12-17-91

DATA EVALUATION RECORD

STUDY 7

CHEM 103601 Glyphosate §165-4

FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 41228301
Forbis, A.D. 1989. Uptake, depuration and bioconcentration of ^{14}C glyphosate to bluegill sunfish (Lepomis macrochirus). Part I: MSL-9304. Laboratory Project No. MSL-9304. R.D. No. 955. Performed by Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO, and submitted by Monsanto Agricultural Company, St. Louis, MO.

STUDY ID 41228302
Ridley, W.P. and K.A. Chott. 1989. Uptake, depuration and bioconcentration of 14C glyphosate to bluegill sunfish (Lepomis macrochirus). Part II: Characterization and quantitation of glyphosate and its metabolites. Laboratory Project No. MSL-9303. R.D. No. 955. Performed and submitted by Monsanto Agricultural Company, St. Louis, MO.

DIRECT REVIEW TIME = 7

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CONCLUSIONS:

Laboratory Accumulation - Fish

1. Studies MRID #41228301 and 41228302 completely satisfy the bioaccumulation in fish (165-4) data requirement for glyphosate.

2. Glyphosate residues did not significantly accumulate in bluegill sunfish exposed to glyphosate at 12 ppm for 35 days. Maximum bioconcentration factors were 0.38X for edible tissues, 0.63X for nonedible tissues, and 0.52X for whole fish. Only parent glyphosate was detected in aqueous extracts from edible and nonedible tissues; glyphosate and aminomethylphosphonic acid were detected in aqueous extracts from whole fish. A significant portion of total sample radioactivity was found to be incorporated into proteins. Residues accumulated by day 35 of the exposure period were depurated gradually with 35% elimination from edible tissues, 57% form nonedible tissues, and 52% from whole fish after 21 days of depuration.

EXCERPT FROM TASK L: REVIEW AND EVALUATION OF INDIVIDUAL STUDIES 12/17/91

METHODOLOGY:

Bluegill sunfish (Lepomis macrochirus; mean weight 8.1 g, mean length 63 mm) were held in culture tanks on a 16-hour daylight photoperiod for at least 14 days prior to initiation of the study. Flow-through aquatic exposure systems were prepared using four 70-L glass aquaria. Aerated well water (pH 7.7-8.1, dissolved oxygen 7.5-9.2 mg/mL, alkalinity 300-320 mg/mL as $CaCO_3$, hardness 238-278 mg/mL as $CaCO_3$, temperature 17-21 C; Table 1) was provided to each aquarium at a rate of approximately 7.0 turnovers per day. The aquaria were placed in a water bath and maintained at 22 ± 1 C.

Bluegill sunfish (110) were placed in each aquarium, and two aquaria were treated with [^{14}C]glyphosate (radiochemical purity 99%, specific activity 81.1 dpm/ug, Monsanto) at 12 ppm. The remaining two aquaria served as untreated controls. Following a 35-day exposure period, the [^{14}C]glyphosate-treated water was replaced with untreated water for a 21-day depuration period. The treated water was sampled prior to introducing the fish, then water samples and fish (10 or 20) were taken from the treated and control aquaria after 0.17 (2-6 hours), 1, 3, 7, 14, 21, 28, and 35 days of exposure. During the depuration period, water and fish were sampled on days 1, 3, 7, 10, 14, and 21. Water and fish samples were stored frozen until analysis.

Radioactivity in the water samples was quantified using LSC; the average detection limit was 0.0209 ppm. Water samples from days 1, 28, and 35 of the exposure period were filtered and evaporated to dryness. The remaining residue was redissolved in deionized water plus 1 drop of 1.2 N hydrochloric acid, then stored frozen at -20 C until HPLC analysis. Aliquots were analyzed by HPLC using an anion exchange column eluted with methanolic potassium phosphate buffer; fractions were collected at 0.5-minute intervals and analyzed using LSC.
Pooled samples (6 fish) from each sampling interval were dissected into edible tissues (body, muscle, skin, skeleton) and nonedible tissues (fins, head, internal organs). Pooled edible and nonedible tissue samples and additional whole fish samples (4 fish/sampling interval) were homogenized with dry ice and analyzed for total radioactivity by LSC following combustion; average detection limits for whole fish, edible tissues, and nonedible tissues were 1.34, 1.35, and 1.33 ppm, respectively.

In order to characterize [^{14}C]residues, 21- and 28-day exposure plus 7-day depuration samples (20 g) of edible, nonedible, and whole fish tissues were extracted twice with 2 mM ethylenediaminetetraacetic acid (EDTA) pH 5.0. The aqueous extracts were combined and partitioned twice with chloroform:isoamyl alcohol:phenol (25:1:24); organic phases were discarded. The aqueous phase was then partitioned four times with chloroform:isoamyl alcohol (24:1); organic phases were again discarded. The remaining aqueous phase was concentrated, adjusted to pH 2-3 with 6 N hydrochloric acid or glacial acetic acid, and applied to a C-18 solid phase column. The sample was eluted with 1% acetic acid, concentrated, and aliquots were analyzed by HPLC as described above. Additional aliquots were applied to an AG 50W-X8 cation exchange column and eluted with deionized water; fractions were collected at 4-minute intervals and analyzed using LSC. Unextractable [^{14}C]residues remaining in the extracted tissues was quantified by LSC following combustion.

Additional samples of edible tissue and whole fish were digested with proteinase K. Tissue samples were homogenized with 50 mM Tris:150 mM sodium chloride:2 mM or 100 mM EDTA (pH 10). The homogenate was

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diluted to 1% in sodium dodecyl sulfate, then incubated with 200 ppm proteinase K for 16 hours at 37 C. The digested homogenate was partitioned with chloroform:isoamyl alcohol:phenol as described above, but attempts to analyze the aqueous fraction using HPLC were unsuccessful.

DATA SUMMARY:

Total [^{14}C]glyphosate residues did not readily accumulate in bluegill sunfish during 35 days of exposure to [^{14}C]glyphosate at 12 ppm in a flow-through system maintained at 22 C. Maximum bioconcentration factors were 0.38, 0.63, and 0.52x in edible tissues (body, muscle, skin, skeleton), nonedible tissues (fins, head, internal organs), and whole fish, respectively (Table 3). Uptake of [^{14}C]glyphosate residues was variable with maximum accumulation of residues occurring between day 21 of the exposure period and day 7 of the depuration period; maximum concentrations of [^{14}C]residues were 4.8 ppm in edible tissues, 7.6 ppm in nonedible tissues, and 13 ppm in whole fish. Water-soluble [^{14}C]residues comprised 17-20.2% of the recovered radioactivity from 21-day exposure whole fish tissues and 7-day depuration edible and nonedible tissues; 14.6-57% was associated with protein/lipid fractions and 34.8-61.1% was unextractable residues (Table 4A). In the edible and nonedible tissues, only parent glyphosate was detected comprising 89.9-91.3% of the radioactivity recovered after cation exchange column chromatography of the water-soluble residues; column recoveries were 62-74% (Table 5). In the whole fish tissues, glyphosate and its degradate aminomethylphosphonic acid (AMPA) were detected comprising 28 and 48.7%, respectively, of the radioactivity recovered after HPLC of water-soluble residues; column recovery was 64%. Incubation of whole fish and edible tissues with proteinase K increased extractability of water-soluble residues indicating incorporation of radioactivity into proteins. Residues accumulated at day 35 were depurated gradually; at day 21 of depuration, [^{14}C]residues were 3.0 ppm in edible tissues, 3.5 ppm in nonedible tissues, and 2.2 ppm in whole fish, representing 35% depuration in edible tissues, 57% in nonedible tissues, and 52% in whole fish (Table 4B). No mortality or abnormal effects were observed during the study.

Throughout the study, the temperature of the treated water was 21-22 C, the pH ranged from 7.8 to 8.2, and the dissolved oxygen content ranged from 6.4 to 8.4 ppm; values were comparable to the control aquarium (Table 8). Total [^{14}C]residues in the treated water ranged from 11 to 13 ppm. Analysis of 1-, 28-, and 35-day water samples from the exposure period found that glyphosate comprised 95-96.6% of the recovered radioactivity and AMPA comprised 1.1-1.9% (Table 2); recovery of initial sample radioactivity ranged from 74 to 98%.

COMMENTS:

1. Uptake of [^{14}C]glyphosate residues was variable with maximum accumulation of residues occurring between day 21 of the exposure period and day 7 of the depuration period. The registrant reported that a previous bioaccumulation study with crayfish (Chott and Livingston; Monsanto Report No. MSL-5019) showed an interspecimen variability in [^{14}C]residue levels of 64%, and attributes the variability to individual variation in the disposition of glyphosate in aquatic organisms.

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2. Only parent glyphosate was detected (comprising 89.9-91.3% of the radioactivity recovered from the column) in aqueous fractions from edible and nonedible tissues analyzed using cation exchange chromatography; however, glyphosate and AMPA were detected (comprising 28 and 48.7%, respectively) in the aqueous fraction from whole fish analyzed using HPLC. It could not be determined if the significant difference in the levels of parent glyphosate detected was due to the different analytical methods used or was a storage stability problem. Fortification of control fish tissues and analysis by HPLC or cation exchange column chromatography was not adequately described to determine if the two methods were equivalent in detection and quantification of glyphosate in the tissue samples. However, since [^{14}C]residues did not significantly accumulate in the fish during 35 days of exposure to [^{14}C]glyphosate, additional characterization and quantification of residues is not required.

3. Except for the 1-, 28-, and 35-day water samples from the exposure period, it was not specified how long samples were stored frozen prior to analysis. To demonstrate storage stability of glyphosate, the 28-day water samples were analyzed for glyphosate and AMPA after 115 and 504 days of storage. At both intervals, glyphosate and AMPA comprised approximately 96 and 2% of the recovered radioactivity, respectively (Table 2); column recoveries were 82.9 and 97.8%. The registrant reported that storage stability analyses for glyphosate and its degradates in the fish tissues were not possible due to difficulties in isolation and characterization of the tissue residues. To determine storage stability in the fish tissues, the registrant should have spiked the tissues with a known amount of technical grade or purer test substance (glyphosate or AMPA), then analyzed for the test substance and its degradates immediately posttreatment and after various lengths of time of frozen storage to determine if any degradation had occurred.

4. A preliminary 6-day toxicity study was conducted to verify that the 12 ppm treatment rate was not lethal to bluegill sunfish. Nominal test concentrations for the toxicity study were 0.93, 1.8, 3.8, 7.5, and 15 ppm; no mortalities or abnormal effects were observed during the study.

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GLYPHOSATE 103601

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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.

REFERENCES

The following studies were reviewed:

Castle, S., L.O. Ruzo, and K. Shepler. 1990. Degradation study: Photodegradation of [^{14}C] glyphosate in a buffered aqueous solution at pH 5, 7 and 9 by natural sunlight. PTRL Report No. 233W-1. PTRL Project No. 233W. R.D. No. 1020. Performed by Pharmacology and Toxicology Research Laboratory, Inc., Richmond, CA, and submitted by Monsanto Agricultural Company, St. Louis, MO. (41689100)

Forbis, A.D. 1989. Uptake, depuration and bioconcentration of ^{14}C glyphosate to bluegill sunfish (Lepomis macrochirus). Part I: MSL-9304. Laboratory Project No. MSL-9304. R.D. No. 955. Performed by Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO, and submitted by Monsanto Agricultural Company, St. Louis, MO. (41228301)

Kesterson, A. and S.B. Jackson. 1990. Aerobic aquatic metabolism of [^{14}C]glyphosate. PTRL Report No. 1300. PTRL Study No. 366. Performed by Pharmacology and Toxicology Research Laboratory East, Inc., Richmond, KY, and submitted by Monsanto Agricultural Company, St. Louis, MO. (41723601)

Kesterson, A. and S.B. Jackson. 1990. Anaerobic aquatic metabolism of [^{14}C]glyphosate. PTRL Report No. 1304. PTRL Study No. 367. Performed by Pharmacology and Toxicology Research Laboratory East, Inc., Richmond, KY, and submitted by Monsanto Agricultural Company, St. Louis, MO. (41723701)

Kesterson, A.L. and R.H. Atkins. 1991. Aerobic metabolism of [^{14}C]glyphosate in sandy loam and silt loam soils with biometer flask. PTRL Report No. 1301. PTRL Study No. 368. R.D. No. 1031. Performed by Pharmacology and Toxicology Research Laboratory East, Inc., Richmond, KY, and submitted by Monsanto Agricultural Company, St. Louis, MO. (41742901)

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. (41543202)

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. (41543201)

Ridley, W.P. and K.A. Chott. 1989 Uptake, depuration and bioconcentration of 14C glyphosate to bluegill sunfish (Lepomis macrochirus). Part II: Characterization and quantitation of glyphosate and its metabolites. Laboratory Project No. MSL-9303. R.D. No. 955. Performed and submitted by Monsanto Agricultural Company, St. Louis, MO. (41228302)

GLYPHOSATE

TASK 1: REVIEW AND EVALUATION OF INDIVIDUAL STUDIES

December 17, 1991

Final Report

Contract No. 68D90058

Submitted to:
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Arlington, VA 22202

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