8-31-00

DATA EVALUATION RECORD

STUDY 9

CHEM 417300	Glyphosate acid	§162-3
CAS No. 1071-83-6		
FORMULATION--00--ACTIVE INGREDIENT		

STUDY ID 44621801

Dix, M. E. 1998. Glyphosate acid - Determination of aquatic metabolism under anaerobic conditions. Springborn Laboratories Study No.: 13582.0795.6101.755. Unpublished study performed by Springborn Laboratories, Inc., Wareham, MA; and submitted by Industria Prodotti Chimici S.P.A., Milan, ITALY.

DIRECT REVIEW TIME = 55 Hours

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CONCLUSIONS

Metabolism - Anaerobic Aquatic

1. Although the study contains some deficiencies, the data suggest ~~This study is not scientifically valid and does not provide~~ useful information on the anaerobic aquatic metabolism of glyphosate acid. The parent data were variable between replicates and over time; thus, the calculated half-life is of questionable validity. Also, the material balances were variable throughout the study and were <90% of the applied radioactivity at the end of the study. (actually 85% is not bad!)

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2. This study does not meet Subdivision N Guidelines for the fulfillment of EPA data requirements on anaerobic aquatic metabolism for the following reasons:

   (i) the analytical method may have been inadequate;

   (ii) a major degradate was not identified; However, identification is underway and

   (iii) degradate data were not reported separately for the sediment and aqueous phases, which is important in determining partition between sediment + the water column.

3. Nonradiolabeled plus phosphonomethylene-labeled [^{14}C]glyphosate acid, at a nominal application rate of 2.78 mg/L, degraded with a registrant-calculated half-life of 199 days ($r^2 = 0.94$) in anaerobic flooded clay loam sediment that was incubated in darkness at 22 ± 1 °C for up to 365 days. However, the results were of questionable validity due to variability in the data. The observed first half-life occurred between 120 and 181 days posttreatment. All data, designated as percentages of the applied radioactivity, represent percentages of the nominal application. In the total sediment/water system, the parent was initially 69.0% (mean of 76.9% and 61.1%) of the applied radioactivity, was 59.2% at 1 day, was a maximum of 83.1% (mean of 87.3% and 78.9%) at 7 days, was 66.2-67.2% at 14-30 days, was 49.0% (mean of 66.3% and 31.7%) at 120 days and 39.8% (mean of 32.9% and 46.7%) at 181 days, and was 20.2% at 365 days posttreatment. In the aqueous phase, the parent was initially 8.1% (mean of 14.4% and 1.7%) of the applied radioactivity, was 0.94-3.2% from 1 to 60 days, and was <0.7% from 90 to 365 days posttreatment. In the sediment phase, the parent was initially 61.0% of the applied radioactivity, was a maximum of 81.5% at 7 days, was 64.1-65.3% at 14-30 days, was 49.0% (mean of 66.3% and 31.7%) at 120 days and 39.8% (mean of 32.8% and 46.7%) at 181 days, and was 20.1% at 365 days posttreatment. Only whole system data were reported for the remaining residues. In the total sediment/water system, the major degradate AMPA was initially (time 0) 8.5% of the applied radioactivity, was a maximum of 21.9% at 60 days, and was 12.2% at 365 days posttreatment. An unidentified major degradate designated as "unknown polar metabolite" accounted for 11.6% of the applied radioactivity at 365 days posttreatment; complete and tabular data were not provided. Nonextractable [^{14}C]residues were 2.8-5.1% of the applied radioactivity from 0 to 30 days, were a maximum of 16.3% at 120 days, and were 9.7% at 365 days posttreatment;

[^{14}C]residues associated with the humic acid, fulvic acid, and humin fractions were 0.7%, 4.6%, and 8.6% of the applied radioactivity, respectively, at 120 days posttreatment. Evolved $^{14}CO_2$ was 9.0% of the applied radioactivity at 60 days, increased to 15.9% by 90 days, and was 19.5-23.9% from 181 to 365 days posttreatment. The distribution ratio of [^{14}C]residues between the sediment and water fractions was not reported; however, the majority of the residues were observed in the sediment phase throughout the incubation period. The respective percentages of the applied radioactivity in the sediment phase *vs.* the aqueous phase were 84.4-99.1% *vs.* 1.4-8.9% at 0-60 days, were 77.4% *vs.* 0.68% at 181 days, and were 55.2% *vs.* 0.96% at 365 days posttreatment.

METHODOLOGY

Samples (50 g) of sieved (2 mm) clay loam sediment (28% sand, 42% silt, 30% clay, 3.4% organic matter, pH 7.7, CEC 29.2 meq/100g; Table 1, p. 30) collected from Mercer County, Ohio, were placed into 250 mL Erlenmeyer flasks (autoclaved) with glass caps and equipped with inlet and outlet ports for air exchange (p. 13). Samples were flooded with 100 mL of pond water (collected from the same site as the sediment; conductivity 500 μmhos, hardness 320 mg/L as $CaCO_3$, alkalinity 88 mg/L as $CaCO_3$, pH and total suspended solids not reported; Table 2, p. 31). The sediment/water systems were sealed and pre-incubated anaerobically (nitrogen atmosphere) in darkness for 24 days (p. 14). Following the pre-incubation period, the sediment/water systems were treated with nonradiolabeled plus phosphonomethylene-labeled [^{14}C]glyphosate acid {N-(phosphonomethyl)glycine; radiochemical purity 95.9%, specific activity 54 mCi/mmol; p. 11}, dissolved in water:acetonitrile (95:5, v:v), at a nominal application rate of 2.78 mg/L. The sediment/water systems were incubated anaerobically (nitrogen atmosphere) in darkness at 22 ± 1 °C (see Comment #7) for up to 365 days. The flasks were purged daily with moist, filter-sterilized (0.2 μm) nitrogen gas and the effluent gas was passed through an ethylene glycol trap and two 1.0 *N* KOH traps connected in series (Figure 1, p. 45). Duplicate sediment/water samples were removed for analysis at 0, 1, 3, 7, 14, 30, 60, 90, 120, 181, 273, and 365 days posttreatment (p. 15). The volatile trap solutions were removed for analysis and replaced at each sampling interval (with the exception of time 0). Anaerobic conditions were monitored by taking redox probe measurements (one measurement) prior to study initiation and at 2, 9, 16, 33, 62, 92, 121, 181, 280, and 365 days posttreatment (Table 4, p. 33).

At each sampling interval, the aqueous phase was separated from the sediment phase by decanting followed by centrifuging and decanting of the supernatant (p. 15). Aliquots of the aqueous phase were analyzed for total radioactivity by LSC before (triplicate) and after (duplicate) centrifugation; the limit of detection was 0.0017 mg/L and the limit of quantitation was 0.0034 mg/L (p. 24). Aliquots of the aqueous phase were analyzed by HPLC (Biorad glyphosate column) using an isocratic mobile phase of methanol:2 mM KH_2PO_4 (pH 2; 4:96, v:v) with UV (wavelength not reported) and radioactive flow detection (p. 18). Samples were co-chromatographed with nonradiolabeled reference standards of the parent and AMPA (p. 11); AMPA was visualized using refractive index detection (p. 22). Eluent fractions were collected at 0.5 or 1.0 minute intervals and analyzed for total radioactivity by LSC. To confirm the identities of the parent and AMPA, selected samples were analyzed by HPLC (Zorbax SB-CN column) using an isocratic mobile phase of acetonitrile:0.025 M cetyltrimethylammonium bromide (20:80, v:v) with radioactive flow detection (p. 19). Eluent fractions were collected and analyzed by LSC.

Prior to extraction, soil subsamples were analyzed for total radioactivity by LSC following combustion (p. 15). Sediment samples were extracted twice by shaking with 0.5 *N* NaOH and centrifuged. The two extracts for each sample collected from 0 to 30 days posttreatment were analyzed by LSC and HPLC separately, and the two extracts were combined for each of the samples collected from 60 to 365 days and analyzed by LSC and HPLC as previously described. Triplicate subsamples of the post-extracted soil were analyzed by LSC following combustion (p. 16). To determine [^{14}C]residues associated with the humic acid, fulvic acid and humin fractions, selected post-extracted sediment samples (days 60-365) were further extracted by shaking with 0.1 *N* NaOH and $CaCl_2$ and centrifuged (p. 16). The caustic supernatant was adjusted to pH 1 (HCl) to precipitate humic acids. Following filtration, triplicate aliquots of the filtrate were analyzed by LSC to determine [^{14}C]residues associated with the fulvic acid fraction. Triplicate aliquots of the filter paper containing the precipitate were analyzed by LSC to determine [^{14}C]residues associated with the humic acid fraction. To determine [^{14}C[residues associated with the humin fraction, triplicate subsamples of the post-extracted sediment were analyzed for total radioactivity by LSC following combustion.

Aliquots of the KOH traps, ethylene glycol trap, and overflow trap solutions were analyzed for total radioactivity by LSC at each sampling interval (pp. 15, 16). To confirm the presence of $^{14}CO_2$ in the KOH trap solutions, aliquots of selected samples were analyzed by HPLC (Interaction ORH-801 column) using an isocratic mobile phase of 10 mM H_2SO_4 with radioactive flow detection (p. 19); the presence of $^{14}CO_2$ was confirmed (p. 24; Figures 19-20; pp. 63-64).

To confirm the presence of anaerobic conditions, the pH and redox potentials of the test samples were measured at selected intervals throughout the incubation period (p. 14); the dissolved oxygen content was not measured. Conditions were moderately reducing to reducing throughout the incubation period. The redox potential was initially -265 mV, was -16.8 to -54.6 mV from 33 to 181 days posttreatment, and was -154.1 mV at 365 days posttreatment (Table 4, p. 33). The pH was 7.1-7.9 throughout the study period.

To determine concurrent recoveries, duplicate samples of pond water were fortified with the parent at 0.22 mg/L or 2.8 mg/L (time 0) and duplicate samples of sediment were fortified at 2.8 mg/kg or 5.6 mg/kg (p. 15). The pond water and sediment samples were extracted and analyzed as previously described. The mean concurrent recovery of the parent from the pond water samples was 95.8 ± 6.6% (Table 8, p. 41). The mean concurrent recovery of the parent from the sediment samples was 94.4 ± 11.7% (1 of 22 samples >120%; Table 7, p. 40).

To confirm sediment viability, sediment samples were extracted with sterilized water (1:99, w:v) and the extract was serially diluted (10^{-3} to 10^{-7}) and plated on nutrient agar plates which were incubated aerobically or anaerobically (p. 19). Aerobic bacteria were present at 3.3 x 10^3 to 9.1 x 10^6 CFU/g soil prior to test initiation to 174 days posttreatment, and anacrobic bacteria were present at 4.4 x 10^5 to 5.3 x 10^6 CFU/g soil from 181 to 365 days posttreatment (Table 3, p. 32).

DATA SUMMARY

Nonradiolabeled plus phosphonomethylene-labeled [^{14}C]glyphosate acid (radiochemical purity 95.9%), at a nominal application rate of 2.78 mg/L, degraded with a registrant-calculated half-life of 199 days ($r^2 = 0.94$) in anaerobic flooded clay loam sediment that was incubated in darkness at 22 ± 1 °C for up to 365 days (p. 23; Figure 17, p. 61). However, the results were of questionable validity due to variability in the data (see Comment #1). The observed first half-life occurred between 120 and 181 days posttreatment. All data, designated as percentages of the applied radioactivity, represent percentages of the nominal application. In the total sediment/water system, the parent compound was initially present at 69.0% (mean of 76.9% and 61.1%) of the applied radioactivity, was 59.2% of the applied at 1 day posttreatment, was a maximum of 83.1% (mean of 87.3% and 78.9%) of the applied at 7 days posttreatment, was 66.2-67.2% of the applied at 14-30 days posttreatment, was 49.0% (mean of 66.3% and 31.7%) at 120 days and 39.8% (mean of 32.9% an 46.7%) at 181 days, and was 20.2% of the applied at 365 days posttreatment (Table 9, p. 42). In the aqueous phase, the parent compound was initially present at 8.1% (mean of 14.4% and 1.7%) of the applied radioactivity, was 0.94-3.2% of the applied from 1 to 60 days posttreatment, and was <0.7% of the applied from 90 to 365 days posttreatment. In the sediment phase, the parent compound was initially present at 61.0% of the applied radioactivity, increased to a maximum of 81.5% of the applied by 7 days posttreatment, was 64.1-65.3% of the applied at 14-30 days posttreatment, was 49.0% (mean of 66.3% and 31.7%) at 120 days and 39.8% (mean of 32.8% and 46.7%) at 181 days, and was 20.1% of the applied at 365 days posttreatment. Only whole system data were reported for the remaining residues (see Comment #4). In the total sediment/water system, the major degradate

aminomethylphosphonic acid (AMPA)

was initially (time 0) detected at 8.5% of the applied radioactivity, decreased to 3.0-3.3% of the applied by 7-14 days posttreatment, increased to a maximum of 21.9% of the applied by 60 days posttreatment, was 12.3-20.0% of the applied from 90-273 days posttreatment, and was 12.2% of the applied radioactivity at 365 days posttreatment. An unidentified major degradate

"unknown polar metabolite"

accounted for 11.6% of the applied radioactivity at 365 days posttreatment (p. 25; see Comment #3); complete and tabular data were not provided. Uncharacterized radioactivity was ≤7.9% of the applied radioactivity throughout the incubation period. Nonextractable [^{14}C]residues were 2.8-5.1% of the applied radioactivity from 0 to 30 days posttreatment, increased to a maximum of 16.3% of the applied by 120 days posttreatment, and were 9.7% of the applied at 365 days posttreatment (Table 6, p. 39); [^{14}C]residues associated with the humic acid, fulvic acid, and humin fractions were 0.7%, 4.6%, and 8.6% of the applied radioactivity, respectively, at 120 days posttreatment (Table 16, p. 43). Evolved $^{14}CO_2$ was 9.0% of the applied radioactivity at 60 days posttreatment, increased to 15.9% of the applied by 90 days posttreatment, and accounted for 19.5-23.9% of the applied from 181 to 365 days posttreatment (Table 9, p. 42).

The distribution ratio of [^{14}C]residues between the sediment and aqueous fractions was not reported; however, the majority of the residues were observed in the sediment phase throughout the incubation period (Table 6, p. 39). The respective percentages of the applied radioactivity in the sediment phase *vs.* the aqueous phase were 84.4-99.1% *vs.* 1.4-8.9% at 0-60 days posttreatment, were 77.4% *vs.* 0.68% at 181 days posttreatment, and were 55.2% *vs.* 0.96% at 365 days posttreatment.

Material balances (based on LSC analysis of individual replicates) were 85.6-105.0% of the applied radioactivity from 0 to 273 days posttreatment and were 72.7-78.6% of the applied at 365 days posttreatment (Table 6, p. 39; see Comment #2).

COMMENTS

1. The registrant-calculated half-life (199 days; $r^2 = 0.94$; Figure 17, p. 61) was of questionable validity due to variability in the data between replicates and over time. The parent was initially present in the total sediment/water system at 69.0% (mean of 76.9% and 61.1%) of the applied radioactivity, was a maximum of 83.1% (mean of 87.3% and 78.9%) at 7 days, was 49.0% (mean of 66.3% and 31.7%) at 120 days and 39.8% (mean of 32.9% an 46.7%) at 181 days, and was 20.2% of the applied at 365 days posttreatment (Table 9, p. 42). In addition, the registrant-calculated half-life does not agree with the observed first half-life which occurred between 120 and 181 days posttreatment, and the registrant-calculated r^2 (0.94) value is unexpectedly high when compared with the graphical representation of the data in Figure 17 (p. 61). Also, the increase in nonextractable [^{14}C]residues (16.0-16.3%) at 120-181 days may have affected the half-life

determination (Table 6, p. 39); only 7.6-13.9% was characterized by organic matter fractionation (Table 10, p. 43). The reviewer noted additional problems with the data which suggest that the analytical method was inadequate. In the aqueous phase, the parent was initially present in the replicate samples at 1.7% and 14.4% of the applied radioactivity (Table 9, p. 42). The data for the degradate AMPA in the total system were variable from 3 to 7 days posttreatment. Also, the material balances were variable throughout the study (also see Comment #2). The study author did not provide an explanation for the observed variability in the data.

2. Material balances (85-110%) were variable throughout the study, with no clear pattern of decline from 0 to 273 days posttreatment, and were 72.7-78.6% of the applied at 365 days posttreatment (Table 6, p. 39). In addition, the reviewer noted several occasions when differences in material balances between replicates or sequential sampling intervals were >10% of the applied radioactivity. Subdivision N Guidelines require that material balances be between 90-110% of the applied radioactivity.

3. A major degradate was not identified. The study author stated that an unidentified major polar metabolite accounted for 11.6% of the applied radioactivity in the total system at 365 days posttreatment (p. 25; also see Comment #4); complete and tabular data were not reported. In contrast, in Appendix I (p. 69), the study author stated that the unknown accounted for 8.3% of the applied at 365 days posttreatment and did not exceed 10% of the applied at any sampling interval; tabular data were not provided. Clarification by the registrant is necessary. The identity of the metabolite was still under investigation at the time the study report was written; in Appendix I (p. 69), the study author stated that the polar unknown was likely methyl-AMPA. Subdivision N Guidelines require that all degradates present at greater than 10% of the applied radioactivity be identified.

4. Complete characterization data were not reported separately for the aqueous and sediment phases. The parent data for the aqueous and sediment phases were reported separately; however, characterization data for AMPA and an unidentified major degradate, and uncharacterized radioactivity were reported only for the total sediment/water system. The study author did not provide an explanation as to why the data were not reported separately for the two phases. Separate data for the aqueous and sediment phases are necessary to determine the degree of partitioning of the parent and its metabolites between the two phases and changes in partitioning over time. In addition, data for a major unidentified degradate ("unknown polar metabolite") were not reported in tabular form. The reviewer noted that [^{14}C]residues in the water phase were <10% of the applied at all sampling intervals (Table 6, p. 39). In the future, aquatic metabolism studies submitted to EPA should include data tables reporting residues of the parent, all degradates, and any remaining [^{14}C]residues in the aqueous and sediment phases.

5. The reviewer notes that the percentage of radioactivity present as the parent compound remained unchanged in the sediment phase from 14 to 30 days posttreatment. The parent

was 81.5% of the applied radioactivity at 7 days, was 64.1-65.3% from 14 to 30 days, and was 49.4% at 60 days posttreatment (Table 9, p. 42). The study author did not provide an explanation for the apparent stability of the parent in the sediment between these two sampling intervals.

6. Residue data were only reported as percentages of the applied radioactivity; concentration data were not reported. In future studies submitted to the EPA, it is necessary that data also be reported in units of concentration, such as ppm.

7. The recorded incubation temperature ranged from 17.2°C to 28.9°C (Table 5, pp. 34-38); however, the reviewer noted that the minimum and maximum temperature observed daily were usually constant (± 1°C) and were generally within 22 ± 1°C. The temperature was reported as such in this DER.

8. The study author stated that test conditions were aerobic from 220 to 241 days posttreatment (p. 26); the redox potentials were not reported for this time period. The study author stated that anaerobic conditions were re-established by increasing the number of purging (nitrogen) intervals and by adding dextrose to all remaining test vessels on day 239. The redox potential was -16.8 mV at 181 days and was -471 mV at 280 days posttreatment (Table 4, p. 33).

9. The maximum application rate of the parent was not reported. The study author stated that the application rate (2.78 mg/L in water; 5.55 mg/kg in sediment) was chosen to sufficiently establish degradation kinetics and to quantify significant metabolites of the parent (p. 14).

10. The proposed metabolic pathway for the anaerobic aquatic metabolism of the parent was presented in Figure 21 (p. 65).

R

glyphosate Metabolism DER

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