DATA EVALUATION RECORD

STUDY 4

CHEM 122990 Mesotrione §162-1
CAS No. 104206-82-8
FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 44373530
Vispetto, A. R. and M. Tovshteyn. 1995. [Cyclohexane-2-^{14}C]ZA1296 aerobic soil metabolism study. Laboratory Study ID: PMS 403. Laboratory Report Nos.: RR 95 047B and WINO 12771. Unpublished study performed by ZENECA Inc., Richmond, CA; and submitted by ZENECA Inc., Wilmington, DE.

STUDY ID 44505130
Vispetto, A. R. and M. Tovshteyn. 1995. Addendum to: [Cyclohexane-2-^{14}C]ZA1296 aerobic soil metabolism study. Laboratory Study ID: PMS 403.. Laboratory Report Nos.: RR 95 047B and WINO 12771. Unpublished study performed by ZENECA Inc., Richmond, CA; and submitted by ZENECA Inc., Wilmington, DE.

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CONCLUSIONS

Metabolism - Aerobic Soil

1. This study is ***acceptable,*** provides useful information on the aerobic soil metabolism of mesotrione and ***partially satisfies data requirements***. However, parts of the reported methodology were questionable in terms of adequacy and accuracy, and leave open questions about the nature and identity of some transformation products. In spite of the discrepancies noted in the Comments section of this report, this study, in conjunction with several other aerobic metabolism studies submitted for mesotrione, is part of a consistent picture of metabolic behavior in aerobic soil. ***In combination with other studies, data requirements for aerobic soil metabolism are satisfied.***

2. The registrant should carefully consider the critical elements in the Comments Section.

3. Cyclohexanedione ring-labeled [2-^{14}C]mesotrione, at a nominal application rate of 0.35 ppm, degraded with a calculated half-life of 13.5 days ($r^2 = 0.99$; 0-30 day data) in silt loam soil (Radford series, see Comment 9) at an intended moisture content of 75% of 0.33 bar and incubated in darkness at 25 ± 1°C for up to 180 days. However, because the soil was not maintained at the required moisture level, the half-life may have been affected. The half-life calculation was based on parent equivalent data which included two compounds that formed during extraction (artifacts); however, a similar half-life was calculated using parent data which did not include the artifacts. Based on HPLC analysis, the parent compound was initially present at 81.3% (0.28 ppm) of the applied radioactivity, decreased to 44.6% (0.17 ppm) by 13 days and 26.0% (0.093 ppm) by 21 days, and was 5.0% (0.018 ppm) at 58 days posttreatment; data were not reported following 58 days.

   Two compounds (referred to as Artifacts 1 and 2) were reportedly formed in the ammonium hydroxide extraction process. Artifact 2 was initially (day 0) present at 4.7% (0.012 ppm) of the applied, increased to a maximum of 15.0% (0.054 ppm) by 1 day, and decreased with variability to 0.63% (0.002) by 58 days posttreatment; data were not reported following 58 days. Artifact 1 was detected only once (day 0; one replicate) at 5.0% (0.02 ppm). Nonextractable [^{14}C]residues were a maximum of 15.9% of the applied at 13 days posttreatment and were 9.2% at 180 days; humic and fulvic acid fractions each accounted for <0.005 ppm. Evolved $^{14}CO_2$ accounted for 2.1% of the applied radioactivity at 1 day, increased to 38.9% by 15 days, and was a maximum of 82.6% at 180 days posttreatment.

METHODOLOGY

MRID 44505130 was an addendum to MRID 44373530; page numbers reported in this DER refer to the addendum report (MRID 44505130).

Subsamples (250 g) of sieved (2 mm) silt loam soil (collected from Walworth County, WI; 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. 54) were weighed into biometer flasks and pre-incubated in darkness at 25 ± 1°C for 12 days (p. 17). The pre-incubated soil was treated by syringe with cyclohexanedione ring-labeled [2-^{14}C]mesotrione {ZA 1296; 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione; radiochemical purity ≥96.7%; specific activity 36.6 mCi/mmol; pp. 13-14}, dissolved in 0.01 M Na_2CO_3 solution, at a nominal rate of 0.35 ppm; the samples were adjusted to a soil moisture content of 75% of 0.33 bar upon application (pp. 14, 17; see Comment #3). Samples were capped and incubated under positive oxygen (air) pressure in darkness at 25 ± 1°C for up to 180 days (pp. 17, 18). The reservoir portion of the biometer flask contained 1.6 *N* NaOH to capture CO_2; a methanol-washed polyurethane foam plug was placed in the connecting arm between the flask and reservoir to capture volatiles (p. 16; Figure 1, p. 40). Duplicate flasks were removed for analysis at 0, 1, 3, 6, 9, 13, 15, 21, 30, 58, and 91 days posttreatment, and single flasks were removed for analysis at 120 and 180 days posttreatment (p. 18); however, percentage of the applied radioactivity data were not reported for the parent following 58 days posttreatment. At each sampling interval, the foam plugs and NaOH solutions from samples designated for that interval were analyzed by LSC; the limit of detection was 0.0004 ppm (Appendix E, p. 57). NaOH solutions from the flasks designated for future sampling intervals were analyzed by LSC and replaced with fresh NaOH solution (p. 18). Foam plugs were quartered and analyzed by LSC (p. 22). Aliquots of the NaOH solution were diluted with water and analyzed by LSC.

At each sampling interval, soil samples were extracted three times by shaking with 0.05 *N* ammonium hydroxide and centrifuged (p. 22; Figure 2, p. 43). Supernatants were decanted and combined. The samples were further extracted with acetone and centrifuged; the supernatant was decanted and stored frozen (<-10°C) until analysis. Aliquots of the ammonium hydroxide and acetone extracts were analyzed by LSC. Selected ammonium hydroxide and acetone extracts (30 days) were analyzed by TLC using Merck silica gel plates developed with chloroform:ethyl acetate:formic acid (20:20:1, v:v:v; p. 20; Figure 8b, p. 50); radioactivity on the plates was quantified using a radioimaging scanner. Samples were co-chromatographed with radiolabeled mesotrione. The ammonium hydroxide and acetone extracts were acidified and centrifuged, reportedly to precipitate out humic and fulvic acids, prior to chromatographic analysis (see Comment #1). The acidified extracts were neutralized prior to HPLC analysis (pp. 22, 23). Ammonium hydroxide and selected acetone extracts were analyzed by reverse-phase HPLC (Alltech, Altima C-18 reverse-phase column) using a mobile phase gradient of

acetonitrile:water with 0.1% H_3PO_4 (Method A; 10:90 to 50:50 to 100:0, v:v) with radioactive flow and UV (254 nm) detection (pp. 20, 21); the limit of detection was <0.001 ppm (p. 20). Samples were co-chromatographed with nonradiolabeled mesotrione (Figures 7, 8a; pp. 48, 49). Selected acetone extracts were analyzed by HPLC as previously described with a different mobile phase gradient (Method B; 10:90 to 25:75 to 50:50 to 100:0, v:v). Eluent factions were collected and analyzed by LSC; the limit of detection was <0.001 ppm (p. 20).

Extracts (unspecified) were further analyzed by LC/MS (Altima C-18 column) using a mobile phase gradient of acetonitrile:0.1% acetic acid (10:90 to 50:50, v:v; Figure 3, p. 44) with radioactive flow and UV (254 nm) detection; MS was performed in the electrospray ionization mode (p. 21).

In an attempt to remove bound residues, selected post-extracted soil samples were further extracted by two separate methods (p. 23). In the first method, subsamples (30 days) were extracted with 0.5 *N* NaOH, and centrifuged. The extract was acidified to pH 1 (HCl) and triplicate aliquots were analyzed by LSC. The acidified extract was partitioned with ethyl acetate, concentrated to dryness under nitrogen, redissolved in acetonitrile and analyzed by HPLC (Method A) as described previously (p. 23). Residual ethyl acetate in the NaOH extract was evaporated under nitrogen and the extract was concentrated by solid phase extraction (SPE, C-18, Bakerbond column; p. 24). The column was eluted with methanol and analyzed by HPLC (Method A) as described previously. In the second method, subsamples (3, 13, 30, 58, and 180 days) were microwave-extracted with 0.1 *N* NaOH by heating at 115°C and 160°C. The sample was cooled to room temperature, centrifuged and the supernatant was decanted. The extract was acidified to pH 1 (HCl) and centrifuged to separate humic and fulvic acids. The extract was neutralized prior to LSC and HPLC analysis as described previously.

Triplicate subsamples of dried, post-extracted soil were analyzed for total radioactivity by LSC following combustion; the limit of detection was 0.0010 ppm (Appendix E, p. 57).

DATA SUMMARY

Cyclohexanedione ring-labeled [2-^{14}C]mesotrione (radiochemical purity ≥96.7%), at a nominal application rate of 0.35 ppm, degraded with a registrant-calculated half-life of 13.5 days (r^2 = 0.99; 0-30 day data) in silt loam soil adjusted to 75% of 0.33 bar moisture content and incubated in darkness at 25 ± 1°C for up to 180 days (p. 27; Figure 4, p. 45). However, an exaggerated application rate was utilized and the soil was not maintained at the required moisture level, both of which may have affected the half-life. The half-life calculation was based on parent equivalent data which included two compounds that formed during extraction; however, a similar half-life was calculated using parent data which did not include the artifacts (see Comment #4). Based on HPLC analysis, the

parent compound was initially present at 81.3% (0.28 ppm) of the applied radioactivity, decreased to 44.6% (0.17 ppm) by 13 days posttreatment and 26.0% (0.093 ppm) by 21 days posttreatment, and was 5.0% (0.018 ppm) at 58 days posttreatment (Table IV, p. 40); data were not reported following 58 days. Two compounds (referred to as Artifacts 1 and 2) were formed in the ammonium hydroxide extraction process (see Comment #4). The compound

3-amino-2-(2-nitro-4-methanesulfonyl benzoyl) cyclohex-2-enone (Artifact 2)

was initially (day 0) present at 4.7% (0.02 ppm) of the applied radioactivity, increased to a maximum of 15.0% (0.054 ppm) by 1 day posttreatment, and decreased with variability to 0.63% (0.002) by 58 days posttreatment; data were not reported following 58 days. Artifact 1 (unidentified) was detected only once (day 0; one replicate) at 5.0% (0.02 ppm; p. 26).

Nonextractable [^{14}C]residues were initially (day 0) 2.7% of the applied radioactivity, increased to a maximum of 15.9% of the applied by 13 days posttreatment, and were 9.2% at 180 days posttreatment (Tables III-A-C, pp. 36-38); humic and fulvic acid fractions each accounted for <0.005 ppm (p. 30). Based on further NaOH (heated) extractions of selected samples, two unidentified minor degradates (metabolites A and B) were detected. At 13 days posttreatment (maximum nonextractables), metabolites A and B were detected at 3.5% and 6.3% of the applied radioactivity, respectively (Table V, p. 41); 4.4% of the applied remained as bound residues. Evolved $^{14}CO_2$ accounted for 2.1% of the applied radioactivity at 1 day posttreatment, increased to 38.9% of the applied by 15 days posttreatment, and was a maximum of 82.6% at 180 days posttreatment. Radioactivity in the polyurethane plugs was detected sporadically at ≤0.45% of the applied radioactivity.

Material balances (based on LSC analysis of individual replicates) were 89.7-102.0% of the applied radioactivity throughout the incubation period; a pattern of decline was not observed over time (Table II, p. 35).

COMMENTS

1. Parts of the reported methodology were questionable and the reviewer could not confirm that the analytical method was adequate. The study authors stated that, following the initial extractions and prior to HPLC analysis, the ammonium hydroxide and acetone extracts were acidified to precipitate humic and fulvic acids from solution (p. 22, 23). The reviewer notes, however, that, by definition, the fulvic acid fraction of soil organic matter is soluble in both acids and bases and, therefore, should not precipitate out in an acidfied 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 not generally removed during initial extractions. Additionally, it is noted that when nonextractable (bound) residues were later removed using NaOH microwave extractions, chromatographic analysis was done following organic extraction of the acidified NaOH extract and the concentration of the aqueous portion of that extract (pp. 23, 24). The study authors stated again that the humic and fulvic acids were precipitated out of the NaOH extract by acidification and stated that two minor degradates (maximums of 4.8% and 7.2% of the applied) were detected by HPLC analysis of the microwave extract. The reviewer questions the statements made by the authors concerning the precipitation of the soluble organic matter fractions. Also, it is unclear why the minor degradates removed during this final analytical step were not removed prior to organic matter fractionation. Clarification by the registrant may be necessary. Generally, soil 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 Soxleht 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 done 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. The reviewer notes that organic matter fractionation was performed (as described here) on the day 30 samples, but was not done on any other samples after it was determined to be ineffective at removing the applied radioactivity from the soil.

2. The study authors stated that the application rate for the present study, 348 g a.i./ha, was in excess of the maximum label rate for a pre-emergence application (280 g a.i./ha; p. 11). The use of exaggerated dose rates may affect the degradation rate of the chemical relative to the degradation rate which would occur under normal use rates. While exaggerated rates may be used to facilitate residue identification, EPA requires that kinetics studies be performed using the proposed maximum application rate (*Pesticide Reregistration Rejection Rate Analysis.* 1993. U.S. EPA Document: EPA 738-R-93-010, pp. 66, 67). However, the maximum application rate given in the currently proposed label is 482 g a.i./ha (0.43 lb/acre).

3. The soil moisture content may not have been maintained at 75% of 0.33 bar during the incubation period. The study authors stated that the soil moisture content was determined following the 12-day pre-incubation period (p. 17); however, soil moisture was not monitored during the 180-day incubation. Subdivision N Guidelines require that aerobic soil metabolism studies be performed at 75% of the soil moisture content at 0.33 bar in order to ensure aerobic conditions and soil viability.

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4. The study authors stated that no discrete degradate of the parent present at greater than 0.01 ppm was detected at any sampling interval (p. 26). However, two compounds were detected in the ammonium hydroxide and acetone extracts at greater than 0.01 ppm (Table IV, p. 40). The study authors stated that these compounds (referred to as Artifacts 1 and 2) were formed in the ammonium hydroxide extraction process, through the formation of an imine intermediate (Shiff's base) and finally to the more stable amine compound (p. 26). *Although the proposed compounds are plausible, the reviewer observes that the chemical structure, pKa of mesotrione, and the selected chromatograms are consistent with the production of the enolate form of mesotrione.* The "artifacts" were reported in parent equivalents and included in the half-life calculation of the parent (p. 30); the reported registrant-calculated half-life was 13.5 days (0 to 30 day data; p. 27). The reviewer-calculated half-life from parent equivalent data (including artifacts) collected from 0 to 30 days posttreatment was 13.6 days ($r^2 = 0.98$). The reviewer-calculated half-life from parent data (without artifacts) collected from 0 to 30 days posttreatment was 13.5 days ($r^2 = 0.97$). Thus, for environmental fate purposes, differences are inconsequential.

5. Method detection limits were reported for LC/MS, HPLC and LSC analyses (p. 20; Appendix E, p. 57); however, limits of quantitation were not reported. Both method limits of detection and quantitation should be reported to allow the reviewer to evaluate the adequacy of the method for the determination of parent and degradate compounds.

6. The study was conducted using cyclohexanedione ring-labeled [2-^{14}C]mesotrione. Additional aerobic soil metabolism studies (MRIDs 44373531, 44505129, and 44505208) conducted with uniformly phenyl ring-labeled [^{14}C]mesotrione were also submitted. Additionally, an aerobic soil metabolism study (MRID 44901714) of the mesotrione degradate AMBA was also submitted.

7. Soil viability throughout the incubation period was not confirmed. The study authors stated that the production of $^{14}CO_2$ indicated that a viable microbial population was present in all flasks and that mineralization of the parent to carbon dioxide is a major route of metabolism (p. 28). Generally, metabolism studies include data demonstrating the viability of the soil microbial population at the start and termination of the study. Measurement of soil respiration and/or use of benchmark compounds are recommended as indicators of soil viability. Such measurements, in effect, normalize results among various metabolism studies, and allow for meaningful comparisons of relative persistence among various chemicals and soils.

8. The study authors stated that MRID 44505130 was an addendum to MRID 44373530 and that changes were made to MRID 44373530 in order to correct typographical errors, label chromatographic figures and clarify statements (p. 5a); no numerical values were affected from the original report. The reviewer utilized the addendum report (MRID 44505130) to write this DER; page numbers reported in this DER refer to the addendum report.

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

10. The reviewer noted that duplicate flasks were removed for analysis at 0, 1, 3, 6, 9, 13, 15, 21, 30, 58, and 91 days posttreatment, and single flasks were removed for analysis at 120 and 180 days posttreatment (p. 18); however, percentages of the applied radioactivity data were not reported for the parent and artifacts following 58 days posttreatment (Table IV, p. 40).

MP 44393530

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