DATA EVALUATION RECORD

STUDY 5

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

STUDY ID 44373531
Subba-Rao, R. V. 1996. [Phenyl-U-^{14}C]ZA1296 - Aerobic soil metabolism study. Laboratory Study ID: PMS 404. Unpublished study performed by ZENECA Inc., Richmond, CA; and submitted by ZENECA Inc., Wilmington, DE.

STUDY ID 45196006
Miller, M. M. 1997. [Phenyl-U-^{14}C]ZA1296: Route and Rate of Degradation in Wisconsin Silt Loam Soil Under Aerobic Laboratory Conditions. European Guidelines (EOEC Annex I Document). Report ID: RR 97-033B. Unpublished study performed by ZENECA Inc., Richmond, CA; and submitted by ZENECA Inc., Wilmington, DE.

DIRECT REVIEW TIME = 70 Hours

REVIEWED BY:	T. Y. Marks, M.S.	Signature:
TITLE:	Scientist	Date:
	M. K. Mahoney, M.S.	
	Scientist	
EDITED BY:	C. A. Sutton, Ph.D.	Signature:
TITLE:	Sr. Scientist/Asst. Project Manager	Date:
APPROVED BY:	P. H. Howard, Ph.D.	Signature:
TITLE:	Project Manager	Date:
ORG:	Syracuse Research Corp. Arlington, VA 22202	
TEL:	703/413-9369	

EDITED, MODIFIED AND APPROVED BY:	Alex Clem, Environmental Scientist
ORG:	ERB III/EFED/OPP
TEL:	703/305-5773

Alex Clem 5/3/01

CONCLUSIONS

Metabolism - Aerobic Soil

1. This first study cited above (MRID 44373531) study is ***acceptable,*** provides useful information on the aerobic soil metabolism of mesotrione in a silt loam soil, and ***partially satisfies data requirements***. However, the presence of high concentrations of unextracted substances by day 6 indicates that the analytical method, specifically extraction, may not have been adequate; thus, the apparent half-life is open to interpretation, and leaves uncertain the nature and identity of some transformation products. In spite of this and other 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 results from other studies, data requirements for aerobic soil metabolism are satisfied.***

   The second study cited above (MRID 45196006, submitted at a later date under another action), although performed under different guidelines and at 20 ^{0}C and different soil moisture conditions, is acceptable under the same general terms and important qualifications as the first. ***Although reviewed, there will be no formally written DER for this second study other than the information provided in this and the following paragraph.*** This is justifiable because of the essential similarity of results to the first study, and because the Agency has other aerobic soil metabolism results for mesotrione for different soils approaching twenty in number. Results of this second study will be incorporated in an appropriate manner with the others. Second study results are as follows:

   Under the conditions and qualifications cited above, in the same soil and with the same radiolabel as the first study, and over a period of 56 days, in this second study mesotrione had a reviewer-calculated first-order regression half-life at *20 ^{0}C and at 50% of saturation water holding capacity* of approximately 18 days. The 95% confidence interval for the 18 day regression half-life is 15-23 days (adjusted r^2 of 0.96). The value which the registrant estimated was 14 days; no error bounds were given. The 50% of saturation water holding capacity is typically far in excess of the EPA standard of 75% of 1/3-bar, and would usually greatly accelerate the metabolism reaction rate. However, the reviewer estimated water content in this study for this particular soil was approximately 23% compared to approximately 20% under the EPA standard; therefore, although it is soil suction (more specifically, the soil moisture characteristic curve which is non-linear and can rapidly change with slight differences in water content) and not soil moisture percentage that strictly governs the energy required to abstract water, we assume that there is little accelerating effect here. In addition, a temperature five degrees lower than the 25 ^{0}C EPA standard usually decreases reaction rates and, therefore, tends to offset any enhancement due to extra water. MNBA and AMBA each ostensibly accounted for up to

6-8% of the phenyl-labeled radioactive dose; carbon dioxide, ostensibly up to 25%; and unextracted soil residues, up to 35 to 40%; however, Fig. 8 appears to be in error for carbon dioxide and AMBA when compared to Tables 5 and 6, respectively. Perhaps the registrant should resolve these differences for themselves, lest such discrepancies affect future study submissions for this or other chemicals. Other minor products (possibly up to 17) ostensibly formed at concentrations ranging from approximately 0.5 to 2.5% of the dose. However, based on other experiments with mesotrione and noted limitations with extraction procedures, such low concentrations may likely be method artifacts, and it is not clear from the study that such precision at lower concentrations is reliable. ***The remainder of the documentation in this DER pertains specifically to the first study cited above.***

2. The registrant should carefully consider the critical elements in the Comments section. For example, failure to report storage stability data, as noted within the third comment, could vitiate results of future study submissions.

3. Uniformly phenyl ring-labeled [^{14}C]mesotrione, at a nominal application rate of 0.31 ppm, degraded with a calculated first-order half-life of 12.1 days (0-30 day data; $r^2 = 0.99$) in silt loam soil (Radford soil series) adjusted to 75% of the soil moisture content at 0.33 bar and incubated in darkness at 25 ± 1°C for up to 121 days. However, based on the high levels of residues not extracted by the analytical methods, the half-life from this study is subjective.

   All data, designated as percentages of the applied radioactivity, represent percentages of the nominal application. All degradate data are reported in parent equivalents. The parent compound was initially present at 97.9% (0.31 ppm) of the applied radioactivity, decreased to 57.4% (0.18 ppm) by 9 days and 46.3% (0.15 ppm) by 13 days, was 19.1% (0.06 ppm) at 30 days, and was 2.2% (0.007 ppm) at 121 days posttreatment. No major degradates were detected. The minor degradate MNBA was a maximum of 7.6% (0.02 ppm) of the applied radioactivity at 6 days posttreatment and was 0.5% (0.001 ppm) at 121 days. The minor degradate AMBA was a maximum of 9.7% (0.03 ppm) of the applied radioactivity at 23 days posttreatment and was 0.9% (0.003 ppm) at 121 days. Nonextractable [^{14}C]residues were reported only as the radioactivity associated with the humin fraction and the humic acid plus fulvic acid fraction of the soil organic matter; total nonextractables were not reported. Nonextractable [^{14}C]residues associated with the humin fraction were16.4% of the applied radioactivity at 13 days posttreatment, were a maximum of 34.1% of the applied at 63 days posttreatment and were 25.9% at 121 days. Nonextractable [^{14}C]residues associated with the humic acid plus fulvic acid fractions were 13.7-16.2% at 9-23 days and were 17.3-23.7% at 30-121 days. An additional 3.1-8.6% of the applied radioactivity removed during the organic matter fractionation procedure was extracted out prior to quantification of the radioactivity associated with the organic matter fractions. Evolved $^{14}CO_2$ was 0.1-0.8% (≤0.02 ppm) of the applied radioactivity at 1-6 days posttreatment, were 15.7% (0.49 ppm) at 23 days, and were

37.6% (0.12 ppm) at 121 days; [^{14}C]organic volatiles were negligible.

METHODOLOGY

Samples (250 g) of sieved (2 mm) silt loam soil (collected from Richmond, WI; 17.1% sand, 57.7% silt, 25.2% clay, 2.7% organic matter, pH 6.2, CEC 12.0 meq/100 g; Appendix E, p. 72) were weighed into biometer flasks, adjusted to 75% of the soil moisture content at 0.33 bar (less 3 g water), and pre-incubated for 12 days at 25°C (p. 16). The soil samples were treated with uniformly phenyl ring-labeled [^{14}C]mesotrione {ZA1296; 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexane dione; radiochemical purity 98.3%, specific activity 30.4 mCi/mmol; pp. 14, 15}, dissolved in 10 mM sodium bicarbonate:ethanol (5:1, v:v; p. 15), at a nominal application rate of 0.31 ppm and incubated in darkness at 25 ± 1°C for up to 121 days (p. 18); six sterilized soil samples were prepared in the same manner (p. 17). Additional soil samples were treated at nominal application rates of 0.62 and 3.1 ppm for metabolite identification (p. 19). The soil was adjusted to 75% of the soil moisture content at 0.33 bar and the soil moisture was maintained at this level by the periodic addition of HPLC grade water throughout the incubation period. Humidified CO_2-free air was pumped through the incubation chamber and into CO_2 (1 *N* NaOH) and organic volatile (polyurethane foam plug) traps (pp. 17, 18; Figure 1, p. 50). Duplicate samples were removed for analysis at 0, 1, 3, 6, 9, 13, 16, 23, 30, 63, 92, and 121 days posttreatment (p. 20); sterilized soil samples were removed for analysis at 16, 63, and 121 days posttreatment. Soil samples were extracted at each sampling interval; extracts were stored frozen for up to 211 days prior to analysis (Appendix K, p. 82; see Comment #3).

At each sampling interval, soil samples were extracted three times by shaking with NH_4OH (p. 25). The extracts were combined and aliquots were analyzed for total radioactivity by LSC. The soil samples were further extracted with acetone followed by centrifugation and filtration; duplicate aliquots of the combined extract were analyzed for total radioactivity by LSC. The NH_4OH and acetone extracts were combined and concentrated by rotary evaporation. The concentrated extract was filtered (0.45 µm) and analyzed by reverse-phase HPLC (Alltima C-18 column) using a mobile phase gradient of either acetonitrile:0.1% phosphoric acid (100:0 to 90:10 to 50:50 to 0:100, v:v; Appendix I, p. 79) or acetonitrile:0.1% acetic acid (95:5 to 50:50 to 0:100, v:v) with UV (wavelength not reported) and radioactive flow detection (p. 23); eluate fractions were collected at half-minute intervals and analyzed by LSC. The limit of quantitation was 0.0037 µg/mL (p. 78). Samples were co-chromatographed with nonradiolabeled reference standards of the parent and the following potential degradates: denitro-ZA1296, 4-OH ZA1296, 5-OH ZA1296, XAN-H4, XAN-2, AMBA, MNBA, and MBA (pp. 15, 31; Appendix D, p. 70). To confirm compound identities, selected soil extracts were analyzed by LC/MS (Alltima C18 column) using a mobile phase of acetonitrile: aqueous 0.1% acetic acid (gradient not reported) with electrospray ionization (p. 31); UV

(254 nm) and radiochemical detection were utilized (p. 24).

To determine radioactivity associated with the soil organic matter, post-extracted soil samples were extracted by shaking with 0.5 *N* NaOH (p. 26). The extracts were centrifuged and duplicate aliquots of the supernatant were analyzed by LSC to determine the radioactivity associated with the fulvic acid plus humic acid fractions. The caustic extracts were acidified to pH 2 (5 *N* HCl) to precipiate humic acids, and the extract was partitioned with ethyl acetate to separate out newly extracted radioactivity; aliquots of the organic extract were analyzed by LSC. The post-extracted soil samples were analyzed by LSC following combustion to determine the radioactivity associated with the humin fraction; data were corrected for combustion efficiency (p. 22; Appendix H, p. 78); the limit of quantitation was 0.0012 µg/g.

The volatile trap solutions were removed for analysis by LSC at each sampling interval (p. 20). Following 64 days of incubation, the NaOH solution in the remaining flasks was replaced with fresh 1 *N* NaOH solution. The presence of $^{14}CO_2$ in the NaOH traps was confirmed by precipitation with $BaCl_2$. The polyurethane foam plugs were removed for analysis at each sampling interval (pp. 21, 25). Duplicate foam plugs collected from 1 to 6 days posttreatment were extracted with ethyl acetate and the extract was analyzed for total radioactivity by LSC (see Comment #8). For the remaining sampling intervals, one foam plug was split into two, and each piece was submerged in scintillation cocktail prior to analysis by LSC; the second foam plug was frozen.

DATA SUMMARY

Uniformly phenyl ring-labeled [^{14}C]mesotrione (radiochemical purity 98.3%), at a nominal application rate of 0.31 ppm, degraded with a calculated first-order half-life of 12.1 days (0-30 day data; $r^2 = 0.99$) in Radford silt loam soil adjusted to 75% of the soil moisture content at 0.33 bar and incubated in darkness at 25 ± 1°C for up to 121 days (Table IX, p. 48; Figure 15, p. 64). However, based on high levels of unextracted residues, the analytical method is questionable and, thus, the half-life is open to interpretation. All data, designated as percentages of the applied radioactivity, represent percentages of the nominal application. All degradate data are reported in parent equivalents. The parent compound was initially present at 97.9% (0.31 ppm) of the applied radioactivity, decreased to 57.4% by 9 days and 46.3% (0.15 ppm) by 13 days, was 19.1% (0.06 ppm) of the applied at 30 days posttreatment, and was 2.2% (0.007 ppm) of the applied at 121 days posttreatment (Tables VI, VII; pp. 45, 46; see Comment #3). No major degradates were detected. The minor degradate 4-(methylsulfonyl)-2-nitrobenzoic acid (MNBA) was initially (day 1) present at 4.1% (0.013 ppm) of the applied radioactivity, increased to a maximum of 7.6% (0.024 ppm) of the applied by 6 days posttreatment, was 1.8% (0.006 ppm) of the applied at 23-30 days posttreatment, and was 0.4-0.5% (0.001 ppm) at 121 days posttreatment. The minor degradate 2-amino-

4-(methylsulfonyl)benzoic acid (AMBA) was initially (day 1) present at 2.2% (0.007 ppm) of the applied radioactivity, increased to a maximum of 9.7% (0.03 ppm) of the applied by 23 days posttreatment, and was 0.9% (0.003 ppm) of the applied at 121 days posttreatment.

Unextracted [^{14}C]residues were only reported as two separate values: (1) the radioactivity associated with the humin fraction and (2) the humic acid plus fulvic acid fraction of the soil organic matter; total unextracted material was not reported. Nonextracted [^{14}C]residues associated with the humin fraction were16.4% of the applied radioactivity at 13 days posttreatment, were a maximum of 34.1% of the applied at 63 days posttreatment and were 25.9% at 121 days (Table I, p. 40). Nonextractable [^{14}C]residues associated with the humic acid plus fulvic acid fractions were 13.7-16.2% at 9-23 days and were 17.3-23.7% at 30-121 days (Tables I, III; pp. 40, 42; designated as "NaOH" in each table). An additional 3.1-8.6% of the applied radioactivity removed during the organic matter fractionation procedure was extracted out prior to quantification of the radioactivity associated with the organic matter fractions.

Evolved $^{14}CO_2$ was 0.1-0.8% ($\leq$0.02 ppm) of the applied radioactivity at 1-6 days posttreatment, increased to 15.7% (0.49 ppm) of the applied by 23 days, and was 37.6% (0.12 ppm) of the applied at 121 days posttreatment; [^{14}C]organic volatiles were negligible (p. 29).

Material balances (based on LSC analysis) were 100.3-108.6% of the applied radioactivity at 0-6 days posttreatment and generally decreased throughout the incubation period to 89.3% of the applied at 121 days posttreatment (Table I, p. 40; see Comment #6).

COMMENTS

1. Nonextracted [^{14}C]residues were high, indicating that the analytical method, specifically extraction, may have been inadequate. Total unextracted material (reviewer-calculated from "NaOH" plus "% Bound" data in Table I, p. 40) were 18.7% of the applied (8.9% as humin plus 9.8% as humic and fulvic acids) by 6 days posttreatment, were 50.6% (26.9% as humin plus 23.7% as humic and fulvic acids) at day 30, and were a maximum of 55.2% (34.1% as humin plus 21.1% as humic and fulvic acids) at day 63. The presence of high concentrations of unextracted components indicates that the analytical method, specifically extraction, may not have been adequate. Without the appropriate extraction procedures to ensure quantitative recovery of the compounds of interest, the validity of the reported half-lives is questionable. The reviewer notes that during the organic matter fractionation procedure, an additional 3.1-8.6% of the applied radioactivity was extracted from the acidified caustic extract and was not considered to be associated with the organic matter; this radioactivity was not further characterized. It is unclear why the

radiolabeled residues removed in this final step were not removed prior to organic matter fractionation. Clarification by the registrant is appropriate.

The reviewer notes that in another submitted aerobic soil metabolism study of the mesotrione degradate AMBA (MRID 44901714), AMBA was detected in both the organic and aqueous fractions of the NaOH extract; *therefore, the initial extraction method in that study (which utilized extraction with NH_4OH and acetone, as did the current study) was not adequate for quantitative extraction of the applied radioactivity.* In the current study, the radioactivity in the NaOH extract was not further characterized other than as radioactivity associated with the organic matter fractions or that removed by extraction with ethylacetate (which was not analyzed by chromatography). 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. Additionally, radioactivity associated with the humic acid and fulvic acid fractions is generally reported separately, rather than as a combined value.

2. The study author stated that the application rate for the present study, 308 g a.i./ha (0.31 ppm), was 10% greater than the proposed maximum label rate for pre-emergence application (280 g a.i./ha; p. 13). 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).

3. Although soil samples were extracted at each sampling interval, the majority of the soil extracts were stored frozen for 78-205 days prior to analysis (p. 27; Appendix K, p. 82); only selected soil extracts (days 23 and 30) were analyzed within 30 days of sampling. The study author stated that the degradate 3-amino-2-(2-nitro-4-methanesulfonyl benzoyl)-cyclohex-2-enone (the enamine of the parent) was detected sporadically throughout the incubation period (pp. 32, 33). Since the enamine was present at as much as 12% of the applied radioactivity at time 0 (Appendix L, p. 83), the study author concluded that the degradate formed as a result of storage in the ammonium hydroxide solution. However, storage stability data were not reported. A storage stability study

should be conducted when samples are stored for longer than 30 days prior to analysis. *A study was performed to verify that the enamine was an artifact of ammonium hydroxide storage (p. 33); the enamine accounted for 17% of the applied radioactivity after 6.5 hours of incubation in ammonium hydroxide. The degradate reverted back to mesotrione under acidic conditions.* The study author reported the concentration of the parent compound based on the sum of the parent compound plus the enamine. The reviewer notes that the table in Appendix K (p. 82) indicates that soil samples were extracted at each sampling interval; however, on page 25, the study author stated that, at sample collection, ammonium hydroxide was added to the soil and the sample was stored frozen prior to extraction. Clarification is in order.

4. Method detection limits were not reported for LSC, LC/MS, or HPLC analyses. Method detection and quantitation limits should be reported to allow the reviewer to evaluate the adequacy of the methods for the determination of the parent compound and its degradates.

5. Soil viability throughout the incubation period was not confirmed. Generally, metabolism studies include data demonstrating the viability of the soil microbial population at the start and termination of the study. The reviewer noted that the parent compound appeared to be relatively stable in the sterilized soil samples from 16 to 21 days posttreatment (see Comment #7).

   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.

6. Material balances generally declined throughout the incubation period (Table I, p. 40). The material balances were 100.3-108.6% of the applied radioactivity at 0-6 days posttreatment, generally decreased to 98.7% by 9 days and 91.1% by 92 days, and were 89.3% of the applied at 121 days posttreatment. The study author did not provide an explanation for this material loss over time.

7. In sterilized soil samples, the parent compound ranged from 84.2% (0.26 ppm) to 77.8% (0.23 ppm) of the applied radioactivity from 16 to 121 days posttreatment (Table VIII, p. 47). The minor degradates MNBA, AMBA, and $^{14}CO_2$ were negligible at 121 days posttreatment. Unextracted residues associated with the humic acid plus fulvic acid fraction were a maximum of 20.8% (0.065 ppm) of the applied radioactivity and unextracted residues associated with the humin fraction were a maximum of 12.0% (0.038 ppm) of the applied radioactivity at 121 days posttreatment (Table II, p. 41).

8. Radioactivity extracted from the foam plugs collected at 1-6 days posttreatment accounted for <0.0005% of the applied radioactivity (p. 25); therefore, only one of the two plugs collected from the remaining samples was analyzed by LSC without extraction.

The second plug was placed in frozen storage for extraction at a later date, if needed.

9. The study was conducted using uniformly phenyl ring-labeled [^{14}C]mesotrione; the compound contained an additional ring structure that was not radiolabeled. The reviewer notes that additional aerobic soil metabolism studies were also submitted (MRIDs 44373531, 44505130, 44505208 and 44901714).

10. 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 MRID 44505128.

11. The proposed degradation pathway of mesotrione is presented in Figure 16 (p. 65).

M+ 44373531

Page ___ is not included in this copy.

Pages 10 through 24 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.