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DATA EVALUATION RECORD

STUDY 3

--CHEM 080803 Atrazine §162-1 and

FORMULATION--00--ACTIVE INGREDIENT

--STUDY ID 42089906

Nelson, D.R. and Schabacker, D.J. 1991. Summary report: Soil metabolism of ^{14}C-atrazine and metabolite characterization/identification. Laboratory Project ID: HLA 6015-185, ABR-91073, ABR-89076, and ABR-90093. Unpublished study performed by Hazleton Laboratories America, Inc., Madison, WI, and Ciba-Geigy Corporation, Greensboro, NC; and submitted by Ciba-Geigy Corporation, Greensboro, NC.

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CONCLUSIONS:

Metabolism - Aerobic Soil

Metabolism - Anaerobic Soil

The results of the two experiments (Ciba-Geigy Laboratory Project Numbers ABR-89076 and ABR-90093) contained in this submission were submitted by the registrant to satisfy data

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requirements for the registration of atrazine in the state of California, and specifically, to address issues raised by the California Department of Food and Agriculture (CDFA) in letters dated June 6, 1989 and April 27, 1990 regarding Ciba-Geigy's submission of reports ABR-113693-E and ABR-89076. The CDFA letters requested: (i) the quantitation of degradates present in the aqueous fractions from aerobic and anaerobic soil metabolism studies of [^{14}C]atrazine; (ii) the tabulation of the R_f values and retention times for the TLC and HPLC system used for the standard and samples; and (iii) the identification of any degradates present at a level ≥0.01 ppm in the organic fractions.

This study is also a supplement to a previous study (MRID 40629303) submitted by the registrant to EPA regarding aerobic and anaerobic soil metabolism of [^{14}C]atrazine, which had been reviewed by Dynamac in a report dated November 18, 1988. According to the Dynamac review, the aerobic soil metabolism experiment, although scientifically sound, did not meet Subdivision N guidelines because degradates comprising up to 7% of the applied (the water-soluble compounds, and one degradate in the organosoluble fraction) were not identified. The anaerobic soil metabolism experiment did not meet Subdivision N guidelines because degradates comprising up to 6.8% of the applied (the water-soluble compounds in soil and water) were not identified. The Dynamac review noted that an acceptable anaerobic aquatic metabolism study (MRID 40431323) was submitted. According to Subdivision N guidelines, an acceptable anaerobic aquatic metabolism study may be used to fulfill data requirements for anaerobic soil metabolism studies.

The current submission (MRID 42089906) included a resubmission of the original study along with data from the supplemental experiment. This data evaluation record pertains to the evaluation of the supplemental experiment for scientific validity and fulfillment of Subdivision N guidelines.

Metabolism - Aerobic Soil

Aqueous extracts: Aqueous soil extracts from the previous active aerobic study (MRID 40629303) were used for this experiment. Because of a limited quantity of the remaining supply, only extracts from days 181, 244, and 300 were analyzed (Table I); these samples had been stored frozen for approximately 636 days.

Aliquots of the aqueous extracts were analyzed for total radioactivity by LSC. Approximately 6.29-6.83% (0.642-0.694 ppm), 6.06-6.44% (0.618-0.657 ppm), and 6.46-6.81 (0.659-0.694 ppm) of the applied radioactivity were found in the aqueous extracts collected from days 181, 244, and 300, respectively (Table IIA). These values are in agreement with values reported from the previous experiment, in which it was reported that the aqueous fraction comprised 6.6%, 6.3%, and 6.7% of the applied from samples collected on days 181, 244, and 300, respectively (Table 6).

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Additional aliquots of aqueous extracts were analyzed by one-dimensional TLC on silica gel plates developed with chloroform:methanol:formic acid:water (140:50:8:4, v:v:v:v). Radioactive areas were located by autoradiography and identified by comparison to reference standards of atrazine and its possible degradates, which had been cochromatographed with the test solution and located under UV light (254 nm). The unlabeled reference standards were visualized in a chlorine chamber (for enhancement of hydroxyatrazines) under UV light (254 nm). Comparison of the R_f values of the standards with the treated samples did not indicate the presence of the parent atrazine. According to the registrant, the major degradates were hydroxy atrazine, the mono- and didealkylated degradates of atrazine, and the monodealkylated degradates of hydroxy atrazine.

In order to confirm the TLC results, aliquots of the aqueous extracts were analyzed for specific atrazine degradates using reverse-phase (C-18 column) HPLC eluted with acetonitrile:0.1% phosphoric acid (95:5, v:v) with UV and radioactivity detection. The retention times of the residues were compared to reference standards of the degradates. Five degradates were identified (Tables IIIA and IVA):

CGA-34048 (2-ethylamino-4-hydroxy-6-isopropylamino-s-triazine)

was 60.43-74.24% (0.382-0.489 ppm) of the total recovered radioactivity in the extracts;

CGA-30033 (2-amino-4-chloro-6-isopropylamino-s-triazine)

was 8.12-14.78% (0.053-0.103 ppm);

CGA-17794 (2-amino-4-isopropylamino-6-hydroxy-s-triazine)

was 6.24-13.65% (0.041-0.095 ppm);

CGA-17792 (2-amino-4-ethylamino-6-hydroxy-s-triazine)

was 2.38-6.41% (0.016-0.042 ppm); and

CGA-28279 (2-amino-4-chloro-6-ethylamino-s-triazine)

was 2.58-5.55% (0.018-0.039 ppm). Approximately 93.07-97.74% of the radioactivity in the aqueous extract was identified; the amount of unaccounted radioactivity was 2.26-6.93% (0.016-0.046 ppm).

<u>Organic extracts</u>: Organic soil extracts from the previous active aerobic study (Hazleton Laboratory Project Number HLA 6015-185; MRID 40629303) were used for this experiment. Because of a

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limited quantity of the remaining supply, only extracts from days 181, 244, and 300 were analyzed (Table I); these samples had been stored frozen for approximately 1,189 days.

Aliquots of the organic extracts were reportedly analyzed for total radioactivity by LSC; the results, however, were not provided. The radioactivity distribution of the organic extract from the original study was reported (Table IIB).

Additional aliquots of organic extracts were analyzed by one-dimensional TLC as described above (see analysis of aqueous extracts). TLC analysis indicated that atrazine accounted for 73.8-86.2% (2.040-3.412 ppm) of the radioactivity in the organic fraction (Tables IIIB and VI). These seven degradates were identified in the organic extracts:

CGA-30033

was 7.4-16.8% (0.293-0.464 ppm) of the total radioactivity recovered in the extracts;

CGA-28279

was 2.9-5.9% (0.115-0.170 ppm);

CGA-28273 (2,4-diamino-6-chloro-triazine)

was 0.4-1.0% (0.011-0.034 ppm);

CGA-34048

was 0.5-1.0% (0.014-0.034 ppm);

CGA-17794

was a maximum of 0.3% (0.012 ppm);

CGA-17792

was a maximum of 0.2% (0.008 ppm); and

CGA-17791 (2,4-diamino-6-hydroxy-s-triazine)

was a maximum of 0.1% (0.004 ppm). Two degradates were unidentified by one-dimensional TLC. Unknown 1 was detected at a maximum of 1.4% of the radioactivity in the extract (0.048 ppm) and exhibited an R_f value between those of CGA-28273 and CGA-28279. Another unknown was a maximum of 0.3% (0.008 ppm).

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In order to confirm the identification of degradates analyzed via one-dimensional TLC, aliquots of the organic extracts were additionally analyzed by two-dimensional TLC (day 181 extracts) and reverse-phase HPLC (day 244 extracts). Two-dimensional TLC analysis was performed on silica gel plates developed with chloroform:methanol:90% formic acid:water (140:50:8:4, v:v:v:v) in the first direction and n-butanol:glacial acetic acid:water (133:33:33, v:v:v) in the second direction. Radioactive areas were located by image scanning and autoradiography, and identified by comparison to standards, which had been cochromatographed with the test solution and located using UV light. HPLC analysis was accomplished using a Du Pont Zorbax Rx reverse phase column and a mobile phase of acetonitrile:0.1% phosphoric acid (95:5, v:v) with UV and radioactivity detection. The retention times of the residues were compared to reference standards of the degradates. The TLC cochromatography and HPLC coelution of degradates and standard confirmed the identity of these degradates.

In order to isolate Unknown 1, additional aliquots of the extracts were combined, concentrated, and analyzed by preparative one-dimensional TLC and two-dimensional TLC as previously described (Figure 14). The two bands (Bands II and III) from the developed TLC plate that corresponded to the area of Unknown I were scraped and extracted with methanol; the extract of each band was analyzed by two-dimensional TLC as described above. Each band was derivatized with diazomethane and n-butanol in HCl and the reaction products were compared to the unreacted samples using one-dimensional TLC. As a result of the comparison of the chromatographic behavior of Unknown 1 with the analytical standards and the results of the derivatization reactions, the registrant proposed two possible chemical structures for Unknown 1 (Figure 29). No additional isolation and characterization procedures were attempted for the other unknown because of insufficient material and because it was found at <0.01 ppm (0.008 ppm).

<u>Organic Extracts from Sterile Aerobic Experiment</u>: Organic soil extracts from the previous sterile aerobic study (MRID 40629303) were used for this experiment. Because of a limited quantity of the remaining supply, only extracts from days 32, 62, 80, and 94 were analyzed; these samples had been stored frozen for approximately 1,189 days.

Aliquots of organic extracts from the sterile aerobic experiment were analyzed by one-dimensional TLC as described above (see analysis of aqueous extracts). Atrazine accounted for 96.1-97.0% (7.743-10.200 ppm) of the radioactivity in the fraction (Tables IVB and VII). The degradates CGA-30033, CGA-28279, CGA-28273, CGA-34048, CGA-17794, CGA-17792, and CGA-17791, which were identified from the active aerobic experiment, were also found in the sterile aerobic experiment; these degradates were present at 0.1-1.8% of the radioactivity in the extract (0.009-0.144 ppm). One degradate (Unknown 2) was unidentified by one-dimensional TLC and was found at a maximum of 0.4% of the radioactivity in the extract (0.032 ppm); Unknown 2 exhibited an R_f value between those of CGA-28273 and CGA-28279.

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The identities of the degradates were confirmed by two-dimensional TLC and reverse-phase HPLC, as previously described. The two-dimensional TLC analysis of day-32 extracts revealed an additional unknown degradate (Unknown 3) which was found at 0.34% of the radioactivity in the sample (ppm value was not reported and could not be calculated by the Dynamac reviewer).

In order to isolate Unknowns 2 and 3, additional aliquots of the extracts were combined, concentrated, and analyzed by preparative one-dimensional TLC and two-dimensional TLC as previously described (Figure 19). The two bands (Bands II and III) from the developed TLC plate that corresponded to the areas of Unknowns 2 and 3 were scraped and extracted with methanol, and the extract of each band was analyzed by two-dimensional TLC as described above. Band II contained Unknown 2 as well as CGA-34048, and Band III contained Unknown 3 as well as CGA-28279. Based on these analyses, the registrant proposed possible chemical structures for Unknowns 2 and 3 (Figure 29).

Overall, this supplemental document along with the previously submitted study (MRID 40629303) adequately addressed all of the issues raised by Dynamac (report dated November 18, 1988) and now meet Subdivision N guidelines for aerobic soil metabolism (§162-1). The registrant analyzed the degradates from the aqueous and organic extracts of the aerobic incubation study.

In summary, uniformly ring-labeled [^{14}C]atrazine (radiochemical purity ≥95.4%, specific activity 20.6 uCi/mg), at 10.2 ppm, degraded with a half-life of 94-181 days in loam soil incubated aerobically in the dark at 25 C and 75% of 0.33 bar moisture; the registrant-calculated half-life was approximately 146 days. [^{14}C]Atrazine decreased from 90.7% of the applied immediately posttreatment to 56.5% at 94 days, 33.1% at 181 days, and 21.2% at 300 days. The major organosoluble degradates were CGA-30033, CGA-28279, CGA-28273, and CGA-34048. The major aqueous degradates were CGA-34048, CGA-30033, CGA-17794, CGA-17792, and CGA-28279.

<u>Metabolism - Anaerobic Soil</u>

<u>Aqueous Extracts</u>: The 0-, 30-, and 62-day samples of aqueous soil extracts from the previous anaerobic incubation study (Hazleton Laboratory Project Number HLA 6015-185; MRID 40629303) were used for this experiment; these samples had been stored frozen for approximately 1,189 days.

Aliquots of the aqueous extracts were reportedly analyzed for total radioactivity by LSC; the results, however, were not provided. The radioactivity distribution of the aqueous extract from the original study (Table IIA) was reported. In the previous experiment, it was reported that the

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aqueous fraction comprised 2.2% (0.224 ppm), 2.5% (0.255), and 3.7% (0.377 ppm) of the applied from samples collected on days 0, 30, and 62, respectively.

Additional aliquots were analyzed by one-dimensional TLC as previously described. Atrazine accounted for 0.9.-2.6% (0.002-0.006 ppm) of the radioactivity in the fraction (Tables V and VIII). The major degradate identified in the fraction was CGA-34048 at 24.2-55.1% (0.091-0.158 ppm) of the radioactivity in the fraction. The following minor degradates were identified:

CGA-30033

was 0.5-1.6% (0.001-0.006 ppm) of the total recovered radioactivity in the extracts;

CGA-28273

was 2.2-6.5% (0.005-0.025 ppm);

CGA-17794

was 8.9-13.7% (0.022-0.052 ppm);

CGA-17792

was 1.1-10.0% (0.003-0.038 ppm); and

CGA-17791

was 1.2-2.8% (0.003-0.011 ppm). Approximately 96.6-99.7% of the radioactivity in the aqueous extract was identified (Table V).

Four degradates were unidentified by one-dimensional TLC. Unknown 4 was detected at 7.8-26.6% of the radioactivity in the extracts (0.018-0.037 ppm) and exhibited an R_f value between those of CGA-17794 and CGA-34048. Two other unidentified degradates (Unknowns 5 and 6) were collectively found at 13.8-25.2% of the radioactivity in the extracts (0.040-0.072 ppm). Another unknown was found at 0.5-2.3% of the radioactivity in the extracts (0.001-0.009 ppm).

In order to isolate Unknowns 4 through 6, additional aliquots of the extracts were combined, concentrated, and analyzed by two-dimensional TLC as previously described (Figure 24). Aliquots of the concentrated extract were characterized by derivatization with n-butanol in HCl followed by two-dimensional TLC. This analytical procedure further revealed two other possible unknowns; the quantitation of these unknowns, according to the study authors, was not possible because of lack of clear separation of the components. Additional aliquots of the concentrated extract were analyzed by preparative HPLC as previously described. Based on

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these analyses, the registrant proposed possible chemical structures for Unknowns 4 through 6 (Figure 29).

Overall, this supplemental document along with the previously submitted study (MRID 40629303) adequately addressed all of the issues raised by Dynamac (report dated November 18, 1988) and now meet Subdivision N guidelines for anaerobic soil metabolism (§162-2). The registrant analyzed the degradates from the aqueous extract of the anaerobic incubation study.

In summary, uniformly ring-labeled [^{14}C]atrazine (radiochemical purity ≥95.4%, specific activity 20.6 uCi/mg) decreased from 6.83 to 5.22 ppm (67 to 51.2% of the applied) in anaerobic (flooded plus N_2 atmosphere) loam soil during 62 days of incubation in the dark at 25 C. The loam soil had been treated with [^{14}C]atrazine at 10.2 ppm and incubated for 32 days under aerobic conditions (in the dark at 25 C and 75% of 0.33 bar moisture) prior to flooding. The degradates isolated from the soil:water system were CGA-30033, CGA-28279, CGA-28273, and CGA-34048. The degradates identified in the aqueous extracts were CGA-34048, CGA-30033, CGA-28273, CGA-17794, CGA-17792, and CGA-17791.

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