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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

MAR 16 1990

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OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCES

Subject: Trifluralin Registration Standard Followup:  
Response to Data Deficiencies in Plant  
Metabolism Studies for Corn and Mustard,  
Submission of February 22, 1990 (MRID Nos.  
41396801 and 41396802, DEB No. 6432, HED  
Project No. 0-0827).

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In response to the review of October 6, 1989, Trifluralin Registration Standard Followup: Response to Residue Chemistry Data Requirements on Plant Metabolism (Elizabeth T. Haeberer), DowElanco has submitted the following supplemental information:

- Addendum to Metabolism of  $^{14}\text{C}$  Trifluralin in Field Corn.  
Note: This study contains photographs.
- Addendum to Characterization and Identification of Radioactivity in Mustard Plants Grown in Soil Treated with  $^{14}\text{C}$  Trifluralin. Note: This study contains photographs.

This addendum attaches three journal articles..

1. "Metabolism and Balance Studies of [<sup>14</sup>C] Monolinuron After Use in Spinach Followed by Cress and Potato Cultures" by Ingolf Schuphan and Winfried Ebing. Pesticide Biochemistry and Physiology, Vol. 9, pp. 107-118, 1978.
2. "Conversion of [<sup>14</sup>C] Pentachloronitro benzene in Onions" by S. Begum et al. Pesticide Biochemistry and Physiology, Vol. 11, pp. 189-200, 1979.
3. "Metabolism and Selectivity of Diclofopmethyl in Wild Oat and Wheat" by Richard H. Shimabukuro et al. J. Agric. Food Chem., Vol. 27, No.3, pp. 615-623, 1979.

#### CONCLUSIONS

1. The nature of the residue in corn is adequately defined. Trifluralin ( $\alpha,\alpha,\alpha$ -Trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) is the predominant residue in forage, with smaller amounts of conjugates C1 (N-[2-Ethyl-1-propyl-5-(trifluoromethyl)-1H-benzimidazol-7-yl]- $\beta$ -D-glucopyranosylamine) and C2 (N-[2-Ethyl-1-propyl-5-(trifluoromethyl)-1H-benzimidazol-7-yl]- $\alpha$ -D-glucopyranosylamine) present, and also metabolite TR-4 ( $\alpha,\alpha,\alpha$ -Trifluoro-5-nitro-N',N'-dipropyltoluene-3,4-diamine). Very little if any residues translocate to grain or cob, and with time, residues on corn plants are converted from nonpolar to polar compounds and subsequently incorporated into insoluble forms including cell wall components.
2. The nature of the residue in mustard leaves is adequately defined. The residue of concern is trifluralin. Although small amounts of trifluralin metabolites have been identified in the metabolism study at the 2.6X rate, it is unlikely that they will occur in mustard leaves from the maximum proposed use rate.
3. The petitioner has submitted data on storage temperatures and length of storage prior to analysis for both corn and mustard. This data deficiency is resolved.

#### RECOMMENDATIONS

The registrant should be informed that the data submitted fulfills the outstanding requirements regarding plant metabolism in corn and mustard.

## DETAILED CONSIDERATIONS

### Corn

The review of October 6, 1989, Trifluralin Registration Standard Followup: Response to Residue Chemistry Data Requirements on Plant Metabolism (Elizabeth T. Haeberer), concluded that the nature of the residue in corn forage and fodder was not adequately defined. Due to low  $^{14}\text{C}$  levels in grain, no characterization was required in that commodity, however, no residues were conclusively identified in fodder, and <50% of the  $^{14}\text{C}$ -residues in forage were characterized. In addition, no data were submitted concerning the storage interval or temperature of storage for the samples in the study, prior to analysis.

The current submission, Addendum to Metabolism of  $^{14}\text{C}$  Trifluralin in Field Corn (MRID No. 41179001), contains a more detailed explanation of the characterization of trifluralin residues in corn, including pictures relevant thin layer chromatography (TLC) plates.

The one week forage sample was selected for the characterization studies because it contained the highest ppm  $^{14}\text{C}$ -residue level in the extracted aqueous fraction and, therefore, the maximum opportunity for residue identification. Twenty percent of the total radioactive residue (TRR) in one week forage was found in the extracted aqueous solution. This solution was chromatographed on an XAD-2 column using the following solvents: water; methanol; dichloromethane; dichloromethane/acetic acid-100/1; methanol/acetic acid-99/1; 1N HCl. Fractions 3 and 4, which were eluted with methanol, represented 70% of the radioactive residue eluted from the column or 14.5% of TRR for one week forage. Fraction 3 was the only fraction which represented more than 2.1% of the TRR. Since fractions 3 and 4 were both methanol soluble, they were combined and subjected to TLC analysis. Three different solvent systems were tried: 80/20 and 50/50 dichloromethane-methanol; 60/25/15 butanol-water-acetic acid. Only the latter gave good chromatographic results. Known trifluralin metabolites TR-6, TR-9, and TR-20 were also spotted on the TLC plate as reference standards. Most of the radioactive residues on the plate ran slower than the reference standards thereby demonstrating their highly polar nature. Sixteen finite sample segments were scraped from the TLC plate and assayed by liquid scintillation counting techniques. No one fraction contained more than 2.5% of the TRR in the one week forage sample. These fractions did not contain sufficient residue to allow further characterization.

The one week extracted forage contained 48.5% of the TRR. This sample portion was subjected to analysis by lignin and cellulose isolation procedures. The lignin portion contained 22.9% of the TRR and the cellulose portion contained 11.6%, leaving 14.0% as uncharacterized radioactive residue. In an attempt to further

characterize the residues in extracted forage an additional sample of one week forage extracted with methanol, containing 47.4% of the TRR, was refluxed with 2N HCl for 1 hour. The petitioner provides a detailed review of additional extractions with ethyl acetate, both acidic and basic, and a rationale for the conclusion that the uncharacterized radioactive residues are represented by 5.0% of the TRR in the acidic ethyl acetate extract and 7.3% of the TRR in the basic ethyl acetate extract, a total of 12.3% of the TRR, very close to the 14% in question. No further attempt was made to characterize these residues since they were both <10% of the TRR.

The petitioner has submitted data concerning the length of storage prior to analysis of samples and also the storage temperatures. All samples were analyzed for TRR as soon after harvest as possible, with the exception of grain and cob which were air dried for 2 weeks, and fodder which was air dried for 3 weeks prior to analysis. With the exception of fodder, all samples were stored at -15°C for a period of 3 weeks to 7.5 months. Fodder was stored at room temperature for 3.5 months prior to analysis. Previously submitted storage stability data indicate that residues of trifluralin are stable at freezer temperatures for up to 10 months, and at room teperature for up to 121 days ( Trifluralin Registration Standard: Residue Chemistry Chapter, July 3, 1985).

The nature of the residue in corn is adequately defined. Trifluralin( $\alpha,\alpha,\alpha$ -Trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) is the predominant residue in forage, with smaller amounts of conjugates C1 (N-[2-Ethyl-1-propyl-5-(trifluoromethyl)-1H-benzimidazol-7-yl]- $\beta$ -D-gluco-pyranosylamine) and C2 (N-[2-Ethyl-1-propyl-5-(trifluoromethyl)-1H-benzimidazol-7-yl]- $\alpha$ -D-gluco-pyranosylamine) present, and also metabolite TR-4 ( $\alpha,\alpha,\alpha$ -Trifluoro-5-nitro-N<sup>4</sup>,N<sup>4</sup>-dipropyltoluene-3,4-diamine). Very little if any residues translocate to grain or cob, and with time, residues on corn plants are converted from nonpolar to polar compounds and subsequently incorporated into insoluble forms including cell wall components.

#### Mustard

The review of October 6, 1989, cited above, concluded that the nature of the residue in mustard greens was inadequately defined. Over 60% of the TRR in leaf tissue was not characterized. In addition, data were needed concerning storage interval and temperature of storage prior to analysis of samples.

The current submission, Addendum to Characterization and Identification of Radioactivity in Mustard Plants Grown in Soil Treated with <sup>14</sup>C Trifluralin (MRID No. 41179002), contains a more detailed explanation of the characterization of the radioactive residues in mustard and photographs depicting the phytotoxic effects of various application rates of trifluralin on mustard plants.

The petitioner states that the maximum trifluralin treatment rate for mustard was determined by treating plants at 1.0, 1.5, 2.0, and 3.0 ppm levels and comparing plant development, including root system, to untreated (control) plants. The photographs included in this submission clearly indicate phytotoxic effects beginning with the 1.5 ppm treatment level and becoming progressively more severe at the higher treatment levels. If the application rate were too high the plants would not survive for the duration of the study. In addition, the metabolism profile obtained from stressed plants could be atypical. The 1.5 ppm treatment level was selected for the study since, in spite of the changes in root morphology, no visible differences were observed in comparing the leaves of these plants to the untreated controls. The actual treatment rate achieved in the study was 1.323 ppm or 2.6X the maximum proposed use rate.

The TRR found in the mustard leaves was 0.126 ppm (limit of detection 0.003 ppm). Samples of mustard root had significantly higher radioactive residue than did leaves, 0.816 ppm, thereby providing a better possibility for identification of metabolites. Dichloromethane extraction of leaves and roots, and column chromatography of these samples indicated a similarity in components in the sample extracts. The major residue in leaves, representing 9.3% of TRR, i.e., 0.01 ppm, was identified as trifluralin. Metabolite TR-22 accounted for 0.9% of TRR and lignin and cellulose fractions for 27.4%. Plant pigments and lower levels of radioactivity prevented further analysis of most leaf samples. Due to the similarities between root and leaf extracts noted by TLC, the root extracts were further analyzed to provide additional identification of residues.

Since the root samples lacked extraneous plant material, residue characterization was possible at lower levels of concentration than in leaves. The least polar fraction consisted of trifluralin, 26.3% of TRR. The following metabolites were found and the percent of TRR calculated: TR-2, 0.9%; TR-3, <0.2%; TR-5, <0.1%; TR-7, <0.1%; TR-9, 2.5%; TR-14, 0.4%; TR-22, <0.5%; TR-28, 1.3%; TR-41, 0.6%; and TR-43, 0.1%. Lignin and cellulose accounted for 38.9% of the TRR in roots. Over 70% of the radioactive residue in roots was characterized.

The mustard leaves and roots were harvested 8 weeks after planting, the roots rinsed with water, the samples chopped, frozen with liquid nitrogen, ground and stored frozen until analysis. Sample analysis was initiated 2 days after harvest.

The nature of the residue in mustard leaves is adequately defined. The residue of concern is trifluralin. Although small amounts of trifluralin metabolites have been identified in the metabolism study at the 2.6X rate, it is unlikely that they will occur in mustard leaves from the maximum proposed use rate.

cc: E. Saito (TOX), J. Burrell (FOD), Trifluralin Registration  
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