conditions (Comment 14).

3. Study Results. Cyclohexanedione ring-labeled [2-^{14}C]mesotrione, at an actual application rate of 0.32 ppm (soil-weight basis), dissipated with a reviewer calculated first-order regression half-life of 4.2 days (95% confidence interval from 3 to 6 days; $r^2 = 0.97$; 0-14 day data) in flooded silt loam soil that was incubated in darkness at 25 ± 2 °C for up to 365 days. However, this half-life is likely to be a modest underestimate because of some loss of radioactivity with increasing time and the existence of an unidentified minor metabolite which may be a pH-dependent, equilibrium form of parent compound. Because of the many study deviations, the reviewer made no attempt to apply hypothetical corrections to the data. Regardless, it is clear that parent was relatively short-lived, and that the half-life results obtained are, within error limits, the same as those obtained in an independent study conducted by different study authors (phenyl-radiolabel, MRID cited above) using the same soil but somewhat different procedures (including more efficient extraction procedures).

   Test samples removed from incubation following >30 days posttreatment were not

analyzed and even the collected 30-day data were not used or presented in suitable form for kinetic analysis. Residue data were only reported for the total soil/water system and were reported for both soil and water on a soil-weight basis. Residue data were not reported for the individual soil and water phases. Date reported below are reviewer-calculated and registrant-calculated (parent data only) means of two replicates. Data reported as percentages of the applied radioactivity represent percentages of the nominal application.

In the total water/sediment system, the parent compound was initially detected at 102% (0.33 ppm) of the applied radioactivity, decreased to 74.5% (0.24 ppm) by 3 days and 39.4% (0.13 ppm) by 7 days, and was 9.3% (0.030 ppm) at 14 days posttreatment. No degradates were detected following the initial extractions. Unextracted [^{14}C]residues were initially (day 0) 4.3% of the applied radioactivity, increased to 20.1% by 3 days and 32.5% by 7 days, and were a maximum of 62.1% at 30 days posttreatment. Following the microwave/caustic extraction of the post-extracted soil samples from days 14 and 30, an additional 29.7% (0.095 ppm) and 30.6% (0.099 ppm) of the applied radioactivity, respectively, was removed from the soil; 11.4-12.2% of the applied radioactivity in the caustic extracts was incorrectly reported to be associated with the humic acid and fulvic acid fractions of the soil organic matter. Following the microwave/caustic extractions, bound [^{14}C]residues were 17.7% (0.52 ppm) of the applied radioactivity at 7 days posttreatment, were 14.3% (0.046) at 14 days and were a maximum of 23.4% (0.075 ppm) at 30 days.

An unidentified major degradate (Metabolite A) was present in the neutralized NaOH extract (single replicates) at 2.5% of the applied radioactivity at 3 days posttreatment, increased to 6.0% by 7 days, and was 9.7% at 30 days; samples collected at >30 days were not analyzed.

Evolved $^{14}CO_2$ accounted for 1.9% of the applied radioactivity at 7 days posttreatment, increased to 5.9% by 14 days, and was 9.7% at 30 days; *volatile [^{14}C]organic residues* were negligible.

Residues increased in the soil phase over time; the soil:water distribution ratio (reviewer-calculated) was approximately 1:2 at day 0, increased to 1:1 by 3 days posttreatment, was 5:1 at 14 days posttreatment, and was 11:1 at 30 days posttreatment.

METHODOLOGY

Samples (250 g) of sieved (2 mm) Radford silt loam soil (17.1% sand, 57.7% silt, 25.2% clay, 2.7% organic matter, pH 6.2, CEC 12.0 meq/100 g; Appendix B, p. 50) collected from Walworth County, WI, were placed in biometer flasks equipped with a sidearm volatile trap containing 1.6 *N* NaOH and fitted with a polyurethane foam plug (p. 15;

Figure 1, p. 39). Samples were flooded with purified water (300 mL) and pre-incubated anaerobically (nitrogen atmosphere) in darkness at 25 ± 2°C for up to 30 days (p. 17). The final soil:water ratio was 1:1.2 (w:v; reviewer-calculated). Following the pre-incubation period, the soil/water systems were treated by syringe with cyclohexanedione ring-labeled [2-^{14}C]mesotrione {ZA1296; E1296; 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione; radiochemical purity ≥97.9%, specific activity 36.6 mCi/mmol; pp. 13, 14}, dissolved in 0.01 M Na_2CO_3 solution, at an actual application rate of 0.32 ppm (soil-weight basis; pp. 14, 16; see Comment #15). Additional treated, sterile soil/water samples were prepared to monitor degradation due to abiotic processes (p. 23); *data were not reported.* The soil/water samples were incubated anaerobically (nitrogen atmosphere) in darkness at 25 ± 2°C for up to 365 days. Duplicate soil/water samples were removed for analysis at 0, 1, 3, 7, 14, 30, 60, 90, 120, 180, 270, and 365 days posttreatment (p. 18); samples removed for analysis at >30 days posttreatment were not analyzed. Sterile soil/water samples were removed for analysis at 30, 180, and 365 days posttreatment. Samples were stored at -10°C for up to six months prior to analysis (p. 21).

At each sampling interval, the water phase was separated from the soil and analyzed for total radioactivity by LSC (p. 18). Aliquots of the water phase were analyzed by reverse-phase HPLC (Alltech, Altima C-18 reverse-phase column) using a mobile phase gradient of acetonitrile:acidified water (0.1% H_3PO_4; 10:90 to 50:50 to 100:0; v:v) with radioactive flow and UV (unspecified wavelength) detection. Eluent fractions were collected at one-minute intervals and analyzed by LSC (pp. 19, 20); the detection limit was reported as <0.001 ppm.

Soil samples were transferred to polyethylene bottles, centrifuged, and then extracted three times by shaking with 0.05 *N* NH_4OH (p. 20); the extracts were combined. The samples were further extracted with acetonitrile or acetone. Both extracts were analyzed for total radioactivity by LSC and HPLC as previously described; the detection limit was twice background (p. 54). The combined NH_4OH extract was acidified (pH 1) to reportedly precipitate humic and fulvic acids, neutralized, centrifuged, and then analyzed by HPLC as previously described (p. 21; see Comment #1). The post-extracted soil samples were dried and analyzed by LSC following combustion (p. 19); data were corrected for combustion efficiency (Appendix F, p. 54). Single samples (for each sampling interval) of post-extracted soil were heated with 0.1 *N* NaOH in a Questron microwave oven (Q Max 4000) at 115°C for 10 minutes and 145°C for 15 minutes, then cooled to room temperature and centrifuged. The 0.1 *N* NaOH solution was separated from the soil, acidified (pH 1; HCl), and centrifuged to reportedly precipitate humic and fulvic acids (see Comment #1). The microwaved extracts were neutralized, passed through a C18 column (Prep Sep-C18), and analyzed by LSC and HPLC as previously described.

At each sampling interval, the NaOH traps and the polyurethane foam plugs (quartered)

were analyzed for total radioactivity by LSC (pp. 18, 20).

To determine the presence of anaerobic conditions, the redox potential of the soil/water systems was measured prior to treatment and at 0, 14, and 30 days posttreatment. Conditions were moderately reducing at day 0 with redox potentials (for individual replicates) of 162 ± 32.5 mV to 165 ± 0.0 mV (Appendix D, p. 52). Redox potentials were -214 ± 1.3 mV (pH 6.8) to -45.6 ± 4.7 mV (pH 6.9) from 14 to 30 days posttreatment. The pH of the soil/water systems was 6.7-7.0 during the incubation period.

DATA SUMMARY

Cyclohexanedione ring-labeled [2-^{14}C]mesotrione (radiochemical purity ≥97.9%), at an actual application rate of 0.32 ppm (soil-weight basis; see Comment #15), dissipated with a first-order half-life of 4.1 days (r^2 = 0.98; 0-14 day data) in anaerobic flooded silt loam soil that was incubated in darkness at 25 ± 2°C for up to 365 days (Figure 3, p. 41). However, the reported half-life is of questionable validity due to the analytical method which left relatively high concentrations of unextracted residues by 3 days posttreatment. Test samples removed from incubation following >30 days posttreatment were not analyzed. Residue data were only reported for the total soil/water system and were reported for both soil and water on a soil-weight basis (see Comment #15). Residue data were not reported for the individual soil and water phases. Reported data are reviewer-calculated and registrant-calculated (parent data only) means of two replicates. Data reported as percentages of the applied radioactivity represent percentages of the nominal application.

In the total water/sediment system, the parent compound was initially detected at 102% (0.33 ppm) of the applied radioactivity, decreased to 74.5% (0.24 ppm) by 3 days and 39.4% (0.13 ppm) by 7 days, and was 9.3% (0.030 ppm) of the applied at 14 days posttreatment (Table IV, p. 35).. No degradates were detected following the initial extractions (p. 27). Unextracted [^{14}C]residues were initially (day 0) 4.3% of the applied radioactivity, increased to 20.1% by 3 days and 32.5% by 7 days, and were a maximum of 62.1% of the applied at 30 days posttreatment (Table III-A, p. 32). Following the microwave/caustic extraction of the post-extracted soil samples from days 14 and 30, an additional 29.7% (0.095 ppm) and 30.6% (0.099 ppm) of the applied radioactivity, respectively, was removed from the soil (Table V, p. 36). Following the microwave/caustic extractions, bound [^{14}C]residues were 17.7% (0.52 ppm) of the applied radioactivity at 7 days posttreatment, were 14.3% (0.046) of the applied at 14 days and were a maximum of 23.4% (0.075 ppm) of the applied at 30 days posttreatment; 11.4-12.2% of the applied radioactivity (in the caustic extracts) was incorrectly reported to be associated with the humic acid and fulvic acid fractions of the soil organic matter (see Comment #1). An unidentified major degradate, designated as

Metabolite A,

was present in the neutralized NaOH extract (single replicates) at 2.5% of the applied radioactivity at 3 days posttreatment, increased to 6.0% of the applied by 7 days posttreatment, and was 9.7% of the applied at 30 days posttreatment (Table VII, p. 38); samples collected at >30 days posttreatment were not analyzed. An unidentified minor degradate (Metabolite B) was present in the neutralized NaOH extract at 2.0% of the applied radioactivity at 3 days posttreatment, increased to a maximum of 2.2% of the applied by 7 days posttreatment, and was 0.64% of the applied at 30 days posttreatment.

Evolved $^{14}CO_2$ accounted for 1.9% of the applied radioactivity at 7 days posttreatment, increased to 5.9% of the applied by 14 days, and was 9.7% of the applied at 30 days posttreatment (Table III-A, p. 32); [^{14}C]organic residues were negligible. Residues increased in the soil phase over time; the soil:water distribution ratio (reviewer-calculated) was approximately 1:2 at day 0, increased to 1:1 by 3 days posttreatment, was 5:1 at 14 days posttreatment, and was 11:1 at 30 days posttreatment.

The material balances (based on LSC analysis of individual replicates) generally decreased with time and were 92.5-110% of the applied radioactivity at 0-7 days posttreatment and 86.3-89.9% of the applied at 14-30 days posttreatment (Table III-A, p. 32; see Comment #9).

COMMENTS

1. Unextracted [^{14}C]residues were relatively high by 3 days posttreatment, indicating that the analytical method, specifically extraction, may have not been adequate. Unextracted [^{14}C]residues were 20.1% at 3 days and 32.5% at 7 days, and were a maximum of 62.1% at 30 days posttreatment. A further attempt to remove bound residues (microwave/caustic extraction) resulted in the removal of an additional 29.7% (0.095 ppm; day 14) and 30.6% (0.099 ppm; day 30) of the applied radioactivity, respectively. The study authors incorrectly stated that 11.4-12.2% of the applied radioactivity in the caustic extracts was associated with the humic acid and fulvic acid fractions of the soil organic matter (also see Comment #2). Following the microwave/caustic extractions, bound [^{14}C]residues were still 14.3% (0.046) at 14 days and were a maximum of 23.4% (0.075 ppm) at 30 days. Without the appropriate extraction procedures to ensure quantitative recovery of the compounds of interest, the validity of the reported half-lives is questionable. Also, when the additional residues were later removed using NaOH microwave/caustic extractions, two degradates were detected at respective maximums 9.7% and 2.2% of the applied radioactivity (Table VII, p. 38; also see Comment #5). It is unclear why the degradates removed during the this final analytical step were not removed prior to the attempted removal of bound residues and the organic matter fractionation. The registrant should clarify or explain why this occurred. Generally, soils samples are extracted

sufficiently to remove any extractable residues, and the initial extracts are analyzed for the primary characterization of the parent and its degradates. Then, soil samples are often further extracted, perhaps using harsh methods such as reflux or Soxhlet extraction, in an attempt to remove bound residues; the harsh extracts are not usually characterized due to the compound-altering effects of the extractants on the residues. Organic matter fractionation is generally performed as a separate, last method in order to associate the remaining radioactivity with the specific fractions of soil organic matter to which the radiolabeled residues have become incorporated. Following extraction with a base to remove humic and fulvic acids, and acidification to precipitate out humic acids, the post-extracted soil is combusted to determine the humin fraction.

2. Parts of the reported methodology were of questionable accuracy. The study authors stated that, following the initial extractions and prior to HPLC analysis, the ammonium hydroxide extracts were acidified (pH 1) to precipitate humic and fulvic acids from solution (p. 21). The reviewer notes, however, that, by definition, the fulvic acid fraction of soil organic matter is soluble in both acids and bases and, therefore, does not precipitate out in an acidified solution. Also, it is unclear whether any radiolabeled material did precipitate out of solution during this step of the analysis, and whether such material was later accounted for in the radioactivity present in the soil organic matter fractions. Organic-matter bound radioactivity is generally not removed during the initial extractions. When additional residues were later removed using microwave/caustic extractions, the study authors again incorrectly stated that the humic and fulvic acids were precipitated out of the NaOH extract by acidification, and reported the data in Table VI (p. 37) as "fulvic and humic acids." The reviewer questions the statements made by the study authors concerning the precipitation of the soluble organic matter fractions. Clarification by the registrant is necessary. Additionally, it is noted that radioactivity associated with the humic acid and fulvic acid fractions is generally reported separately, rather than as a combined value.

3. Concentration data (in ppm) reported for the aqueous phase are questionable in terms of their usefulness in determining expected environmental (aqueous) concentrations since they were reported on a soil-weight basis (p. 16), as opposed to a per-volume basis (e.g.: g/mL) which is generally used to report aqueous concentration data. Data reported on a soil-weight basis for the aqueous phase do not accurately depict the concentration of the parent and degradates in solution. Additionally, the data are not directly convertible (using a 1:1 conversion factor) to ppm data since the soil:water ratio of the test system was not 1:1.

4. Residue data for the parent compound were not reported for the separate soil and water phases, but were reported for the total soil/water system (Table IV, p. 35). Residue data reported in Table III-B (p. 33) as concentrations of the parent compound in the separate soil and water phases appear to actually represent the total radioactivity in the phases, as *they do not agree with the parent data in Table IV.* It is necessary that residue data for

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the parent compound for reported for both phases to allow the reviewer to determine the distribution of [^{14}C]residues between the phases. Kinetic analysis included data only through 14 days (Table IV), although 30-day data were apparently collected (as indicated by other tables). Furthermore the 30-day data were not reported in suitable form in other tables for reviewer kinetic analysis, as were not individual duplicate data values even through 14 days (ostensibly, Table IV data are averages of duplicate samples collected through 14 days).

5. The patterns of degradate formation and decline were not established by study termination. Additionally, a major degradate was not identified. Although samples were removed for analysis for up to 365 days posttreatment, those removed from incubation following >30 days posttreatment were not analyzed. Two degradates (maximums of 9.7% and 2.2% of the applied) were detected by HPLC analysis of the microwave/caustic extract. An unidentified major degradate, designated as Metabolite A, was detected in the neutralized NaOH extract at 2.5% of the applied radioactivity at 3 days posttreatment, increased to 6.0% of the applied by 7 days posttreatment, and was 9.7% of the applied at 30 days posttreatment (the last sampling interval for which samples were analyzed; Table VII, p. 38). Subdivision N Guidelines require that all major degradates (i.e., those approaching or reaching >10% of the applied) be identified and that metabolism studies be conducted until the patterns of formation and decline of all major degradates are established.

6. Purified water, rather than natural water, was utilized to flood the soil samples (p. 16). Subdivision N Guidelines require that the test water used to flood the soil be representative of the intended use site.

7. The soil extracts were stored frozen for up to 6 months prior to analysis (p. 21). Storage stability data were not reported. A storage stability study should be conducted when samples are stored longer than 30 days prior to analysis. A valid frozen storage stability analysis utilizes test soil which is fortified separately with the parent and degradates at known concentrations and analyzed periodically for up to and including the maximum length of time for which the test samples were stored. The reviewer notes, however, that it was the soil extracts (rather than the soil samples) which were stored frozen. Data were not presented to demonstrated the stability of the parent and degradates in the extracts.

8. The material balances were not within the reasonable range of 90-110% of the applied radioactivity at 14 and 30 days posttreatment. Recoveries generally decreased with time; material balances were initially 105-110% (for replicate samples), and were 86.3-89.9% of the applied radioactivity at 14-30 days posttreatment (Table II, p. 31). The study authors did not provide an explanation for this loss of material. The reviewer notes that the half-life (4.1 days) of the parent compound was calculated using data (days 7 and 14) affected by the loss.

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9. The incubation temperature was not held constant at ± 1°C as required by Subdivision N Guidelines. The study authors reported a mean incubation temperature of 25 ± 2°C (p. 17); raw temperature data were not reported.

10. Method detection limits were reported, but quantitation limits were not. Both limits of detection and quantitation should be reported to allow the reviewer to evaluate the adequacy of the method for the determination of the test compound and its degradates.

11. The study was conducted with cyclohexane ring-labeled [2-^{14}C]mesotrione. The compound contained an additional ring structure (phenyl) that was not radiolabeled. An anaerobic aquatic metabolism in silt loam soil study of phenyl ring-labeled [^{14}C]mesotrione was also submitted (MRID 44505131). Similar parent half-lives were observed in the two studies.

12. The reviewer noted high data variability between replicates of the NaOH traps at 30 days posttreatment (Table III-A, p. 32). Evolved $^{14}CO_2$ was 16.1% and 3.4% of the applied radioactivity for the two replicates.

13. The study authors inadvertently referred to the NaOH Fraction as the MeOH Fraction in Table VII (p. 38).

14. The reviewer noted that conditions during the pre-incubation period were only moderately reducing to moderately oxidizing; redox potentials ranged from 158 ± 17.7 mV to 237 ± 23.8 mV (Appendix D, p. 52). Conditions were only moderately reducing at day 0, with redox potentials of 162 ± 32.5 mV to 165 ± 0.0 mV. Subdivision N Guidelines require that the test systems be anaerobic (reducing conditions) at the time of treatment. The reviewer notes that the samples were flooded with water and pre-incubated under a nitrogen atmosphere for 30 days prior to treatment in order to create an anaerobic environment (p. 17); this method is generally considered to be adequate for achieving anaerobic conditions.

15. The study author stated that the application rate for the present study, 322 g/ha (0.32 ppm; soil-weight basis, apparently assuming a soil incorporation depth in the field of 7.5 cm), was in excess (>10%) of the proposed maximum label rate for pre-emergence application (280 g/ha; p. 12). The use of exaggerated dose rates may affect the degradation rate of the chemical relative to the degradation rate that would occur under normal use rates. While exaggerated dose rates may be used to facilitate residue identification, EPA requires that kinetics studies be performed using the proposed maximum application rate (US EPA. 1993. *Pesticide Reregistration Rejection Rate Analysis: Environmental Fate.* EPA 738-R-93-010, p. 67). *However, the maximum application rate given in the currently proposed label is 482 g a.i./ha (0.43 lb/acre)*, which, with the EFED standard 6-inch (15 cm) soil incorporation, is approximately equivalent to a concentration of 0.2 ppm.

16. The reviewer determined that the silt loam soil utilized in this study was of the Radford soil series; the soil characterization data were identical to data reported in MRID 44505128.

M# 44505132

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Pages 11 through 26 are not included in this copy.

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