DATA EVALUATION RECORD

STUDY 18

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STUDY ID 44901714
Marth, J. L. 1997. [^{14}C]AMBA, a metabolite of ZA1296: Rate of degradation in soil under aerobic laboratory conditions. Laboratory Project ID: RR97-032B. Unpublished study performed by Zeneca Ag Products, Richmond, CA; and submitted by Zeneca Inc., Wilmington, DE.

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CONCLUSIONS

Metabolism - Aerobic Soil

1. Portions of this study provide useful information and ***partially satisfy data requirements*** for the aerobic soil metabolism for AMBA, a ZA1296 (mesotrione) metabolite in three test soils (one U.S. and two English).

   There were numerous deficiencies in this study, as noted in the Comments section of this report, which are similar to deficiencies in other aerobic and anaerobic soil metabolism submissions for parent mesotrione. However, this study, in conjunction with other metabolism studies and other fate studies, is part of a consistent picture of the environmental behavior of mesotrione and its transformation products. ***In combination with results from this and other studies, no additional aerobic metabolism data are needed for the metabolite AMBA at this time.***

2. The registrant should carefully consider the critical elements in the Comments section, and, where possible, reply with plausible explanations or additional information. The cited study discrepancies could vitiate future study results. For example, the presence of high concentrations of unextracted residues by day 3 for each of three tested soil indicates that the analytical method, specifically extraction, may not have been adequate for the determination of AMBA and transformation products (see Comment 1); if so, the half-live determinations could be somewhat longer and information on the nature of the metabolites incomplete.

3. The mesotrione degradate AMBA was relatively short-lived in the three tested soils. Reviewer calculated simple, first-order kinetics half-lives in clay (English soil), silt loam (Wisconsin soil), and sandy loam (English soil), respectively, were approximately 16 ($n=9$, $r^2=0.73$), 27 ($n=9$, $r^2=0.74$), and 20 ($n=9$, $r^2=0.62$) days, with an average value for the three soils of 21 $\pm$ 5 days and an upper 90% confidence limit on the mean of 31 days. The only metabolite identified was carbon dioxide which accounted for approximately 43, 14, and 17% of total radioactivity, respectively, after 56 days of incubation. Unextracted soil residues reached maximum values of approximately 37, 48, and 60% of the dose after approximately 28-56 days of incubation (see below). The maximum values for polar degradates varied from approximately 3-15% of the dose. There was no attempt to trap gaseous or volatile compounds other than carbon dioxide.

   Uniformly phenyl-ring labeled [^{14}C]AMBA was incubated at approximately *40% of maximum soil water-holding capacity* and in darkness at *20 $\pm$ 2°C* for up to 56 days. Nominal application rates were 0.213 ppm in clay soil (English) and silt loam soil (Wisconsin, U.S.) and 0.187 ppm in a sandy loam soil (English). Registrant-calculated DT_{50}s were based on equations derived from a two-compartment, first-order model; the values were 3, 6, and 2 days, respectively. The two-compartment model fit the data well,

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but clearly shows that DT_{50}s cannot be substituted for simple first-order half-lives in order to approximate residuals at times near the end of the test period or beyond. However, the presence of relatively high concentrations of unextracted residues by day 3 (for each soil) indicates that the analytical method may not have been adequate (Comment 1); therefore, effective "half-lives" or DTs could be somewhat longer. Also, the registrant could not report true time data because degradation was rapid. The first sampling interval was reported as day 0.1 posttreatment and time zero was estimated as the expected concentration based on the nominal application rate. All data, designated below as percentages of the applied, represent percentages of the nominal application. Reported residue concentration data (in ppm) were reviewer-calculated based on the actual application rates and the percentages of the recovered radioactivity; degradate concentrations were reported in parent equivalents. The test compound, AMBA (a mesotrione degradate), is referred to as "the parent compound" throughout this review for ease of reporting.

In the ***clay soil***, the *parent compound* was initially present (day 0.1) at 72.2% (0.15 ppm) of the applied radioactivity, decreased to 40.1% (0.085 ppm) by 3 days and 30.3% (0.065 ppm) by 7 days posttreatment, was 19.2% (0.041 ppm) at 14 days, and was 7.8% (0.017 ppm) at 28 and 56 days. *Unidentified polar radioactivity* (comprised of an undetermined number of degradates) was initially (day 0.1) 1.6% (0.0034 ppm) of the applied radioactivity, generally increased to a maximum of 14.7% (0.031 ppm) by 14 days posttreatment, and was 3.2% (0.0068 ppm) at 56 days. *Unextracted [^{14}C]residues* were initially (day 0.1) 8.5% of the applied radioactivity, were 20.9-27.6% from 3 to 21 days posttreatment, and were a maximum of 37.2% at 56 days. *Evolved $^{14}CO_2$* was 7.6% of the applied radioactivity at 3 days posttreatment, increased to 17.2% by 7 days and 33.4% by 21 days, and was 42.7% at 56 days.

In the ***silt loam soil***, the *parent compound* was initially present (day 0.1) at 82.8% (0.18 ppm) of the applied radioactivity, decreased to 41.5% (0.088 ppm) by 10 days and 37.4% (0.080 ppm) by 14 days, and was 20.2% (0.043 ppm) at 56 days posttreatment. *Unextracted [^{14}C]residues* were initially (day 0.1) 1.6% of the applied radioactivity, increased to 26.0% by 3 days posttreatment, were a maximum of 47.5% at 28 days, and were 42.8% at 56 days. *Evolved $^{14}CO_2$* was 2.5% of the applied radioactivity at 3 days posttreatment, was 6.8% at 14 days, and was 13.9% at 56 days.

In the ***sandy loam soil***, the *parent compound* was initially present (day 0.1) at 90.8% (0.17 ppm) of the applied radioactivity, decreased to 45.6% (0.085 ppm) by 3 days and 28.4% (0.053 ppm) by 7 days, and was 11.1% (0.021 ppm) at 56 days posttreatment. *Unextracted [^{14}C]residues* were initially (day 0.1) 1.1% of the applied radioactivity, increased to 41.3% by 3 days posttreatment, were 53.6-59.4% from 7 to 21 days, increased to a maximum of 60.2% by 28 days, and were 53.4% at 56 days. *Evolved $^{14}CO_2$* was 0.54% of the applied radioactivity at 3 days posttreatment, was 7.5% at 14 days, and was 17.1% at 56 days.

METHODOLOGY

Samples (60 g) of air-dried, sieved (2 mm) clay soil (collected from West Sussex, England; 15.8% sand, 39.9% silt, 44.4% clay, 3.1% organic matter, pH 4.9, CEC 10.5 meq/100 g; Table 3, p. 29), silt loam soil (collected from Delavan, WI; 16.8% sand, 61.5% silt, 21.8% clay, 2.4% organic matter, pH 6.4, CEC 12.1 meg/100 g), and sandy loam soil (collected from Suffolk, England; 71.9% sand, 9.4% silt, 18.8% clay, 2.5% organic matter, pH 7.9, CEC 8.5 meq/100 g) were weighed into centrifuge bottles, adjusted to 1 gram less than the 40% maximum water-holding capacity, and pre-incubated in darkness at 20 ± 2°C for up to 3 days prior to treatment (p. 17; see Comment #3). Following the pre-incubation period, the samples were treated with uniformly phenyl ring-labeled [^{14}C]AMBA {2-amino-4-(methylsulfonyl) benzoic acid; radiochemical purity ≥98.1%, specific activity 38.9 mCi/mmol; p. 14}, dissolved in $NaHCO_3$ solution, at a nominal application rate of 0.213 ppm (clay and silt loam soils) and 0.187 ppm (sandy loam soil; Table 4, p. 30). Following treatment, the sample bottles were shaken, then uncapped sample bottles were placed inside a desiccator and incubated in darkness at 20 ± 2°C for up to 56 days (p. 18; Figure 2, p. 41). Moist air was passed through the system and into a CO_2 (1 M NaOH) trap. Single soil samples were removed for analysis at 0.1, 3, 7, 10, 14, 21, 28, and 56 days posttreatment for all soils. Volatile trap solutions were collected for analysis and replaced with 1 M NaOH at each sampling interval.

At each sampling interval, soil samples were extracted three times by shaking with 0.05 M NH_4OH followed by a single extraction with acetonitrile, and then centrifuged (p. 19; Figure 3, p. 42). The extracts were combined, acidified to pH 3.5 (formic acid) to precipitate extracted pigments, and centrifuged, and the supernatant was decanted. Extracts were analyzed for total radioactivity by LSC (p. 18); the limit of detection was twice the background (Appendix 2, p. 56). The acidified extracts were partitioned twice with ethyl acetate. The aqueous and organic fractions were concentrated by rotary evaporation, reconstituted in 0.1% H_3PO_4 and acetonitrile, and filtered (0.45 µm). The extract was adjusted to pH 3.5 (HCl or NaOH) and the organic fraction was evaporated under a nitrogen stream. Selected (cloudy) samples were further filtered (0.02 µm) prior to HPLC analysis. The samples were analyzed for the presence of AMBA, parent mesotrione, and MNBA (Table 1, p. 27). Aliquots of the sample extracts were analyzed by HPLC (Ultima C^{18} column) using a mobile phase gradient of water:acetonitrile (modified with 0.1% H_3PO_4; 98:2 to 50:50 to 0:100, v:v; p. 15) with UV (254 nm) and radioactive flow detection (Appendix 3, p. 57); the limit of quantitation was 101 cpm. Samples were co-chromatographed with nonradiolabeled and radiolabeled reference standards. Eluent fractions were collected at one-minute intervals and analyzed for total radioactivity by LSC. A confirmatory method of analysis was not performed on the soil extracts.

In an attempt to remove bound residues, post-extracted soil samples were solubilized by shaking with 0.5 M NaOH followed by 1 M NaOH and centrifuged (p. 19). The caustic extracts were acidified (pH 3.5) and partitioned with ethyl acetate. The fractions were concentrated and analyzed by HPLC as previously described. The remaining post-extracted soil samples were air dried and analyzed for total radioactivity by LSC following combustion.

At each sampling interval, volatile trap solutions were analyzed for total radioactivity by LSC (p. 14); the method used to confirm the presence of $^{14}CO_2$ was not reported.

To determine the viability of the soils, soil samples were analyzed for biomass carbon at the beginning and end of the incubation period (p. 16). Results indicated that the soils were viable (Table 3, p. 29).

DATA SUMMARY

Uniformly phenyl-ring labeled [^{14}C]AMBA (radiochemical purity ≥98.1%), at a nominal application rate of 0.213 ppm (clay and silt loam soils) and 0.187 ppm (sandy loam soil), degraded with respective registrant-calculated DT_{50}s of 3, 6, and 2 days in the clay, silt loam and sandy loam soils adjusted to 40% of the maximum water-holding capacity, and incubated in darkness at 20 ± 2°C for up to 56 days (pp. 19, 24; Figure 10, p. 49; see Comment #4). Degradation was biphasic in all three soils. However, the presence of unacceptably high unextracted residues by day 3 (for each soil) indicates that the analytical method may not have been adequate and renders the validity of the half-lives as questionable. Also, true time zero data were not reported, as degradation was rapid and the first sampling interval was reported as day 0.1 posttreatment; time zero was estimated as the expected concentration based on the nominal application rate (see Comment #5). All data, designated as percentages of the applied, represent percentages of the nominal application. Reported residue concentration data (in ppm) were reviewer-calculated based on the actual application rates and the percentages of the recovered radioactivity; degradate concentrations were reported in parent equivalents. The test compound, AMBA (a mesotrione degradate), is referred to as "the parent compound" throughout this review for ease of reporting.

Clay soil

The parent compound was initially present (day 0.1) at 72.2% (0.15 ppm) of the applied radioactivity, decreased to 40.1% (0.085 ppm) of the applied by 3 days and 30.3% (0.065 ppm) by 7 days posttreatment, was 19.2% (0.041 ppm) of the applied at 14 days posttreatment, and was 7.8% (0.017 ppm) of the applied at 28 and 56 days posttreatment (Table 6, p. 33). Unidentified polar radioactivity (comprised of an undetermined number of degradates, p. 23) was initially present (day 0.1) at 1.6% (0.0034 ppm) of the applied radioactivity, generally increased to a maximum of 14.7% (0.031 ppm) of the applied by

14 days posttreatment, and was 3.2% (0.0068 ppm) of the applied at 56 days posttreatment. Unidentified minor degradates each accounted for ≤7% (≤0.015 ppm) of the applied radioactivity throughout the incubation period (p. 23; tabular data not reported). Unextracted [^{14}C]residues were initially (day 0.1) 8.5% of the applied radioactivity, were 20.9-27.6% of the applied from 3 to 21 days posttreatment, and were a maximum of 37.2% of the applied at 56 days posttreatment (Table 5, p. 31). Evolved $^{14}CO_2$ was 7.6% of the applied radioactivity at 3 days posttreatment, increased to 17.2% by 7 days and 33.4% by 21 days, and was a 42.7% of the applied at 56 days posttreatment.

The material balances (based on LSC analysis) were 91.3%-99.9% of the applied radioactivity throughout the incubation period (Table 5, p. 31).

Silt loam soil

The parent compound was initially present (day 0.1) at 82.8% (0.18 ppm) of the applied radioactivity, decreased to 41.5% (0.088 ppm) by 10 days and 37.4% (0.080 ppm) by 14 days, and was 20.2% (0.043 ppm) of the applied at 56 days posttreatment (Table 8, p. 36). Unidentified polar radioactivity (comprised of an undetermined number of degradates, p. 23) was initially present (day 3) at 2.8% (0.0060 ppm) of the applied radioactivity, increased with variability to a maximum of 5.0% (0.011 ppm) of the applied by 21 days posttreatment, and was 1.4% (0.0030 ppm) of the applied at 56 days posttreatment. Unidentified minor degradates each accounted for ≤7% (≤0.015 ppm) of the applied radioactivity throughout the incubation period (p. 23; tabular data not reported). Unextracted [^{14}C]residues were initially (day 0.1) 1.6% of the applied radioactivity, increased to 26.0% of the applied by 3 days posttreatment, were a maximum of 47.5% of the applied at 28 days posttreatment, and were 42.8% of the applied at 56 days posttreatment (Table 7, p. 34). Evolved $^{14}CO_2$ was 2.5% of the applied radioactivity at 3 days posttreatment, was 6.8% of the applied at 14 days posttreatment, and was 13.9% of the applied at 56 days posttreatment.

The material balances (based on LSC analysis) were 90.6-97.9% of the applied radioactivity throughout the incubation period (with the exception of 87.0% at 56 days posttreatment; Table 7, p. 34); a pattern of loss was not observed.

Sandy loam soil

The parent compound was initially present (day 0.1) at 90.8% (0.17 ppm) of the applied radioactivity, decreased to 45.6% (0.085 ppm) by 3 days and 28.4% (0.053 ppm) by 7 days, and was 11.1% (0.021 ppm) of the applied at 56 days posttreatment (Table 10, p. 39). Unidentified polar radioactivity (comprised of an undetermined number of degradates, p. 23) was initially present (day 3) at 1.5% (0.0028 ppm) of the applied radioactivity, increased with variability to a maximum of 3.3% (0.0062 ppm) of the

applied by 21 days posttreatment, and was 0.6% (0.0011 ppm) the applied at 56 days posttreatment. Unidentified minor degradates each accounted for ≤7% (≤0.013 ppm) of the applied radioactivity throughout the incubation period (p. 23; tabular data not reported). Unextracted [^{14}C]residues were initially (day 0.1) 1.1% of the applied radioactivity, increased to 41.3% of the applied by 3 days posttreatment, were 53.6-59.4% of the applied from 7 to 21 days posttreatment, increased to a maximum of 60.2% of the applied by 28 days posttreatment, and were 53.4% of the applied at 56 days posttreatment (Table 9, p. 37). Evolved $^{14}CO_2$ was 0.54% of the applied radioactivity at 3 days posttreatment, was 7.5% of the applied at 14 days posttreatment, and was 17.1% of the applied at 56 days posttreatment.

The material balances (based on LSC analysis) were 90.5-97.5% of the applied radioactivity throughout the incubation period (with the exception of 89.6% and 88.0% at 21 and 56 days, respectively; Table 9, p. 37); a pattern of loss was not observed.

COMMENTS

1. Unextracted [^{14}C]residues were high for all three soils by 3 days posttreatment (Tables 5, 7, 9, pp. 31, 34, 37). Unextracted [^{14}C]residues in the clay, silt loam, and sandy loam soils were 27.6%, 26.0% and 41.3% of the applied radioactivity, respectively, by day 3 and were respective maximums of 37.2%, 47.5% and 60.2%. The presence of these high concentrations of unextracted residues by day 3 (for each soil) indicates that the analytical method, specifically extraction, may not have been adequate for the determination of AMBA. Without the appropriate extraction procedures to ensure quantitative recovery of the compounds of interest, the validity of the reported half-lives is questionable. Following preliminary extractions, soil samples were subjected to caustic (NaOH) extraction followed by acidification (pH 3.5) and partitioning with ethyl acetate (p. 19). The reviewer noted that AMBA was detected in both the organic and aqueous fractions of the NaOH extract; therefore, the initial extraction method was not adequate for quantitative AMBA extraction. Based on the remaining residues, it is possible that the caustic extractions were also inadequate for recovery of the compounds of interest. Unless appropriate extraction procedures are utilized, it cannot be confirmed that additional parent material or major degradates were not present in the fraction of the applied radioactivity that was classified as nonextractable (labeled "unextracted" in the data tables). Additionally, despite the use of methods which would have allowed for the collection of such data, organic matter fractionation data were not reported. 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

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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. Duplicate samples were not utilized in the study. The use of single test samples is generally not considered to be good laboratory practice; at a minimum, duplicate samples should be utilized for each sampling interval and each treatment. *However, single samples at each time interval in each of three soils is preferable to duplicates in the presently required one test soil.*

3. The reviewer could not confirm that the soil moisture was maintained at 75% of the soil moisture at 0.33 bar during the incubation period. The study author reported that the test system was initially adjusted to 1 gram less than the 40% maximum water-holding capacity prior to the pre-incubation (p. 17). At the time of treatment, the 40% maximum water-holding capacity was reached with the addition of the test material in 1 mL of aqueous $NaHCO_3$. *However, the study author did not report the relationship between the 75% of 0.33 bar and that at 40% of maximum water-holding capacity.* Clarification is desirable. Subdivision N Guidelines require that aerobic soil metabolism studies be performed at 75% of the soil moisture at 0.33 bar in order to ensure aerobic conditions and soil viability. The reviewer notes that it is likely that the moisture content used was suitable for aerobic metabolism.

4. The reported "DT_{50}s" and other DTs are said to be based on a two-compartment regression model with first-order kinetics applied to each compartment (p. 19; p. 24; Figure 10, p. 49).

5. The study author reported that a treated sample from each soil was analyzed by 2 hours posttreatment (p. 18). Degradation was rapid during this interval; therefore, the study author reported the data as "day-0.1" rather than as "time 0." The radiochemical purity of the test solution was reported as time 0 data (p. 20); however, this is not adequate for the determination of a half-life because it does not confirm that the soil samples received the appropriate amount of the treatment solution. Additionally, data were not provided which indicated that all of the radioactivity present in the treatment solution was present as AMBA. Radiochemical purity is more correctly used to describe the fraction of the compound of interest which is radiolabeled, rather than the fraction of total test material which is present as the compound of interest.

6. It could not be determined whether the clay soil was representative of the intended use area of the parent compound. It is preferred that the soil used in aerobic soil metabolism studies be either a sandy loam or silt loam or representative of the intended use area. The study author stated that the soils used in the study were from areas of agricultural use and were "representative of compound use locations with a wide range of soil characterization parameters" (p. 20).

7. The incubation temperature was not held constant at 25 ± 1°C, as required by Subdivision N Guidelines. Instead, it was maintained at 20 ± 2°C throughout the incubation period.

8. Residue data were reported only as percentages of the nominal application rate; concentration data were not provided. All concentration data (in ppm based on parent equivalents) were reviewer-calculated from the nominal application rate and the reported percentages of the applied radioactivity (Tables 4, 6, 8, 10; pp. 30, 33, 36, 39). In future studies submitted to the EPA, it is necessary that data be reported as both percentages of the applied radioactivity and in units of concentration, such as ppm.

9. The study author stated that the target application rate (192.8 g/ha) was based on the maximum amount of AMBA expected in the samples (p. 17); the expected amount was determined using the highest percentage of AMBA found in other metabolism studies of the parent mesotrione (38% AMBA in an anaerobic aquatic study, MRID 44505131) and an application rate of 400 g/ha for mesotrione (p. 17). The reviewer notes that the nominal application rate in the study was 212 g/ha (0.213 ppm) for the clay and silt loam soils, and 187 g/ha (0.187 ppm) for the sandy loam soil.

10. The limit of detection was not reported for HPLC analysis, and the limit of quantitation was not reported for LSC analysis. Both limits of detection and quantitation should be reported for each analytical method utilized to allow the reviewer to evaluate the adequacy of the methods for determination of the parent and degradates.

11. The study author reported that two failures occurred in the air system during the incubation period for the clay and silt loam soils (pp. 20, 21). The malfunctions occurred on May 17, 1995 (5 days posttreatment) and on June 25, 1995. The study author reported that material balances remained above 90% during the malfunction at 5 days posttreatment and the second malfunction occurred after the DT_{50} was observed.

12. The study author reported that unidentified polar radioactivity was detected in the clay soil system at a maximum of 14.7% (day-14) and was likely comprised of several degradates (p. 23). It was not specified whether any of the degradates comprised at least 10% of the applied radioactivity.

13. The soil series name for the silt loam soil was not reported. The clay and sandy loam soils were foreign soils.

14. Sterilized test systems were not prepared and incubated along with the treated test system as controls. Sterile controls would have helped to quantify the extent of abiotic degradation occurring in the test systems.

15. Microbial biomass after 57-58 days of incubation decreased to 68-77% (loss of 23-32%) of the values 3-4 days after start of incubation (Table 3, p. 29).

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