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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

OCT 6 1989

Subject: Trifluralin Registration Standard Followup: Response to Residue Chemistry Data Requirements on Plant Metabolism (MRID Nos. 41179001, 41179002, DEB Nos. 5644, 1989)

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Attached is a review of a followup to the Trifluralin Registration Standard prepared by Dynamac Corp. under supervision of Dietary Exposure Branch (DEB). This review has undergone secondary review and revision in the Dietary Exposure Branch and reflects current Branch policies.

If you need additional input, please advise us.

cc with attachment: PMSD/ISB, Trifluralin Standard File (Boodee)
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eth

DYNAMAC
CORPORATION

Final Report

# TRIFLURALIN
# Task 4: Registrant's Response to Residue Chemistry Data Requirements

**Contract No. 68-D8-0080**

September 29, 1989

**Submitted to:**
Environmental Protection Agency
Arlington, VA 22202

**Submitted by:**
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# TRIFLURALIN

## REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

### Task - 4

### BACKGROUND

The Residue Chemistry Chapter dated July 3, 1985 for the Trifluralin Registration Standard concludes that the metabolism of trifluralin in plants is not adequately understood and requires additional data as follows:

> "Data depicting the distribution and nature of residues of $^{14}C$-trifluralin (ring-labeled) in mature, fresh and dry corn kernels, and in forage harvested at intervals following an over-the-top spray application at a rate sufficiently high to permit $^{14}C$-residue characterization.
>
> Data depicting the distribution and nature of residues of $^{14}C$-trifluralin (ring-labeled) in a mature leafy or Brassica leafy vegetable (i.e. celery, cabbage, collards, etc.) following a preplant soil application at a rate sufficiently high to permit $^{14}C$-residue characterization."

In response to these data requirements, Elanco Products Company has submitted two volumes of data (DEB No. 5644, 1989; MRIDs 41179001 and 41179002) which are reviewed here for their adequacy in fulfilling the outstanding data requirements.

### Deficiencies Remaining to be Resolved

The remaining residue chemistry data gaps on animal metabolism; storage stability; residue analytical methods; residue data for corn grain, sorghum grain, corn forage and fodder, alfalfa hay, flax straw, and peppermint and spearmint oil; tolerance proposals and supporting residue data for sorghum hay, alfalfa forage, cotton forage, peanut vines and hay, sugarcane forage, and sunflower forage; and processing data for potatoes, sugar beets, soybeans, citrus fruits, wheat grain, corn grain, alfalfa, cottonseed, peanuts, peppermint or spearmint hay, sugarcane, and sunflower seed that were identified in the Registration Standard have not been addressed in this submission and are still unresolved.

The Conclusions and Recommendations stated below apply only to the plant metabolism data contained in this submission. The other data gaps indicated above are not included again here but still remain outstanding.

CONCLUSIONS

1. The nature of the residue in corn forage and fodder is inadequately defined. Due to low $^{14}C$ levels in grain, no characterization is required. However, no residues were conclusively identified in fodder, and <50% of the $^{14}C$-residues in forage were characterized. In addition, no data were submitted concerning the storage interval or temperature of storage for the samples in this current study, prior to analysis. These data are needed.

2. The nature of the residue in mustard greens is inadequately defined. Over 60% of the total radioactive residue in leaf tissue was not characterized. The registrant has characterized over 70% of the TRR in root tissue and contends that, since the radioactive residues in root and leaf extracts exhibit similar elution patterns during column chromatography, the data from root extracts is sufficient to describe metabolism in leaves. However, in light of the large number of possible metabolites, potential differences in root and leaf metabolism (roots contained ca. 3x the amount of trifluralin found in leaves), and the possibility of the existence of the conjugated residues C1 and C2 (found in corn forage), we conclude that further characterization of radioactive residues in or on leaves of mustard plants is necessary. In addition, no data were submitted concerning the storage interval or temperature of storage for the samples in this current study, prior to analysis. These data are needed.

RECOMMENDATIONS

The registrant should be informed that the submitted data do not fulfill outstanding requirements regarding plant metabolism in corn and that residues in or on corn fodder, and the insoluble and aqueous fractions of corn forage need to be characterized more specifically. The petitioner should submit a postulated metabolic pathway. If an additional metabolism study is needed on corn to fullfil the outstanding data requirements, it should be at >2X level, if possible, to generate residues of sufficient magnitude in corn grain to permit characterization.

In addition, we recommend that the registrant be instructed to characterize radioactive residues in mustard leaves, including those released by hydrolysis of aqueous and insoluble fractions.

Table 1. Trifluralin and its known and putative metabolites in plants[a].

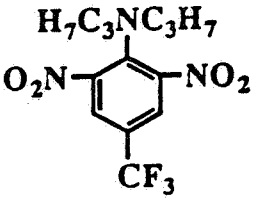
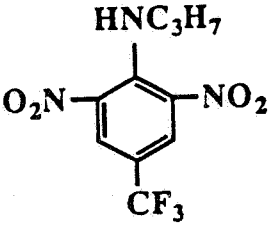
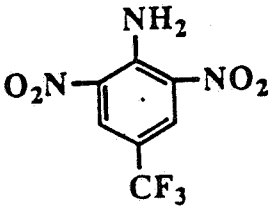
| Code | Chemical name<br>Structure | Substrate | MRID[b]<br>Common name |
|---|---|---|---|
| I | α, α, α-Trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine | | |
| |  | Carrot root | 00093553 |
| | | Carrot top | 00093553 |
| | | Corn forage | 41179001 |
| | | Mustard leaf | 41179002 |
| | | Mustard root | 41179002 |
| | | Peanut plant | None |
| | | Sweet potato | None |
| | | | **Trifluralin (TR-1)** |
| II | α,α,α-Trifluoro-2,6-dinitro-N-propyl-p-toluidine | | |
| |  | Carrot root | 00093553 |
| | | Carrot top | 00093553 |
| | | Corn forage | 41179001 |
| | | Mustard root | 41179001 |
| | | Peanut plant | None |
| | | | **TR-2** |
| III | α,α,α-Trifluoro-2,6-dinitro-p-toluidine | | |
| |  | Corn forage | 41179001 |
| | | Mustard root | 41179002 |
| | | | **TR-3** |

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Table 1. Trifluralin and its metabolites (continued).

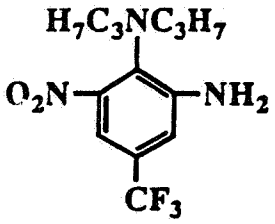
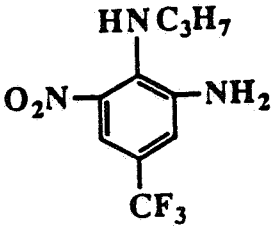
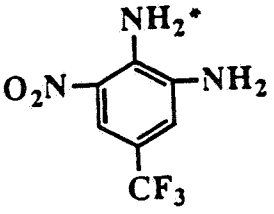
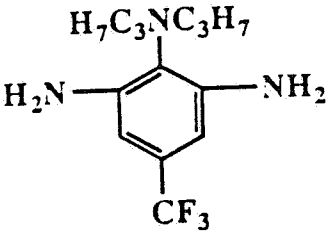
| Code | Chemical name<br>Structure | Substrate | MRID[b]<br>Common name |
|---|---|---|---|
| IV | α,α,α-Trifluoro-5-nitro-$N^4$,$N^4$-dipropyltoluene-3,4-diamine | Corn forage | 41179001 |
| |  | Peanut plant | None |
| | | | TR-4 |
| V | α,α,α-Trifluoro-5-nitro-$N^4$-propyltoluene-3,4-diamine | Carrot root | 00093553 |
| | | Corn forage | 41179001 |
| |  | Mustard root | 41179002 |
| | | | TR-5 |
| VI | α,α,α-Trifluoro-5-nitrotoluene-3,4-diamine | Corn forage | 41179001 |
| | | Peanut plant | None |
| |  | Sweet potato | None |
| | | | TR-6 |
| VII | α,α,α-Trifluoro-$N^4$,$N^4$-dipropyltoluene-3,4,5-triamine | Mustard root | 41179002 |
| |  | | TR-7 |

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Table 1. Trifluralin and its metabolites (continued).

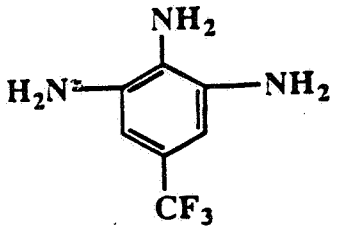
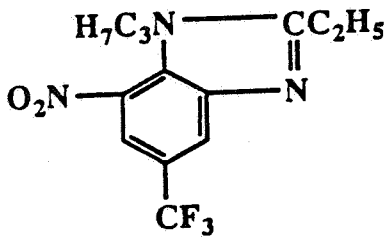
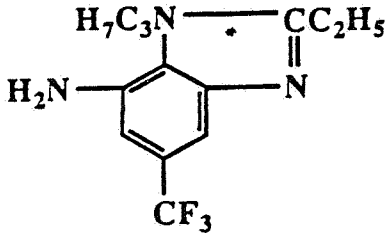
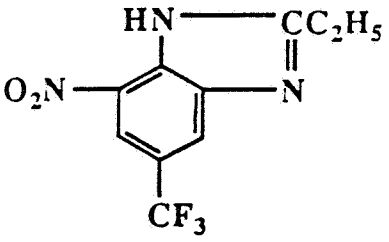
| Code | Chemical name<br>Structure | Substrate | MRID[b]<br>Common name |
|---|---|---|---|
| VIII | α,α,α-Trifluoro-toluene-3,4,5-triamine | Cow feces | None |
| | | Mustard root | 41179002<br>TR-9 |
| |  | | |
| IX | 2-Ethyl-7-nitro-1-propyl-5-(trifluoro-methyl)-benzimidazole | (putative) | TR-13 |
| |  | | |
| X | 7-Amino-2-ethyl-1-propyl-5-(trifluoromethyl)benzimidazole | Mustard root | 41179002<br>TR-14 |
| |  | | |
| XI | 2-Ethyl-7-nitro-5-(trifluoromethyl)benzimidazole | Corn forage | 41179001<br>TR-15 |
| |  | | |

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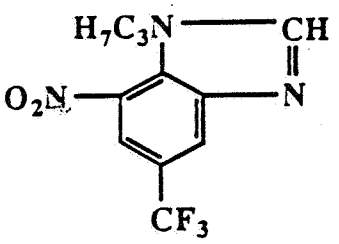
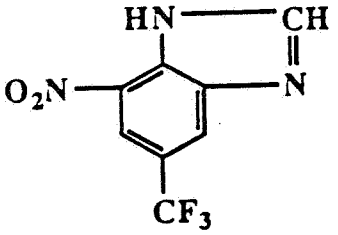
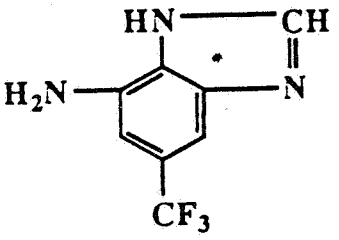
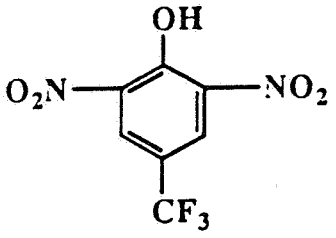
Table 1. Trifluralin and its metabolites (continued).

| Code | Chemical name<br>Structure | Substrate | MRID[b]<br>Common name |
|---|---|---|---|
| XII | 7-Nitro-1-propyl-5-(trifluoromethyl)benzimidazole | Mustard root | 41179002<br>TR-17 |
| |  | | |
| XIII | 7-Nitro-5-(trifluoromethyl)-benzimidazole | Corn forage<br>Corn forage | 41179001<br>41179001<br>TR-18 |
| |  | | |
| XIV | 7-Amino-5-(trifluoromethyl) benzimidazole | (putative) | TR-19 |
| |  | | |
| XV | α,α,α-Trifluoro-2,6-dinitro-p-cresol | Corn forage | 41179001<br>TR-20 |
| |  | | |

(Continued)



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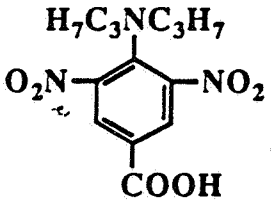
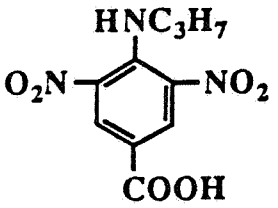
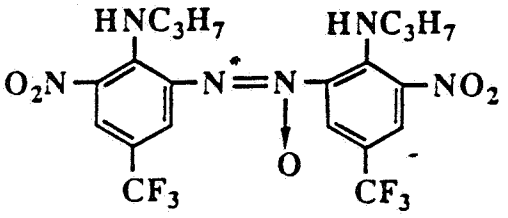
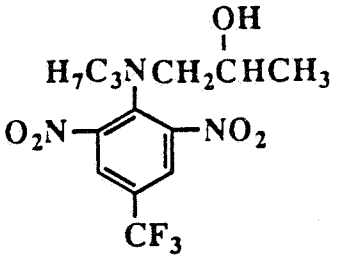
Table 1. Trifluralin and its metabolites (continued).

| Code | Chemical name<br>Structure | Substrate | MRID[b]<br>Common name |
|---|---|---|---|
| XVI | 4-(Dipropylamino)-3,5-dinitrobenzoic acid<br>  | Carrot root<br>Corn forage | 00093553<br>41179001<br>**TR-21** |
| XVII | 3,5-Dinitro-4-(propylamino)-benzoic acid<br>  | Mustard leaf<br>Mustard root | 41179002<br>41179002<br>**TR-22** |
| XVIII | 2,2'-Azoxybis(α,α,α-trifluoro-6-nitro-N-propyl-p-toluidine)<br>  | Mustard root | 41179002<br>**TR-28** |
| XIX | α,α,α-Trifluoro-2,6-dinitro-N-(propan-2-ol)-N-propyl-p-toluidine<br>  | Corn forage<br>Mustard root | 41179001<br>41179002<br>**TR-41** |

(Continued)



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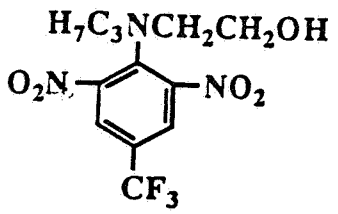
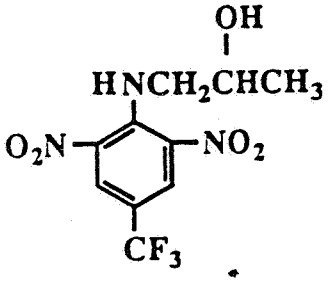
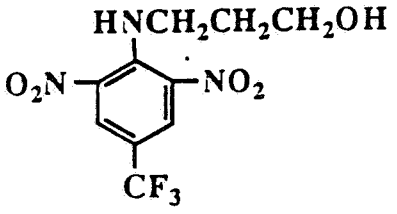
Table 1. Trifluralin and its metabolites (continued).

| Code | Chemical name<br>Structure | Substrate | MRID[b]<br>Common name |
|---|---|---|---|
| XX | α,α,α-Trifluoro-2,6-dinitro-N-(propan-3-ol)-N-propyl-p-toluidine<br>  | Mustard root | 41179002<br>TR-42 |
| XXI | α,α,α-Trifluoro-2,6-dinitro-N-(propan-2-ol)-p-toluidine<br>  | Corn forage<br>Mustard root | 41179001<br>41179002<br>TR-43 |
| XXII | α,α,α-Trifluoro-2,6-dinitro-N-(propan-3-ol)-p-toluidine<br>  | (putative) | TR-44 |

(Continued)



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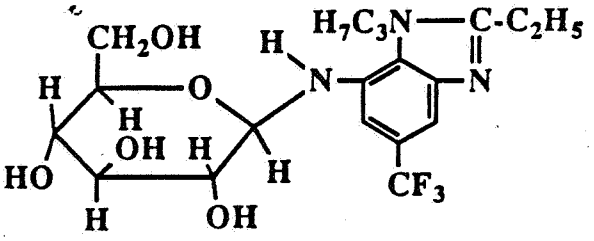
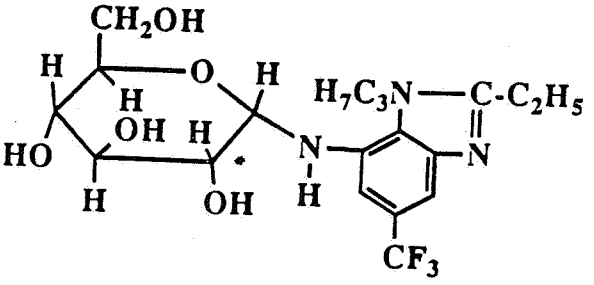
Table 1. Trifluralin and its metabolites (continued).

| Code | Chemical name<br>Structure | Substrate | MRID[b]<br>Common name |
|---|---|---|---|
| XXIII | N-[2-Ethyl-1-propyl-5-(trifluoromethyl)-1H-benzimidazol-7-yl]-ß-D-gluco-pyranosylamine | | |
| | | Corn fodder | 41179001 |
| | | Corn forage | 41179001 |
| | | Corn silage | 41179001 |
| | | | C-1 |



| | | | |
|---|---|---|---|
| XXIV | N-[2-Ethyl-1-propyl-5-(trifluoromethyl)-1H-benzimidazol-7-yl]-α-D-gluco-pyranosylamine | | |
| | | Corn fodder | 41179001 |
| | | Corn forage | 41179001 |
| | | Corn silage | 41179001 |
| | | | C-2 |



| | | | |
|---|---|---|---|
| XXV | α,α,α-Trifluoro-$N^4$,$N^4$-di-(n-propyl)-toluene-3,4,5-triamine | | |
| | | Peanut plant | None |
| | | Sweet potato | None |

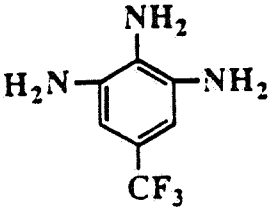


Table 1. Trifluralin and its metabolites (continued).

| Code | Chemical name<br>Structure | Substrate | MRID[b]<br>Common name |
|---|---|---|---|
| XXVI | Phenol derivative | Peanut plant | None |
| | | Sweet potato | None |
| XXVII | Benzoic acid derivative | Peanut plant | None |
| | | Sweet potato | None |

[a] This table icludes findings from studies reviewed in the Trifluralin Residue Chemistry Chapter dated 7/12/85 in addition to the studies discussed in this review.

[b] Where no MRID number is cited, the finding of the metabolite in the substrates listed was attributed, in the Residue Chemistry Chapter, to a published study by B.K. Biswas and W. Hamilton, 1969 (Weed Sci. 17:206-221).

## DETAILED CONSIDERATIONS

### Qualitative Nature of the Residue in Plants

The Trifluralin Residue Chemistry Chapter dated 7/3/85 concludes that the metabolism of trifluralin in plants is not adequately understood and requires additional data reflecting the metabolism of ring-labeled [$^{14}$C]trifluralin in mature fresh and dry corn kernels, in corn forage, and in a mature leafy or Brassica leafy vegetable. In response to these data requirements, Elanco Products Company has submitted metabolism studies for field corn and mustard plants. These are discussed below. The molecular structures and chemical names of trifluralin and its metabolites are given in Table 1.

Field Corn: Elanco Products Company (1989; MRID 41179001) submitted data pertaining to the metabolism of trifluralin in field corn. Two plots of corn plants (1.5-2 feet tall) were sprayed over the top with an EC formulation of uniformly ring-labeled [$^{14}$C]trifluralin (8.59 $\mu$Ci/mg specific activity, radiochemical purity 99.6%) and unlabeled trifluralin (final specific activity 1.0 $\mu$Ci/mg) at 0.75 lb ai/A (1x the maximum registered rate) and 1.5 lb ai/A (2x the maximum registered rate). Forage was sampled on days 0, 7, 14, and 29 posttreatment; silage was sampled on day 63 posttreatment; mature corn ears were harvested 82 days posttreatment; and fodder was harvested 106 days posttreatment. Forage, silage, cobs, and mature grain were ground and stored frozen until analysis; the storage intervals and temperatures were not specified. Fodder was ground and stored at room temperature for an unspecified period of time until analysis.

### Total Radioactive Residues

Samples were analyzed for total radioactivity by combustion/liquid scintillation spectrometry (LSS). The limits of detection and the distribution of the total radioactive residues (TRR) in corn forage, silage, cobs, grain, and fodder are presented below in Table 2. In addition, samples of mature grain were ground, extracted with n-hexane, filtered, and dried to obtain a "flour" fraction. Aliquots of dry corn flour and concentrated hexane extract (containing corn oil) were analyzed for total radioactivity by LSS directly or following combustion. $^{14}$C-Residues were below the limit of detection in processed corn flour or corn oil; the reported limit of detection in processed fractions ranged from 32 to 37 counts per minute (cpm).

Table 2. Average TRR in forage, silage, grain, cobs, and fodder (four samples/commodity) of field-grown corn treated with [$^{14}$C]trifluralin at 0.75 lb ai/A (1x) and 1.5 lb ai/A (2x).

| Commodity | Sampling interval (days) | Detection limit (cpm) | TRR (ppm) 1x | TRR (ppm) 2x |
|---|---|---|---|---|
| Forage | 0 | 35.3 | 48.2 | 107.0 |
| | 7 | 44.2 | 2.27 | 4.59 |
| | 14 | 33.2 | 0.851 | 2.12 |
| | 29 | 31.2 | 0.332 | 0.658 |
| Silage | 63 | 30.2 | 0.126 | 0.444 |
| Grain | 82 | 29.6 | ND | 0.020 |
| Cob | 82 | 32.5 | ND | 0.020 |
| Fodder | 106 | 38.7 | 0.500 | 0.932 |

Extraction

Forage and silage: Samples were extracted with methanol, filtered, and the remaining plant tissue was dried and analyzed for nonextractable radioactivity by combustion/LSS. The methanol extracts were diluted with water (50:50, v/v), partitioned into methylene chloride, and the methanol was evaporated and the remaining aqueous phase partitioned into ethyl acetate. Aliquots of the aqueous, methylene chloride, and ethyl acetate phases were analyzed for total radioactivity by LSS.

Fodder: Samples were extracted with methanol:water (75:25, v/v), filtered, and the remaining plant tissue was dried and analyzed for nonextractable radioactivity by combustion/LSS. The methanol was evaporated and the remaining aqueous extracts were adjusted to pH 2 with hydrochloric acid and partitioned into ethyl acetate. Aliquots of the ethyl acetate and aqueous phases were analyzed for total radioactivity by LSS. The distribution of total radioactivity in extracts of forage, silage, and fodder (2x-treated corn) is presented in Table 3.

Table 3. Distribution of total radioactivity in extracts of forage, silage, and fodder of field-grown corn treated with [$^{14}$C]trifluralin at 1.5 lb ai/A (2x).

| Commodity | Sampling interval (days) | Percent of total radioactivity | | | |
|---|---|---|---|---|---|
| | | methylene chloride | ethyl acetate | aqueous | non-extractable |
| Forage | 0 | 97.8 | 0.4 | 1.2 | 0.6 |
| | 7 | 13.5 | 17.9 | 20.0 | 48.5 |
| | 14 | 9.9 | 4.3 | 30.3 | 55.5 |
| | 29 | 3.7 | 7.8 | 30.6 | 58.0 |
| Silage | 63 | 8.1 | 4.7 | 15.7 | 71.5 |
| Fodder | 106 | -- | 13.7 | 10.2 | 76.1 |

Isolation Of Lignin and Cellulose Fractions From Forage and Fodder

The lignin and cellulose fractions from fodder and 7-day forage samples were isolated using published methods (Loomis and Shull, 1937 and Van Soest and Wine, 1968) and analyzed for total radioactivity by combustion/LSS. The lignin and cellulose fractions in 7-day forage accounted for 22.9 and 11.6% of the total plant $^{14}$C-residues, respectively. In fodder, lignin and cellulose accounted for 34.9 and 10.0% of the total plant $^{14}$C-residues, respectively.

Hydrolysis of 7-day forage fractions: A sample of forage harvested 7 days following treatment at 2x was extracted using the procedures described previously; following extraction, 23.3% of the TRR partitioned into the methylene chloride/ethyl acetate fraction, 29.3% was in the aqueous extract, and 47.4% remained in the extracted solids.

The extracted plant tissue was refluxed with 2 N hydrochloric acid, filtered, and the extract was partitioned into ethyl acetate. The aqueous extract was adjusted to pH 12 with sodium hydroxide and partitioned into ethyl acetate. The remaining aqueous phase and the acidic and basic ethyl acetate phases were analyzed for total radioactivity by LSS. Following acid extraction, the remaining plant tissue was refluxed with 1N sodium hydroxide, filtered, and the aqueous extract was partitioned into ethyl acetate. The aqueous extract was adjusted to pH 2 with hydrochloric acid, partitioned into ethyl acetate, and the remaining aqueous phase was centrifuged and the precipitate removed. The precipitate, base-extracted forage, and aqueous and ethyl acetate phases were analyzed for total

radioactivity by LSS directly or following combustion. In addition, following initial extraction of 7-day forage (described previously), the final aqueous phase (after ethyl acetate partitioning) was refluxed with hydrochloric acid and partitioned into ethyl acetate. The remaining aqueous phase was neutralized, refluxed with sodium hydroxide, partitioned into ethyl acetate, adjusted to pH 2 with hydrochloric acid, and partitioned again into ethyl acetate. The aqueous phase and ethyl acetate extracts were analyzed for total radioactivity by LSS.

The pooled ethyl acetate fractions from the hydrolyzed insoluble and aqueous fractions contained 45.4% of the TRR in 7-day forage tissue; 15.5% remained in aqueous extracts following ethyl acetate partitioning, 11% was in the lignin precipitate, and 5.1% remained in unextracted solids. The combined ethyl acetate fractions were analyzed using silica gel chromatography and TLC.

Characterization of residues

Residues in the combined methylene chloride and ethyl acetate extracts of forage, silage, and fodder, and the ethyl acetate extracts from the hydrolyzed forage fractions were fractionated using medium-bore silica gel chromatography eluting with a nonlinear gradient of solvents of increasing polarity (hexane, toluene, ethyl acetate, and methanol with water or glacial acetic acid).

Radioactive fractions from the extracts of 7-day forage and the pooled ethyl acetate extracts of hydrolyzed insoluble and aqueous fractions of 7-day forage were analyzed further using one-dimensional thin-layer chromatography (TLC). Radioactive zones were located by autoradiography, identified by cochromatography of known standards, and radioassayed by LSS. The standard compounds used in this study were trifluralin, metabolites designated TR-1 through TR-6, TR-9, TR-14, TR-15, TR-18, TR-20, TR-21, TR-41, TR-43, C1, and C2 (these compounds are depicted in Table 1).

Seven-day forage: Trifluralin and TR-4 were identified by TLC (using hexane:methanol, 97:3, as the solvent system) as radioactive residues in one of the less polar fractions from silica gel column chromatography, comprising 4.1 and 0.2%, respectively, of the TRR. (The figure of 4.7% for trifluralin reported on page 35 of the submission is a typographical error, since the value of 4.1% calculated on page 46 appears to be correct.) Conjugated metabolites C1 (3.4% of the TRR) and C2 (2.7%), not found in previous metabolism studies, were identified as components in one of the more polar fractions (containing 14.7% of the TRR in forage), using methylene chloride:methanol, 80:20 as the TLC solvent system. The identities of C1 and C2 were confirmed by fast atom bombardment mass spectrometry. TR-6,



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TR-15, and TR-21 were isolated and comprised 0.3, 0.4%, and 0.7%, respectively, of the TRR. Diffuse radioactive zones appeared on the TLC plates cochromatographing with TR-2, TR-3, TR-5, TR-18, TR-20, TR-41, and TR-43 and accounted for <0.1-1.6% of the TRR; these zones were not well defined and could not be identified conclusively.

Hydrolyzed extracts of 7-day forage: It was reported in the submission that the radioactivity in one of the fractions from silica gel chromatography consisted of trifluralin accounting for 0.6% of the TRR; data from TLC were not presented to support this claim. Two additional fractions from silica gel chromatography containing 11.8 and 16.9% of the TRR were subjected to TLC. The TLC resulted in broad diffuse zones of radioactivity that could not be identified or quantified. Only one TLC plate was shown for each fraction (solvent system was unspecified) and whether or not alternate solvent systems had been used was not reported.

Forage, silage, and fodder: Trifluralin was reportedly identified by TLC of zero-day and 14-day forage, and the conjugated metabolites C1 and C2 were reported to have been identified by TLC in 14-day and 1-month forage samples; supporting chromatographic data were not presented. C1 and C2 were theorized to be present in all samples of forage, silage, and fodder since extracts of each of these commodities contained a radioactive fraction with the same elution behavior in silica gel column chromatography exhibited by the 7-day forage samples. TLC analysis was not performed with extracts of 1-month forage, silage, or fodder.

Conclusions for Corn Study: Total $^{14}C$-residues remaining on corn forage 2 weeks after application represented 2% of 0 time samples. Trifluralin accounted for only 2% of total radioactive residue (TRR) in the two week forage samples. No trifluralin was detected in any samples collected after 2 weeks. Only a small percentage of the TRR was identified as specific metabolic products. For all samples, except 0 time, the majority of plant $^{14}C$ was presented in the two fractions designated "extracted aqueous" and "extracted tissue." Additional characterization of these metabolites is needed.

Characterization of residues in or on grain was not pursued due to low levels of radioactivity (0.02 ppm). Following hexane extraction, residues in the soluble and insoluble fractions were reported as nondetectable. Since this an early season herbicide use, the plant metabolism requirement for corn may be satisfied by adequate data for forage and fodder, where significant levels of $^{14}C$-residue occur. Residues in or on fodder need to be further characterized; it should not be assumed that the residues are the same as those in forage based on the elution profiles seen in column chromatography. If an additional metabolism study on corn

is needed in order to fullfil the data requirements for forage and fodder, the study should be conducted at >2X level, if possible, in order to generate residues of sufficient magnitude in corn grain to allow characterization.

The relatively harsh procedures used to hydrolyze aqueous and insoluble residues in forage may have resulted in the degradation of residues and, thus, the absence of identifiable zones of radioactivity on thin-layer chromatograms of the extracts. Furthermore, the results of TLC of the hydrolyzed residues was reported for only one solvent system (not specified). The registrant should make additional efforts to characterize hydrolyzable residues by employing additional solvent systems for TLC. Also, the registrant should consider attempting to release bound residues by using a less rigorous, more specific procedure such as enzymatic hydrolysis.

The available data, though inadequate, indicate that with time following foliar application of trifluralin, residues in or on corn plants are converted from nonpolar to polar residues and subsequently into insoluble forms including cell wall components. Very little residue appears to be translocated to the grain or cobs. Trifluralin is the predominant residue in forage collected on the day of treatment. In 7-day forage, trifluralin and the conjugated residues designated C1 and C2 are detected as is a small amount of TR-4 and residues cochromatographing with TR-6, TR-15, and TR-21. Evidence exists for the presence of several other metabolites accounting for <0.1-1.6% of the TRR (the molecular structures and chemical names of trifluralin and its metabolites are summarized in Table 1). Since C1 and C2 are glucosyl conjugates of TR-14, we believe that TR-14 is an intermediate in trifluralin metabolism, although none has been isolated or identified. C1 and C2 are the only residues identified in plant samples collected at posttreatment intervals longer than 2 weeks. Significant amounts of radioactivity are associated with the isolated cell wall components cellulose and lignin in 7-day forage and in fodder.

<u>Mustard Plants</u>: Elanco Products Company (1989; MRID 41179002) submitted data pertaining to the metabolism of trifluralin in greenhouse-grown mustard plants. Mustard seed was planted in soil treated with a solution of uniformly ring-labeled [$^{14}$C]trifluralin (8.59 $\mu$Ci/mg specific activity, radiochemical purity 99.6%) and unlabeled trifluralin (final specific activity 4.01 $\mu$Ci/mg) in methanol at 1.323 ppm (2.6x the maximum registered rate). Mustard leaves and roots were harvested 8 weeks after planting and the roots were rinsed with water. Samples were chopped, frozen with liquid nitrogen, ground, and stored frozen until analysis; the storage intervals and temperatures were not specified.

Total Radioactive Residues

Samples were analyzed for total radioactivity by combustion/LSS. Total radioactive residues (TRR) in mature mustard leaves and roots harvested 8 weeks after planting were 0.126 and 0.816 ppm, respectively; the method detection limit and number of samples analyzed were not reported.

Extraction

Mustard leaves and roots were extracted with methanol, filtered, refluxed with methanol, and the filtrate concentrated. The combined extracts were diluted with water, partitioned into methylene chloride, and the methanol was evaporated. The aqueous phase was partitioned into ethyl acetate, adjusted to pH 2 with hydrochloric acid, and partitioned again into ethyl acetate. The methylene chloride and combined ethyl acetate extracts (designated EtOAc-1) were analyzed for total radioactivity by LSS. The methylene chloride and ethyl acetate fractions were pooled for analysis by silica gel column chromatography.

Hydrolysis

The aqueous fraction was refluxed in 3 N hydrochloric acid for 2 hours at an unspecified temperature, the pH was adjusted to 2 with sodium hydroxide, and the residues partitioned to ethyl acetate (designated EtOAc-2); the remaining aqueous phase was designated Aqueous-1. The solid plant residue was refluxed in 1 N hydrochloric acid at an unspecified temperature for 18 hours. The extract was filtered, the filtrate partitioned with ethyl acetate at pH 2 (EtOAc-3) and pH 10 (EtOAc-4), and the remaining solids subjected to lignin and cellulose isolation procedures. The remaining aqueous solution (Aqueous-2), Aqueous-1, and the four EtOAc extracts were each radioassayed by LSS. The aqueous fractions were combined and further fractionated using Amberlite XAD-2 chromatography. The ethyl acetate solutions were pooled for analysis by silica gel column chromatography.

Isolation of lignin and cellulose

Lignin and cellulose were isolated as described above for corn tissues.

The distribution of radioactivity in the fractions of leaves and roots obtained from extraction and hydrolysis procedures is illustrated in Table 4.

Table 4. Distribution of total radioactivity in extracts of mustard leaves and roots grown in soil treated with [$^{14}$C]trifluralin at 2.6x.

| Fraction | Percent of TRR (ppm) Leaves | Roots |
|---|---|---|
| (methanol extraction) | | |
| methylene chloride | 17.3 (0.022) | 29.1 (0.238) |
| ethyl acetate (EtOAc-1) | 15.4 (0.019) | 10.7 (0.087) |
| (acid hydrolysis) | | |
| EtOAc-2 | 7.5 (0.01) | 1.2 (0.01) |
| EtOAc-3 | 12.9 (0.016) | 11.8 (0.096) |
| EtOAc-4 | 1.2 (0.001) | 1.3 (0.01) |
| Aqueous-1 | 5.8 (0.007) | 1.2 (0.01) |
| Aqueous-2 | 12.5 (0.016) | 5.8 (0.047) |
| Lignin | 20.2 (0.026) | 36.1 (0.295) |
| Cellulose | 7.2 (0.009) | 2.8 (0.023) |

Characterization of residues

Organic solvent extracts were fractionated using silica gel column chromatography performed as explained above for corn tissues. Fractions eluting in solvents of similar polarity were pooled for further analysis by thin-layer chromatography (TLC).

Nonpolar fractions of the initial organic-solvent extracts were analyzed by TLC using hexane:methanol (97:3, v/v) and more polar fractions were separated using chloroform:methanol:acetic acid (90:10:2, v/v). Radioactive zones were located by autoradiography and identified by cochromatography with known standard compounds, and radioactivity was quantified by scraping the zones into scintillation fluid and analyzing using LSS. The standard compounds used in this study were trifluralin, metabolites designated TR-1 through TR-7, TR-9, TR-13 through -15, TR-17 through -19, TR-22, TR-28, TR-41, TR-42, TR-43, and TR-44 (these compounds are depicted in Table 1).

More than 80% of the radioactivity in the combined methylene chloride and ethyl acetate fractions from the initial extraction of leaves was detected, following column chromatography, in a nonpolar fraction accounting for 9.9% of the TRR and two more polar fractions accounting for 9.1 and 7.7% of the TRR. The nonpolar fraction from leaves was revealed by TLC to consist of

trifluralin, accounting for 9.3% of the TRR. The more polar fractions from initial leaf organic extracts were not characterized further. One fraction from column chromatography of the combined ethyl acetate extracts made during hydrolysis of leaf aqueous and insoluble fractions was analyzed by TLC; the only metabolite identified was TR-22 accounting for 0.9% of the TRR. Lignin and cellulose fractions accounted for 27.4% of the TRR in leaves.

The radioactivity in organic solvents from root extraction resolved into the same fractions as did that from leaves following column chromatography, with 29.2% of the TRR in the nonpolar fraction and 2.9 and 2.4% in two more polar fractions. The least polar fraction consisted of trifluralin, identified by TLC, accounting for 26.3% of the TRR. Also identified by TLC, each in of one or more of the six other fractions of this extract, were TR-2 (0.9% of the TRR), 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%). Ethyl acetate extracts from hydrolyzed root fractions contained TR-14 (0.4%), TR-17 (0.9%), TR-22 (0.2%), TR-41 (<0.1%), and TR-42/43 (0.2%). Lignin and cellulose contained 38.9% of the TRR in roots. (A radioactive zone labeled "-12" appeared on one of the TLC plates, although this designation does not correspond to any of the standard compounds tested.)

Residue remaining in aqueous solutions following hydrolysis were fractionated using Amberlite XAD-2 resin eluted sequentially with water; 20, 40, 60, and 80% methanol in water; methanol; acetone; ethyl acetate; methanol; and 0.1 N sodium hydroxide. Fractions from leaves contained 0-5% of the TRR in leaves and 0-2% of the TRR in roots. None of these fractions was characterized further.

Conclusions for Mustard Study: Over 60% of the total radioactive residue in leaf tissue was not characterized. The registrant has characterized over 70% of the TRR in root tissue and contends that, since the radioactive residues in root and leaf extracts exhibit similar elution patterns during column chromatography, the data from root extracts is sufficient to describe metabolism in leaves. However, in light of the large number of possible metabolites, potential differences in root and leaf metabolism (roots contained ca. 3x the amount of trifluralin found in leaves), and the possibility of the existence of the conjugated residues C1 and C2 (found in corn forage), we conclude that further characterization of radioactive residues in or on leaves of mustard plants is necessary. In addition, no data were submitted concerning the storage interval or temperature of storage for the samples in this current study, prior to analysis. These data are needed.

In summary, the nature of the residue in plants remains inadequately delineated for the following reasons: (i) no

residues were conclusively identified in or on corn fodder; (ii) <50% of the $^{14}C$-residues in corn forage were characterized; (iii) the radioactive residues released by hydrolysis of insoluble and aqueous fractions of corn forage were not identified; and (iv) over 60% of the total radioactive residue in mustard leaf tissue was not characterized.