DATA EVALUATION RECORD

STUDY 8

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

STUDY ID 44505131
Carley, S. E. 1996. [Phenyl-U-^{14}C]ZA1296 Anaerobic aquatic soil metabolism. Laboratory Study No.: PMS-395. Report No.: RR 96-033B. Unpublished study performed by ZENECA Inc., Richmond, CA; and submitted by ZENECA Inc., Wilmington, DE.

DIRECT REVIEW TIME = 55 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 5/03/01

CONCLUSIONS

Metabolism - Anaerobic Aquatic

1. This study provides useful information and ***partially satisfies data requirements*** for anaerobic aquatic (flooded) metabolism. With supporting data available from other fate studies, the deficiencies noted in this study do not preclude 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 study conducted using cyclohexane ring-labeled [2-^{14}C]mesotrione (MRID 44505132). However, this study, in conjunction with the aforementioned metabolism study and other fate studies, is part of a consistent picture of the environmental behavior of mesotrione. ***In combination with results from this and other studies, data requirements for anaerobic aquatic metabolism are satisfied. No additional data are needed at this time.***

2. The registrant should carefully consider the critical elements in the Comments section, and provide brief explanations or corrections as requested. The many study discrepancies would, in most cases, vitiate future study results. For example, the material balance was incomplete (Comment 1), and concentration data reported for the aqueous phase do not accurately depict actual aqueous concentrations since they were reported on a soil-weight basis (Comment 2). There were also irregularities in the measurement and maintenance of anaerobic conditions (Comment 7).

3. Study Results: Uniformly phenyl ring-labeled [^{14}C]mesotrione, at an actual application rate of 0.34 ppm (soil-weight basis), degraded with a reviewer calculated system first-order half-life of 3.9 days (0-30 day data, 95% confidence interval half-life from 3 to 5 days, $r^2 = 0.94$). The system was a flooded Radford series silt loam soil under moderately oxidizing to moderately reducing conditions (day 0-30 Eh ranged from approx. +190 mV to -90 mV) with an average day 3 to day 30 pH of 6.9 $\pm$ 0.1. Incubation was in darkness at 25 $\pm$ 1°C for up to 365 days. Test samples removed from incubation following >59 days post-treatment were not analyzed. All data (aqueous and soil phases) reported below are on a soil-weight basis; therefore, water phase data do not depict the actual aqueous concentration of the compounds of interest.

   In the *total soil/water system*, the parent compound was initially present at 0.31 ppm, decreased to 0.21 ppm by 3 days and 0.12 ppm by 7 days, and was last detected at 0.021 ppm at 14 days post-treatment.

   In the *aqueous phase* (system floodwater), the parent compound was initially present at 0.31 ppm, decreased to 0.16 ppm by 3 days, and was last detected at 0.009 ppm at 14 days post-treatment. The *major aqueous degradate* AMBA was a maximum of 0.031 ppm at 14 days [reviewer calculated maximum of 66% of aqueous phase radioactivity (not 42%

as stated in the study Summary section, see Comment 2) and a reviewer calculated 11% of system total radioactivity], and was 0.027 ppm at 59 days post-treatment.

In the soil, the parent compound was initially present at 0.0015 ppm, was a maximum of 0.062 ppm at 7 days, and was last detected at 0.012 ppm at 14 days post-treatment. The *major soil degradate AMBA* was initially (day 3) present at 0.012 ppm (one of two replicates), was a maximum of 0.13 ppm at 30 days (approx. 68% of soil radioactivity or 49% of system total radioactivity), and was 0.048 ppm at 59 days. *Unextracted [^{14}C]residues* were 11.5% of the applied radioactivity at 3 days, were 14.5% at 30 days, and were a maximum of 16.9% at 59 days post-treatment (reviewer-calculated means of two replicates).

Evolved $^{14}CO_2$ was anomalous, accounting for 0.01-0.38% of the applied radioactivity from 0 to 90 days, 2.7-4.5% at 120-183 days, 11.2% at 275 days, and 2.5% at 365 days post-treatment (reviewer-calculated means of two replicates). There was no explanation for the apparent irregular results for the last two intervals. *[^{14}C]organic volatiles* were negligible.

Residues increased in the soil phase over time; the soil:water distribution ratio (reviewer-calculated) was approximately 1:3 at day 0, changed to 1:1 by 3 days, was 5:1 at 14-30 days, and was 4:1 at 59 days post-treatment.

METHODOLOGY

Samples (245.4 g) of sieved (3 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; Table II, p. 30), collected from Walworth County, Wisconsin, were weighed into biometer flasks equipped with a side-arm volatile trap containing 1 *N* NaOH and fitted with a polyurethane foam plug (p. 15; Figure 1, p. 38). Samples were flooded with purified water (300 mL; p. 14) and pre-incubated anaerobically (nitrogen atmosphere) in darkness at 25 ± 1°C for 29 days prior to treatment. The final soil:water ratio was 1:1.2 (w:v; reviewer-calculated). Three additional flasks were prepared as in the manner described, and then autoclaved and treated with sodium azide to serve as sterile control samples. Following the pre-incubation period, the soil/water systems were treated with uniformly phenyl ring-labeled [^{14}C]mesotrione {ZA1296; 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione; radiochemical purity 97.2%; specific activity 30.4 mCi/mmol; p. 13; Table I, p. 27}, dissolved in 0.01 M sodium bicarbonate solution, at an actual application rate of 0.34 ppm (soil-weight basis; p. 16; see Comment #2); the parent compound was enriched with ^{13}C in the carbonyl position. Additional soil/water samples were treated with [^{14}C]mesotrione at an exaggerated rate of 3.4 ppm for use in metabolite identification. The soil/water systems were incubated anaerobically (nitrogen atmosphere) in darkness at 25 ± 1°C for up to 365 days. Duplicate soil/water samples were removed for analysis at

0, 1, 3, 7, 14, 30, 59, 90, 120, 183, 275, and 365 days post-treatment (p. 17); samples removed for analysis at >59 days post-treatment were not analyzed. Sterile samples were removed for analysis at 30, 183, and 365 days post-treatment. Samples prepared for metabolite identification were removed for analysis at 14, 30, and 183 days post-treatment. The volatile traps were removed for analysis at each sampling interval (through 365 days post-treatment).

At each sampling interval, the soil/water systems were centrifuged and the aqueous layer was decanted (p. 18). Duplicate aliquots of the aqueous phase were analyzed for total radioactivity by LSC. Aliquots of the aqueous phase were also analyzed by reverse-phase HPLC (Alltima C18 column) using a mobile phase gradient of acetonitrile:water (0.1% H_3PO_4; 2:98 to 100:0, v:v) with UV (wavelength not reported) and radioactive flow detection (pp. 19, 23). Samples were co-chromatographed with non radiolabeled reference standards. The extracts were further analyzed by normal-phase TLC on silica gel plates developed in toluene:methanol:triethylamine (60:30:10, v:v:v; see Comment #9). To confirm the identity of the degradate AMBA, a sample from the exaggerated treatment rate was analyzed by LC/MS in the electrospray ionization mode (pp. 20, 24).

Soil samples (days 7-60) were extracted, in sequence, three times with 0.05 M NH_4OH, once with acetonitrile, four times with 0.05 M NaOH, and once with acetonitrile (p. 18). Soil samples from days 0, 1, and 3 were extracted by shaking with NH_4OH and acetonitrile only. Duplicate aliquots of each extract were analyzed for total radioactivity by LSC. The extracts were concentrated and analyzed (individually or combined) by HPLC and TLC as described previously. Post-extracted soil samples were analyzed by LSC following combustion (p. 14).

At each sampling interval, duplicate aliquots of the NaOH trap solutions were analyzed for total radioactivity by LSC (p. 17). One polyurethane foam plug was quartered at each sampling interval and analyzed for total radioactivity by LSC (p. 14).

To determine the presence of anaerobic conditions, the redox potential and pH were measured prior to treatment and at each sampling interval (pp. 15, 18). The redox potentials for individual replicates ranged from -6.4 ± 0.28 mV to 45.3 ± 4.4 mV from 7 to 14 days, were -220.2 ± 0.99 mV to -109.9 ± 1.3 mV from 59 to 275 days post-treatment, and were -61.5 ± 1.3 mV to -44.7 ± 12.7 mV at 365 days post-treatment (Table IV, p. 32). In the sterile control samples, the redox potential ranged from -150.7 mV to +238.8 ± 1.1 mV. The pH was 6.5-7.0 throughout the incubation period (with the exception of pH 7.4 at 59 days post-treatment), with no observed pattern of decline; the pH was 6.3-7.1 in the sterile control samples.

DATA SUMMARY

Uniformly phenyl ring-labeled [^{14}C]mesotrione (radiochemical purity 97.2%), at an actual application rate of 0.34 ppm (soil-weight basis; see Comment #2), degraded with a calculated half-life of 3.6 days (0-30 day data; $r^2 = 0.93$; p. 22) in anaerobic flooded silt loam soil that was incubated in darkness at 25 ± 1°C for up to 365 days (Figure 3, p. 40). Test samples removed from incubation following >59 days post-treatment were not analyzed. All data (aqueous and soil phases) reported below are on a soil-weight basis (see Comment #2).

In the *total soil/water system*, the parent compound was initially present at 0.31 ppm, decreased to 0.21 ppm by 3 days and 0.12 ppm by 7 days, and was last detected at 0.021 ppm at 14 days post-treatment (Table VII, p. 35).

In the *aqueous phase*, the parent compound was initially present at 0.31 ppm, decreased to 0.16 ppm by 3 days post-treatment, and was last detected at 0.009 ppm at 14 days post-treatment. The *degradate 2-amino-4-(methylsulfonyl)benzoic acid (AMBA)* was initially (day 3) present at 0.005 ppm, increased to a maximum of 0.031 ppm by 14 days post-treatment, and was 0.027 ppm at 59 days post-treatment (Table VIII, p. 36).

In the soil, the parent compound was initially present at 0.0015 ppm, increased to a maximum of 0.062 ppm by 7 days post-treatment, and was last detected at 0.012 ppm at 14 days post-treatment (Table VII, p. 35). The *major degradate AMBA* was initially (day 3) present at 0.012 ppm (one of two replicates), increased to a maximum of 0.13 ppm by 30 days post-treatment, and was 0.048 ppm at 59 days post-treatment (Table IX, p. 37). *Unextracted [^{14}C]residues* were 11.5% of the applied radioactivity at 3 days post-treatment, were 14.5% of the applied at 30 days post-treatment, and were a maximum of 16.9% of the applied at 59 days post-treatment (reviewer-calculated means of two replicates; Table V, p. 33).

Evolved $^{14}CO_2$ accounted for 0.01-0.38% of the applied radioactivity from 0 to 90 days post-treatment, was 2.7-4.5% of the applied at 120-183 days post-treatment, accounted for 11.2% of the applied at 275 days post-treatment, and was 2.5% of the applied at 365 days post-treatment (reviewer-calculated means of two replicates). There was no explanation for the seemingly anomalous values for the last two intervals. *[^{14}C]organic volatiles* were negligible.

Residues increased in the soil phase over time; the soil:water distribution ratio (reviewer-calculated) was approximately 1:3 at day 0, changed to 1:1 by 3 days post-treatment, was 5:1 at 14-30 days post-treatment, and was 4:1 at 59 days post-treatment.

Material balances (based on LSC analysis of individual replicates) were 82.8-101.4% of the applied radioactivity from 0 to 30 days post-treatment, with no observed pattern of decline, then decreased to 53.5-58.7% of the applied by 59 days post-treatment (Table V, p. 33; see Comment #1). Material balances could not be determined for samples removed

beyond 59 days post-treatment because only volatile phase data were collected.

COMMENTS

1. The material balances were not within the reasonable range of 90-110% as required by Subdivision N Guidelines. Material balances were 82.8-101.4% of the applied radioactivity from 0 to 30 days post-treatment, with no observed pattern of decline, and then decreased to 53.5-58.7% of the applied by day 59 (the last sampling interval for which samples were analyzed; Table V, p. 33). The study author did not provide an explanation for this loss of material. The reviewer notes that the half-life (3.6 days; p. 22) of the parent occurred well before the observed decline in the material balance. The reviewer noted that evolved $^{14}CO_2$ decreased from 11.2% to 2.5% of the applied radioactivity from 275 to 365 days post-treatment; an explanation for this loss also was not provided.

2. 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. Data were not reported as percentages of the applied radioactivity. The reviewer defined major degradates as those degradates present at ≥10% of the applied radioactivity (0.034 ppm). In future studies submitted to the EPA, in addition to being reported in units of concentration data (such as ppm), the data should be reported as percentages of the applied radioactivity.

   Based on Tables V and VIII, as interpreted by the reviewer, the maximum aqueous phase concentration for the AMBA metabolite which occurred at day 14 should correspond to 66% of the floodwater radioactivity or 11% of the total system radioactivity. In contrast, the study author reported AMBA as comprising 42% of the floodwater radioactivity, and did not report the AMBA percentage of total system radioactivity. For the soil phase the reviewer calculates that AMBA comprised 68% of the soil radioactivity, instead of 66%. Although the 2% difference in the soil value is inconsequential, since the same tabular numbers are used, identical results (except for roundoff error) should be calculated by all. An explanation or correction is in order.

3. Purified water, rather than natural water, was utilized to flood the soil samples (p. 14). Subdivision N Guidelines require that the test water used to flood the soil be representative of the intended use site.

6

4. Duplicate soil/water samples were also removed for analysis at 90, 120, 183, 275, and 365 days post-treatment; however, these samples were not analyzed due to the short half-life of the parent (Table V, p. 33). The reviewer notes that, based on the reported data (Tables VIII-IX, pp. 36, 37), the patterns of formation and decline of the degradate AMBA were established by 59 days post-treatment (the last sampling interval for which samples were analyzed). However, evolved $^{14}CO_2$ did not reach its maximum (11.2% of the applied radioactivity) until 275 days post-treatment.

5. The study was conducted using uniformly phenyl ring-labeled [^{14}C]mesotrione. An anaerobic aquatic metabolism study of cyclohexadione ring-labeled [^{14}C]mesotrione in silt loam soil was also submitted (MRID 44505132; p. 11). Similar half-lives were observed between the two studies.

6. Method detection limits were not reported. Limits of detection and quantitation should be reported to allow the reviewer to evaluate the adequacy of methods for determination of the test compound and its degradates.

7. The reviewer noted that conditions during the pre-incubation period were moderately reducing to moderately oxidizing; the mean redox potential was 192.7 ± 35.6 mV (Table IV, p. 32). Conditions from 7 to 14 days post-treatment were only moderately reducing, with redox potentials of -6.4 ± 4.4 mV to 45.3 ± 4.4 mV; redox potentials were not reported for 0 to 3 days post-treatment. 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 in a nitrogen atmosphere for 29 days prior to treatment in order to create an anaerobic environment (p. 15); this method is generally considered to be adequate for achieving anaerobic conditions.

8. The soil was sieved using a 3-mm screen size (p. 13). Subdivision N Guidelines require that soils be sieved at 2 mm, the upper limit for sand-sized particles. The presence of soil particles of >3-mm diameter would effectively lead to greater macroporosity in a soil sample, resulting in altered soil physical and, perhaps, biological properties. However, because the ratio of air- to water-filled pore space would not affect the outcome of an aquatic metabolism study, the reviewer concluded that the effect of this method inadequacy was likely to be negligible.

9. The study author did not state whether the TLC analysis was used to quantitate residues and/or to confirm compound identities; TLC data were not reported.

10. The aqueous solubility of the parent compound was not reported. The solubility of the test compound in the treatment water should be reported to allow the reviewer to accurately assess whether the compound was available in solution for degradation.

11. In the sterile control samples, the parent was present in the aqueous phase at 51.1% and

37.7% of the applied radioactivity at 30 and 365 days post-treatment, respectively. In the soil phase, the parent compound was present at 31.1% and 29.3% of the applied radioactivity at 30 and 365 days, respectively (Table VI, p. 34). Nonextractable [^{14}C]residues were 4.8% and 19% of the applied radioactivity at 30 and 365 days post-treatment, respectively. Evolved $^{14}CO_2$ was <1.0% of the applied at 30 and 365 days post-treatment; [^{14}C]organic volatiles were negligible. Material balances were 87.1% and 86.0% of the applied at 30 and 365 days post-treatment, respectively. The sterility of the control sample prepared for analysis at 183 days post-treatment was not maintained throughout the incubation (Table VI, p. 34). In addition, conditions for the day-30 sterile sample were moderately oxidizing, with redox potentials of 238.8 ± 1.1 mV (Table IV, p. 32).

12. 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.

13. The study author stated that the application rate (0.34 ppm; soil-weight basis) was equivalent to the expected use rate (340 g/ha) of mesotrione (p. 11).

M# 44505131

Page ___ is not included in this copy.

Pages 9 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.