DATA EVALUATION RECORD UNDATED

STUDY 5

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CHEM 103601 Glyphosate §165-1

FORMULATION--15--SOLUBLE CONCENTRATE (SC/L)

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STUDY ID 41543201
Nicholls, R.G. 1990. Confined rotational crop study of glyphosate. Part I: In-field portion. Pan-Agricultural Project No. Amended Report EF-88-22. R.D. No. 991. Monsanto Report No. MSL-9810. Performed by Pan-Agricultural Laboratories, Inc., Madera, CA, and submitted by Monsanto Agricultural Company, St. Louis, MO.

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STUDY ID 41543202
McMullan, P.C., J.L. Honegger, and E.W. Logusch. 1990. Confined rotational crop study of glyphosate. Part II: Quantitation, characterization, and identification of glyphosate and its metabolites in rotational crops. (Part I: MSL-9810). Laboratory Project No. MSL-9811. R.D. No. 991. Performed by and submitted by Monsanto Agricultural Company, St. Louis, MO.

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DIRECT REVIEW TIME = 10

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CONCLUSIONS:

Confined Accumulation - Rotational Crops

1. Studies MRID #41543201 and 41543202 do not satisfy the confined accumulation in rotational crop (165-1) data requirement at this time for the following reasons:

a) Freezer storage stability data on glyphosate and its degradates in the plant and soil substrates must be provided.

b) The test substance was incompletely characterized.

2. $^{14}$C-Glyphosate residues accumulated in lettuce, carrots, and barley planted 30, 119, and 364 days after sandy loam soil was treated with glyphosate at 3.71 lb ai/A. Accumulation decreased as the length of the rotation increased. In crops planted at 30 days posttreatment, [$^{14}$C]residues at harvest were 0.097 ppm in lettuce, 0.051 and 0.037 ppm in carrot tops and roots, respectively, and 0.188 and 0.175 ppm in barley grain and straw, respectively. In immature lettuce harvested at 40 and 60



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days postplanting, [$^{14}$C]residues were 0.108 and 0.048 ppm, respectively (Table 7). In crops planted at 119 days posttreatment, [$^{14}$C]residues at harvest were 0.037 ppm in lettuce, 0.028 and 0.017 ppm in carrot tops and roots, respectively, and 0.078 and 0.056 ppm in barley grain and straw, respectively. In immature lettuce harvested at 28 and 48 days postplanting, [$^{14}$C]residues were 0.059 and 0.055 ppm, respectively (Table 7). In crops planted at 364 days posttreatment, [$^{14}$C]residues at harvest were 0.028 ppm in lettuce, 0.018 and 0.0096 ppm in carrot tops and roots, respectively, and 0.047 and 0.061 ppm in barley grain and straw, respectively. In immature lettuce harvested at 35 and 61 days postplanting, [$^{14}$C]residues were 0.057 and 0.043 ppm, respectively; in barley forage harvested at 48 days postplanting, [$^{14}$C]residues were 0.056 ppm.

METHODOLOGY:

[$^{14}$C]Glyphosate (radiochemical purity 96.5%, specific activity 1.31 mCi/mmole, Monsanto) was formulated as the isopropylamine salt, a tallowamine surfactant was added, and the substance was prepared to the same formulation as Roundup herbicide (SC/L, final purity unspecified). The formulated test substance was applied as a spray at 3.71 lb ai/A to three plots (each 16.6 x 2 feet with a 3-foot buffer zone between each plot) of seedling ryegrass (height 4-6 inches) growing on sandy loam soil (62-70% sand, 21-29% silt, 9% clay, 0.3-0.5% organic matter, pH 6.2-7.3, CEC 3.6-4.4 meq/100 g) located in Madera, California on April 1, 1988. Three additional plots (same size) of seedling ryegrass were treated with unlabeled formulated glyphosate to serve as controls. At 7 days posttreatment, the treated and control plots were rototilled to a 4-inch depth, then planted to soybeans. Soybeans were harvested when immature (23, 63, and 112 days postplanting; 30, 70, and 119 days posttreatment) and at maturity (175 days postplanting; 182 days posttreatment).

At 30, 119, and 364 days posttreatment, one each of the treated and control plots was planted to lettuce, carrots, and barley; each crop covered one-third of a plot (Figure 3). Prior to planting the rotational crops, soybean foliage (if present) was collected and the plot was rototilled to a depth of 4 inches. Lettuce was harvested twice when immature (28-40 and 48-61 days postplanting) and at maturity (62-91 days postplanting); carrots and barley were harvested at maturity (91-124 and 95-195 days postplanting, respectively). An additional immature barely forage sample was harvested at 48 days postplanting from the 364-day rotational planting. Crop plants were separated into their various components and stored frozen until analysis. Nine or eighteen soil cores (1-inch diameter, 0- to 12-inch depth) were taken using a zero-contamination probe prior to and immediately posttreatment, when the soybeans were planted (7 days posttreatment), when rotational crops were planted (30, 119, and 364 days posttreatment), and at all rotational crop harvest intervals. Cores were divided into 0- to 6- and 6- to 12-inch segments and stored frozen until analysis.

Plant samples were ground to a fine powder with dry ice, then subsamples were analyzed for total radioactivity by LSC following combustion. Additional subsamples were extracted with water for 5 minutes; chloroform was added and the sample extracted an additional 5 minutes. Following centrifugation, the aqueous phase was removed, filtered, concentrated, and analyzed for radioactivity by LSC. Additional aliquots of the aqueous phase were analyzed using HPLC with a cation exchange column and radioactivity detection; for quantitation of radioactivity, fractions were collected at 0.3-minute intervals and analyzed by LSC. Unextractable [$^{14}$C]residues remaining

in the extracted plant tissue were quantified by LSC following combustion.

Aliquots of the aqueous extract from 30-day rotational lettuce was used for degradate isolation and characterization. The degradate aminomethylphosphonic acid (AMPA) was identified by coelution of reference standard AMPA with lettuce extract using HPLC with a cation exchange column. Metabolite I was isolated following repeated injections on the HPLC using the cation exchange column; aliquots of isolated metabolite I were concentrated, purified, and analyzed by HPLC using an anion exchange column, C18 reverse phase column, and a Bio-Rad organic acids column (carbohydrate analysis); reference glucose, fructose, and sucrose standards were also analyzed to compare retention times. Aliquots of purified metabolite I were also analyzed by NMR and applied to a Bio-Gel P-2 column (molecular weight range 100-1800); glucose, fructose, and sucrose standards were analyzed for comparison.

To further characterize unextractable [$^{14}$C]residues, extracted 105-day lettuce, and 125-day barley grain and barley straw tissues were homogenized with 5.0 M ammonium hydroxide; resulting extracts were concentrated and analyzed using HPLC with a cation exchange column. The extracted tissue was heated at 100 C for 16 hours with dimethyl sulfoxide (DMSO) to remove starch and lignin; the resulting extract was concentrated and an aliquot analyzed by LSC. Remaining DMSO extract was lyophilized and the resulting solid residue was incubated with amyloglucosidase in sodium acetate buffer (pH 4.5) at 55 C for 6 hours to release glucose from the starch. DMSO-extracted barley straw tissue was washed with water, then incubated with cellulase in a sodium acetate buffer (pH 5.0) at 37 C for 16 hours to release glucose from cellulose. Amyloglucosidase- and cellulase-treated samples were centrifuged and the supernatants were concentrated and applied to a sizing column; collected fractions were analyzed by LSC and with Chemstrips for enzymatic detection of glucose.

Soil cores were thawed, homogenized, and analyzed for total radioactivity by LSC following combustion. Subsamples (20 g) were composited according to depth and sampling interval; an aliquot (50 g) of the composite sample was extracted twice by shaking with 0.5 M ammonium hydroxide. Following centrifugation, extracts were combined, concentrated, and analyzed for total radioactivity using LSC and for glyphosate and its degradates using HPLC with a cation exchange column as described above. Glyphosate and AMPA were identified by coelution of reference standards using HPLC with a cation exchange column and an anion exchange column. Metabolite A was isolated from 147-day soil extract following repeated injections on the HPLC using the cation exchange column; aliquots of isolated metabolite A were concentrated and analyzed by HPLC using a cation exchange column, C18 reverse phase column, Bio-Rad organic acids column, and a sizing column (molecular weight range 100-1800). Unextractable [$^{14}$C]residues remaining in the extracted soil were quantified by LSC following combustion.

DATA SUMMARY:

[$^{14}$C]Glyphosate residues accumulated in lettuce, carrots, and barley planted 30, 119, and 364 days after formulated [$^{14}$C]glyphosate (Roundup, SC/L, final purity unspecified) was applied at 3.71 lb ai/A to seedling ryegrass growing on sandy loam soil located in Madera, CA. Accumulation decreased as the length of the rotation increased; the concentration of [$^{14}$C]residues in crops from the 30-day rotation

was approximately 1 to 3x greater than the concentration in crops from the 119-day rotation (designated as 120 days by the study authors) and the concentration of [$^{14}$C]residues in crops from the 119-day rotation was approximately 1 to 2x greater than the concentration in crops from the 364-day rotation (designated as 365 days by the study authors; Table 6).

In crops planted at 30 days posttreatment, [$^{14}$C]residues at harvest were 0.097 ppm in lettuce, 0.051 and 0.037 ppm in carrot tops and roots, respectively, and 0.188 and 0.175 ppm in barley grain and straw, respectively. In immature lettuce harvested at 40 and 60 days postplanting, [$^{14}$C]residues were 0.108 and 0.048 ppm, respectively (Table 7).

In crops planted at 119 days posttreatment, [$^{14}$C]residues at harvest were 0.037 ppm in lettuce, 0.028 and 0.017 ppm in carrot tops and roots, respectively, and 0.078 and 0.056 ppm in barley grain and straw, respectively. In immature lettuce harvested at 28 and 48 days postplanting, [$^{14}$C]residues were 0.059 and 0.055 ppm, respectively (Table 7).

In crops planted at 364 days posttreatment, [$^{14}$C]residues at harvest were 0.028 ppm in lettuce, 0.018 and 0.0096 ppm in carrot tops and roots, respectively, and 0.047 and 0.061 ppm in barley grain and straw, respectively. In immature lettuce harvested at 35 and 61 days postplanting, [$^{14}$C]residues were 0.057 and 0.043 ppm, respectively; in barley forage harvested at 48 days postplanting, [$^{14}$C]residues were 0.056 ppm.

In all crops, water-soluble residues ranged from 15.3 to 56.4% of the recovered radioactivity and unextractable residues ranged from 45.3 to 83.2% (Table 7). In the water-soluble fraction,

glyphosate

was only detected in lettuce (0.0028-0.0041 ppm), barley grain (0.0184 ppm), and barley straw (0.0018 ppm) from the 30-day rotation and in lettuce (0.0009 ppm) from the 119-day rotation (Table 8). The degradate

aminomethylphosphonic acid (AMPA)

was detected in all crops; 0.0027-0.0158 ppm in lettuce, 0.0003-0.0007 and 0.0014-0.0041 ppm in carrot tops and roots, respectively, 0.0093 ppm in barley forage, and 0.0074-0.0336 and 0.0047-0.0065 ppm in barley grain and straw, respectively. Also detected in all crops was metabolite I, identified as a component containing primarily

glucose, but may also contain fructose.

Metabolite I was detected at 0.0079-0.0327 ppm in lettuce, 0.0049-0.0136 and 0.0039-0.0151 ppm in carrot tops and roots, 0.0082 ppm in barley forage, and 0.0031-0.0144 and 0.0046-0.0294 ppm in barley grain and straw (Table 8). Further extraction and characterization of the nonwater-soluble [$^{14}$C]residues comprising 45.3 to 83.2% of the recovered radioactivity indicated that the radioactivity was associated with glucose incorporated into starch, cellulose, and lignin.

In the/ 0- to 6-inch soil depth, total [$^{14}$C]residues were 0.711-0.738 ppm at 0-7 days posttreatment, 0.518 ppm at 30-76 days, then erratically decreased to 0.172-0.179 ppm by 455-482 days (Table 1).

In the 6- to 12-inch depth, [$^{14}$C]residues were 0.0453 ppm at day 0 posttreatment, 0.0088 ppm at 7 days, then ranged from <0.0006 to 0.0036 ppm up to 482 days. [$^{14}$C]Residues were identified only in the 0- to 6-inch soil depth. Glyphosate decreased from 0.6431 ppm at day 0 posttreatment to 0.1189-0.1453 ppm at 30-76 days and was ≤0.0456 ppm from 90-482 days (Table 4). The primary degradate was AMPA, detected at a maximum 0.3014 ppm at 125 days posttreatment, then ranged from 0.0757 to 0.2442 ppm between 105 and 482 days. Also detected was metabolite A tentatively identified as component containing glucose and a glucose derivative. Metabolite A was detected at a maximum 0.202 ppm at day 0 posttreatment, 0.0066-0.0483 ppm at 30-147 days, and was 0.0057-0.0176 ppm at 154-482 days.

During the study (4/1/88 to 7/27/89), rainfall plus irrigation totaled 168.31 inches, air temperatures ranged from 24 to 105 F, and soil temperatures ranged from 35 to 119 F at the 2-inch depth and 39 to 104 F at the 8-inch depth.

COMMENTS:

1. It was not specified how long samples were stored frozen prior to analysis. For storage stability of glyphosate and AMPA in crop matrices, 30-day rotational lettuce, barley straw, and carrot tops were extracted at 5-7 months and again at 12-15 months after receipt (samples were shipped within 1-2 weeks after sampling). The registrant provided HPLC profiles of the extracts and reported that no significant differences existed between the respective profiles; quantitative data were not provided (Figure 10). Similarly, for storage stability of soil residues, it was reported that the 76-day soil sample was extracted and analyzed by HPLC at 7 and 17 months after receipt (sample was shipped one month after sampling). The registrant reported that HPLC profiles indicate that glyphosate, AMPA, and metabolite A were present in roughly the same quantities in the two extracts, but no quantitative data were provided (Figure 9).

   This is not an appropriate method for determining the freezer storage stability of glyphosate and AMPA in the various plant matrices and soil. The samples were not initially analyzed until after 5-8 months of storage; significant degradation could have already occurred as indicated by the day 0 soil extract which contained 0.6431 glyphosate, 0.0334 ppm AMPA, and 0.202 ppm metabolite A. The appropriate method for determining the freezer storage stability of a test substance in a particular matrix involves spiking the matrix with a known amount of technical grade or purer test substance (glyphosate or AMPA), then analyzing for the test substance and its degradates immediately posttreatment and after various lengths of time of frozen storage to determine if any degradation has occurred.

2. Mylar film squares (2 x 2 inches) were used to determine the amount of spray solution that intercepted the soil. It was reported that analysis of the film squares indicated that an average of 42.1-46.2% (range 30.7 to 78.0%) of the total expected radioactivity was applied to the test plots; individual data were not provided.

3. The test substance was formulated (final purity unspecified) and, therefore, was not analytical grade or purer. It was reported the relative proportions of glyphosate, isopropylamine, and surfactant in the formulated test substance were the same as in the formulation of Roundup, but those proportions were not specified. It was assumed that the formulation was a water-soluble concentrate, because the test substance was diluted prior to spray application; typical

formulations of glyphosate are aqueous solution, water-soluble liquid, soluble liquid, and water soluble concentrate.

4. Pretreatment samples were not taken to confirm that the site had not been contaminated with the test substance prior to the study, and data from analysis of the control soil and crop samples were not provided. It was reported that the site had been fallow for eight years prior to initiation of the study, and no known pesticide applications had ever been made to the test site.

5. Immature carrots were not analyzed; immature barley was only harvested and analyzed for the 365-day rotation.

6. The registrant refers to 30-, 120-, and 365-day rotations in the text and tables. The rotations were actually 30, 119, and 364 days.

7. Diazinon was applied at 15 lb/A on June 29, 1988 to the 119- and 364-day plots for ant control. Fertilizer (15-15-15) was applied at 30-150 lb/A seven times; March 18 and 21, April 8, May 1, July 29, and September 2, 1988 and June 9, 1989.

8. Data concerning residues in the primary crop (soybeans) were not reviewed as they are not pertinent to Subdivision N guidelines.

9. The control plot was located 275 feet west of the treated plot, there was a 0.03% slope declining to the east, and the area contained no subsurface drainage system.

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