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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

DATE: 18-MAY-2000

SUBJECT: PP# 9F05066. Tetraconazole use on Sugar Beets. **Evaluation of Residue Data and Analytical Methods**. MRID#s 447513-11 thru -18, 450684-04, and 450684-05. Barcode D254411. Chemical# 120603. Case# 289222. Submission# S537973.

FROM: William H. Donovan, Ph.D., Chemist
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Health Effects Division (HED) (7509C)

THRU: George F. Kramer, Ph.D., Chemist
Melba Morrow, D.V.M., Branch Senior Scientist
RAB1/HED (7509C)

TO: Mary Waller/Lisa Jones, PM Team 21
Registration Division (7505C)

Sipcam Agro USA, Inc., formerly Sostram Corporation (c/o Landis, International, Inc.), has submitted a petition for the establishment of permanent tolerances for residues of the fungicide, tetraconazole [(±)-2-(2,4-dichlorophenyl)-3-(1H-1,2,4-triazol-1-yl)propyl-1,1,2,2-tetrafluoroethyl ether], in/on the commodities listed below as a result of the petitioner's request to register use of the fungicide on sugar beets:

Sugarbeet roots	0.1 ppm
Sugarbeet tops	7 ppm
Sugarbeet pulp (dried)	0.3 ppm
Sugarbeet molasses	0.3 ppm
Sugarbeet refined sugar	0.01 ppm
Milk	0.02 ppm
Cattle meat	0.01 ppm
Cattle meat byproducts	2 ppm
Cattle fat	0.1 ppm

Tetraconazole is a new synthetic fungicide and is a member of the conazole class of pesticides. Other members of this class include hexaconazole and propiconazole. On sugar beets, tetraconazole is intended to control fungal diseases such as Cercospora leaf spot and powdery mildew.

The attached contractor's document has been reviewed and revised to reflect HED policy.

Executive Summary of Chemistry Deficiencies

- Revised Section B
- New sugar beet metabolism studies using phenyl- and tetrafluoroethyl-labeled tetraconazole
- Final identification of residues of concern in plants, livestock, and rotational crops
- Sample storage information/storage stability data supporting the sugar beet and goat nature of the residue studies
- Radiovalidation of the plant and livestock analytical methods
- Agency validation of the plant and livestock analytical methods
- Confirmatory method
- Independent laboratory validation (ILV) of triazole method for livestock commodities*
- Multiresidue testing results
- Triazole storage stability data supporting storage intervals of cattle milk and tissue samples*
- Bovine feeding study with a minimum dose rate equivalent to 6.2 ppm tetraconazole
- Storage stability data supporting the rotational crop study
- New rotational crop study using phenyl-labeled tetraconazole
- Revised Section F

* Contingent on findings of HIARC meeting

RECOMMENDATIONS

The residue chemistry database does not presently support the establishment of tolerances for residues of tetraconazole *per se* in/on the raw and processed commodities of sugar beets, or the establishment of tolerances for residues of tetraconazole and triazole in the milk and edible tissues of ruminants. The petitioner should address the deficiencies discussed in Conclusions 1, 2a, 2b, 3a, 3b, 4b, 4c, 5b, 5c, 6, 8, 11, 13a, 13b, and 13c, and submit a revised Section F to correct commodity definitions and/or adjust tolerance levels as appropriate (Conclusions 9b, 9d, 10b, 10c, 12a, 12b, 12c, and 12d). HED will initiate a human health risk assessment of the proposed uses on tetraconazole on sugar beets when the above deficiencies have been resolved.

Attachment 1- contractor review

cc: PP#9F05066, W. H. Donovan, O. Odiott
RDI: G.F. Kramer (17-MAY-2000), M. Morrow (18-MAY-2000), RAB1 Chemists (04-MAY-2000), ChemSAC (17-MAY-2000)
W.H. Donovan:806R:CM#2:(703)305-7330:7509C:RAB1

TETRACONAZOLE
PC Code 120603
(DP Barcode D254411)

PP#9F05066; EVALUATION OF RESIDUE CHEMISTRY DATA TO SUPPORT PERMANENT TOLERANCES FOR USE OF TETRACONAZOLE ON SUGAR BEETS

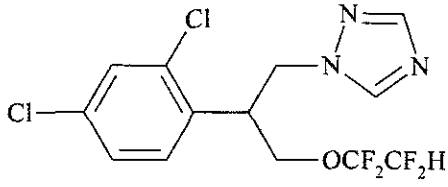
January 11, 2000

Contract No. 68-D4-0010

Submitted to:
U.S. Environmental Protection Agency
Arlington, VA

Submitted by:
Dynamac Corporation
The Dynamac Building
2275 Research Boulevard
Rockville, MD 20850-3268

TETRACONAZOLE



PP#9F05066: EVALUATION OF RESIDUE CHEMISTRY DATA

TO SUPPORT PERMANENT TOLERANCES FOR USE OF

TETRACONAZOLE ON SUGAR BEETS

PC CODE NO. 120603

(DP BARCODE D254411)

INTRODUCTION

Sipcam Agro USA, Inc., formerly Sostram Corporation (c/o Landis, International, Inc.), has submitted a petition for the establishment of permanent tolerances for residues of the fungicide, tetraconazole [(±)-2-(2,4-dichlorophenyl)-3-(1H-1,2,4-triazol-1-yl)propyl-1,1,2,2-tetrafluoroethyl ether], in/on the commodities listed below as a result of the petitioner's request to register use of the fungicide on sugar beets:

Sugarbeet roots	0.1 ppm
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Sugarbeet refined sugar	0.01 ppm
Milk	0.02 ppm
Cattle meat	0.01 ppm
Cattle meat byproducts	2 ppm
Cattle fat	0.1 ppm

Tetraconazole is a new synthetic fungicide and is a member of the conazole class of pesticides. Other members of this class include hexaconazole and propiconazole. On sugar beets, tetraconazole is intended to control fungal diseases such as Cercospora leaf spot and powdery mildew.

Time-limited tolerances for residues of tetraconazole *per se* have recently been established [40 CFR §180.557(b)] in/on sugar beet roots at 0.10 ppm, sugar beet tops at 6.0 ppm, sugar beet dried pulp at 0.20 ppm, sugar beet molasses at 0.30 ppm, cattle fat at 0.60 ppm, cattle kidney at 0.20 ppm, cattle liver at 6.0 ppm, cattle meat at 0.030 ppm, cattle meat byproducts (except kidney and liver) at 0.030 ppm, and milk at 0.050 ppm [FR Vol. 64, No. 233, pp.68046-68052, 12/6/99]. These time-limited tolerances were established in connection with an emergency exemption under FIFRA Section 18 authorizing use of tetraconazole on sugar beets in North Dakota and Minnesota. The tolerances will expire on December 31, 2001. Information concerning exposures and risks related to this Section 18 exemption request was summarized by HED in a 3/18/99 memorandum (DP Barcodes D252214 and D252213, W. Dykstra, L. Cheng, and S. Tadayon).

In addition to the present sugar beet petition, Sipcam Agro has concurrently requested the establishment of a tolerance for residues of tetraconazole *per se* in/on imported bananas (PP#7E04830) and permanent tolerances for residues of tetraconazole *per se* in/on commodities of peanuts (PP#9F06023). Residue chemistry data associated with these two petitions are the subject of separate reviews but are referenced in this document where appropriate.

The HED Metabolism Assessment Review Committee (MARC) considered the results of the available tetraconazole metabolism studies in two meetings held 07- and 14-MAR-2000 (D264157, W. Donovan and D. Nixon, 19-APR-2000). Conclusions reached and data gaps identified by the MARC are also included in this document. The MARC determined that triazole (a tetraconazole metabolite) should be considered by the HIARC for endpoint selection and confirmation of the need to include it in the tetraconazole tolerance expression and risk assessment. This chemistry review was prepared under the assumption that the HIARC will confirm the MARC's concern about triazole. Should the HIARC determine that triazole is not of concern, then the conclusions in this review pertaining to triazole will need to be modified.

CONCLUSIONS

OPPTS GLN 860.1200: Proposed Uses

1. No rotational crop restrictions are included on the submitted label. Based on the results of a confined rotational crop study submitted in support of the peanut petition PP#9F06023 (D259321, W. Donovan, in preparation), a revised Section B is required to incorporate the following crop restrictions: "Peanuts and sugar beets may be rotated at any time. Rotation to all other crops is prohibited."

OPPTS GLN 860.1300: Nature of the Residue in Plants

Sugar beet

2a. The submitted sugar beet metabolism study is inadequate. The study was conducted using only triazole-labeled tetraconazole and did not include information pertaining to sample storage intervals (from harvest to the final TLC analyses of extracts). The HED MARC determined that data from sugar beet studies using phenyl-labeled tetraconazole and tetrafluoroethyl-labeled tetraconazole is needed to fully assess the nature of the residue in sugar beets (D264157, W. Donovan and D. Nixon, 19-APR-2000). Moreover, the submitted study used an application rate of approximately 0.4x the maximum seasonal rate, resulting in no detectable residues in sugar beet roots. The additional studies should be conducted at exaggerated rates (see discussion in OPPTS 860.1300) so that tetraconazole and metabolites can be adequately identified/characterized in sugar beet roots and tops.

2b. The HED MARC tentatively determined that the residue of concern in sugar beets, peanuts, and bananas is tetraconazole *per se*. However, this conclusion cannot be finalized until the MARC considers the results of additional data as specified in the MARC results memo (D264157, W. Donovan and D. Nixon, 19-APR-2000).

OPPTS GLN 860.1300: Nature of the Residue in Livestock

Ruminants

3a. The goat metabolism studies are acceptable provided the petitioner submits supporting storage stability data for the total toxic residues of tetraconazole in goat milk and tissues. It appears that milk and tissues samples may have been stored frozen for up to 351 days prior to study completion. The petitioner is required to provide evidence that the identity of residues did not change during the period between collection and final chromatographic analysis. Typically, this can be achieved by analyses of a representative substrate early in the study and at its completion. Such analyses should show that the basic profile of radiolabeled residues has not changed during that time.

3b. The HED MARC tentatively determined that the residues of concern in livestock commodities are tetraconazole and triazole. However, before this conclusion can be finalized, the Committee must consider the findings from a scheduled Hazard Identification Assessment Review Committee (HIARC) meeting on triazole and evaluate data from a poultry nature of the residue study (D264157, W. Donovan and D. Nixon, 19-APR-2000).

OPPTS GLN 860.1340: Residue Analytical Method - Plant Commodities

4a. The petitioner utilized a gas chromatography/electron capture detection (GC/ECD) method for the determination of tetraconazole residues in/on samples of sugar beet commodities that were collected from the field, processing, and storage stability studies. The validated method limits of quantitation (LOQs) were 0.010 ppm for sugar beet roots, molasses, and refined sugar, 0.10 ppm for sugar beet tops, and 0.20 ppm for sugar beet dry pulp. The method validation and concurrent method recovery data indicate that this method is adequate for data collection. In addition, the petitioner submitted an independent

laboratory validation (ILV) of the GC/ECD method, demonstrating adequate recoveries from fortified samples of peanut, peanut oil, banana, and refined sugar.

4b. The registration requirements for residue analytical methods in plants remains unfulfilled. The GC/ECD method should be subjected to radiovalidation using samples from the plant metabolism studies to determine whether the method recovers total toxic residues of tetraconazole from weathered plant matrices. The GC/ECD plant method has been forwarded to the Agency laboratories for petition method validation (PMV) (D264681, W. Donovan, 07-APR-2000). Conclusions about the adequacy of the analytical method for enforcement purposes will be deferred until completion of the PMV.

4c. The GC/ECD method should be supplemented by a confirmatory method that is significantly different (such as mass spectrometry (MS)). If the petitioner proposes a confirmatory method which employs MS, then an interference study is not necessary (chromatograms and spectra of fortified samples should be submitted along with the limit of quantitation (LOQ)).

OPPTS GLN 860.1340: Residue Analytical Method - Livestock Commodities

5a. The petitioner utilized a GC/ECD method for the determination of tetraconazole residues in/on samples of cattle milk and tissues that were collected from the dairy cattle feeding study. The validated LOQ of the GC/ECD method was 0.01 ppm for all cattle matrices. A gas chromatography/flame ionization detection (GC/FID) method was used for the determination of triazole residues in/on the same samples; the validated LOQs of the GC/FID method were 0.015 ppm in milk and 0.020 ppm in fat, liver, and muscle. The concurrent method recovery data indicate that these methods are adequate for data collection. In addition, the petitioner submitted an ILV of the GC/ECD method, demonstrating adequate recoveries from fortified samples of milk, eggs, muscle, fat.

5b. The petitioner has indicated that the GC/ECD method may also be used for enforcement of tetraconazole tolerance levels in livestock commodities. The method should be subjected to radiovalidation using samples from the ruminant metabolism studies to determine whether the method recovers total toxic residues of tetraconazole from weathered livestock matrices. The GC/ECD livestock method has been forwarded to the Agency laboratories for petition method validation (D264681, W. Donovan, 07-APR-2000). Conclusions about the adequacy of the analytical method for enforcement purposes will be deferred until completion of the PMV.

5c. Because the HED MARC tentatively determined that triazole is a residue of concern in livestock commodities, an enforcement method is needed to detect triazole residues in livestock commodities. Accordingly, if the HIARC confirms the decision to regulate triazole, the petitioner should have an ILV study conducted on the GC/FID method for

determination of triazole residues in livestock commodities. If the results of the ILV are acceptable, the method will be forwarded to the Agency laboratory for PMV.

OPPTS GLN 860.1360: Multiresidue Method

6. Data concerning the recovery of tetraconazole residues of concern using FDA's multiresidue method protocols (PAM Vol. I) have not been submitted but are required for this tolerance petition request.

OPPTS GLN 860.1380: Storage Stability Data

7. The storage intervals and conditions for sugar beet commodities collected from the field and processing studies are supported by adequate storage stability data. Residues of tetraconazole are stable under frozen storage conditions (-20 C) in/on sugar beet roots and tops for up to 70 days (~2.5 months) and in the processed commodities of sugar beet (dry pulp, molasses, and refined sugar) for up to 34 days (~1 month). Samples from the field and processing studies were stored frozen for up to 78 and 35 days, respectively.

8. All livestock matrices collected from the dairy cattle feeding study were stored frozen for less than 37 days (~1 month) prior to analysis for residues of tetraconazole. Data to support the storage intervals and conditions for milk and tissue samples from the feeding study are not required because samples were analyzed for tetraconazole residues within approximately one month. Separate subsamples of milk and tissues, stored for up to 101 days (3.5 months), were also analyzed for triazole residues. The petitioner indicated that a storage stability study of triazole residues in livestock commodities is ongoing at Isagro Ricerca, and reported that preliminary data suggest that residues of triazole are stable in cattle milk for up to 1 year and in cattle tissues for up to 3 months. HED will verify these statements when the petitioner submits the final storage stability report for triazole.

OPPTS GLN 860.1500: Crop Field Trials

Beets, sugar

9a. In support of this petition, 11 trials reflecting the maximum proposed use pattern for sugar beets were conducted. For the establishment of tolerances on sugar beet commodities, Tables 1 and 5 of OPPTS GLN 860.1500 specify that 12 field trials should be conducted in Regions 5 (5 trials), 7 (1 trial), 8 (1 trial), 9 (1 trial), 10 (2 trials), and 11 (2 trials). HED will not require the petitioner to conduct an additional field trial in Region 11 because there does not appear to be wide variability in residues obtained in the current submission.

9b. The submitted field trial data indicate that residues of tetraconazole will not exceed the proposed tolerance of 7 ppm in/on sugar beet tops, when the 1 lb/gal SC formulation of tetraconazole is applied according to the maximum proposed use pattern. However, the field trial data indicate that the proposed tolerance of 0.1 ppm for tetraconazole residues

in/on sugar beet roots should be increased to 0.15 ppm. Residues of tetraconazole were 0.0132-0.103 ppm and 1.13-5.90 ppm, respectively, in/on sugar beet roots and tops harvested 14 days following the last of six sequential broadcast applications of the 1 lb/gal SC formulation at 0.107 lb ai/A/application (1x the maximum proposed single and seasonal application rates).

9c. The residue decline data suggest that residues of tetraconazole dissipated from 3.21 ppm (0-day pre-harvest interval (PHI)) to 0.869 ppm (60-day PHI) in/on sugar beet tops. A meaningful decline trend was not observed in sugar beet roots.

9d. The petitioner should submit a revised Section F to correct the commodity definitions for tetraconazole tolerances for sugar beet roots and tops to "beet, sugar, roots" and "beet, sugar, tops."

OPPTS GLN 860.1520: Processed Food/Feed

Beets, sugar

10a. The submitted sugar beet processing data are adequate for the purposes of this petition. Residues of tetraconazole did not concentrate in refined sugar but concentrated 2.1x in dry pulp and 2.8x in molasses processed from sugar beet roots bearing detectable residues.

10b. The maximum expected residue of tetraconazole in sugar beet dry pulp and molasses are 0.181 and 0.242 ppm, calculated by multiplying the highest average field trail (HAFT) residue (0.0864 ppm; see sugar beet field trial) and the observed concentration factors (2.1x in dry pulp and 2.8x in molasses). Based on this calculation, the proposed tolerances of 0.3 ppm for residues of tetraconazole in sugar beet dry pulp and molasses are appropriate. The petitioner should submit a revised Section F to correct the commodity definitions for tetraconazole tolerances for dry pulp and molasses to "beet, sugar, dried pulp" and "beet, sugar, molasses."

10c. The proposed tolerance for residues of tetraconazole in sugar beet refined sugar is not required. Expected tetraconazole residues in refined sugar do not exceed the proposed tolerance for the raw agricultural commodity (RAC). The petitioner should delete this commodity (sugar beet refined sugar) from the requested Section F revision.

OPPTS GLN 860.1480: Meat, Milk, Poultry, Eggs

Milk and edible tissues of ruminants

11. The submitted dairy cattle feeding data are adequate for the purpose of establishing a tolerance for secondary transfer of tetraconazole and triazole residues in dairy cattle milk, but not in tissues. The submitted feeding study had a maximum feed rate equivalent to 3.4 ppm tetraconazole, which covers the MTDB for dairy cattle. However, the MTDB of beef

cattle is 6.2 ppm. Thus, in order to determine the appropriate tolerance levels in cattle tissues, a feeding study with a feed rate equivalent to at least 6.2 ppm tetraconazole is needed. With the data presently available and applying a multiplication factor of (6.2/3.4) = 1.82, the tolerance levels that follow may be derived. However, these levels are subject to change once the data from the requested feeding study are submitted and once the HIARC has issued recommendations about triazole.

12a. The combined maximum residues of tetraconazole and triazole were 0.071 ppm in whole milk, 0.024 ppm in skimmed milk, and 0.420 ppm in cream. These data suggest that the proposed tolerance of 0.02 ppm for milk is inadequate and that a specific tolerance value should be established for milk fat. The petitioner should submit a revised Section F to propose a tolerance for residues of tetraconazole and triazole in "milk, fat (0.08 ppm in whole milk) at 2.5 ppm".

12b. The combined maximum residues of tetraconazole and triazole were 0.227 ppm in subcutaneous fat and 0.219 ppm in peritoneal fat. These data suggest that the proposed tolerance of 0.1 ppm for cattle fat is inadequate. For the purposes of conditional registration/temporary tolerances, the petitioner should submit a revised Section F to propose tolerances for residues of tetraconazole and triazole in the "fat of cattle, goats, hogs, horses, and sheep at 0.50 ppm".

12c. The combined maximum residues of tetraconazole and triazole were 0.101 ppm in kidney and 1.879 ppm in liver. These data suggest that separate tolerances should be established for kidney and liver because of the 18x difference in the magnitude of the expected residues. For the purposes of conditional registration/temporary tolerances, the petitioner should submit a revised Section F to propose tolerances for residues of tetraconazole and triazole in the "meat byproducts (except liver) of cattle, goats, hogs, horses, and sheep at 0.20 ppm" and in the "liver of cattle, goats, hogs, horses, and sheep at 3.5 ppm".

12d. The combined maximum residues of tetraconazole and triazole were <0.030 ppm in muscle. These data suggest that the proposed tolerance of 0.01 ppm for cattle meat is inadequate. For the purposes of a conditional registration/temporary tolerances, the petitioner should submit a revised Section F to propose a tolerance for residues of tetraconazole and triazole in the "meat of cattle, goats, hogs, horses, and sheep at 0.060 ppm".

<u>OPPTS GLNs 860.1850 and 860.1900: Confined/Field Accumulation in Rotational Crops</u>

13a. The petitioner submitted a confined rotational crop study in conjunction with the peanut petition (PP#9F06023, D259321, W. Donovan, in preparation). Pending submission of storage stability data to validate the storage conditions and intervals of rotational crop commodities, the submitted confined rotational crop study for triazole-labeled tetraconazole is adequate. However, as the triazole-labeled study showed evidence for

cleavage of tetraconazole occurring between the phenyl and triazole rings, a rotational crop study using phenyl-labeled tetraconazole is needed to determine whether this moiety is translocated into the rotational crops.

13b. Although the petitioner has not proposed plantback restrictions for rotational crops on the product label, rotational restrictions are required. Subject to change based on the results of the requested phenyl-labeled tetraconazole rotational crop study, the rotational restrictions are specified in the "OPPTS GLN 860.1200: Proposed Uses" section of this document. If the petitioner wishes to have rotational restrictions other than those specified in this document, then the petitioner should submit limited field trial data depicting tetraconazole residues of concern in/on rotational crops at the plantback interval(s) the petitioner wants to support.

13c. The HED MARC tentatively determined that the residue of concern in rotational crops is tetraconazole *per se*. However, before this conclusion can be finalized, the MARC requires review of the requested rotational crop study using phenyl-labeled tetraconazole, and consideration of the HIARC deliberations on triazole (D264157, W. Donovan and D. Nixon, 19-APR-2000).

Codex Issues

14. There are no established Codex, Canadian, or Mexican limits for residues of tetraconazole in/on plant or livestock commodities. Therefore, no compatibility issues exist with regards to the proposed tolerances discussed in this petition review.

RECOMMENDATIONS

The residue chemistry database does not presently support the establishment of tolerances for residues of tetraconazole *per se* in/on the raw and processed commodities of sugar beets, or the establishment of tolerances for residues of tetraconazole and triazole in the milk and edible tissues of ruminants. The petitioner should address the deficiencies discussed in Conclusions 1, 2a, 2b, 3a, 3b, 4b, 4c, 5b, 5c, 6, 8, 11, 13a, 13b, and 13c, and submit a revised Section F to correct commodity definitions and/or adjust tolerance levels as appropriate (Conclusions 9b, 9d, 10b, 10c, 12a, 12b, 12c, and 12d). HED will initiate a human health risk assessment of the proposed uses on tetraconazole on sugar beets when the above deficiencies have been resolved.

DETAILED CONSIDERATIONS

OPPTS GLN 860.1200: Proposed Uses

Information pertaining to the proposed uses of tetraconazole on sugar beets was obtained from the administrative materials submitted for the peanut petition (PP#9F06023).

A soluble concentrate (SC) formulation [Product Name = Eminent™ 125 SL; EPA File Symbol No. 60063-RE] containing 11.6% or 1 lb ai/gal of tetraconazole is proposed for use on sugar beets for the control of Cercospora leaf spot and powdery mildew. The formulation is proposed for up to six foliar spray applications at 13 fl oz. product per application (equivalent to 0.102 lb ai/A/application) for a total seasonal rate of 0.612 lb ai/A. Application is to be made beginning when conditions are favorable for disease development and repeated at 14- to 21-day retreatment intervals, if needed. Ground or aerial equipment may be used, and applications may be made in a minimum of 20 gal of water/A for dilute spray and 5 gal of water/A for concentrate spray. The label recommends that application of the product be alternated with a non-triazole fungicide registered for use on sugar beets. Combination with other pesticides, fertilizers, or surfactants is not recommended unless prior use has demonstrated the tank mix to be compatible and effective. The proposed preharvest interval (PHI) is 14 days and the proposed restricted entry interval is 24 hours. No rotational crop restrictions are included on the label.

Conclusions: No rotational crop restrictions are included on the submitted label. Based on the results of a confined rotational crop study submitted in support of the peanut petition PP#9F06023 (D259321, W. Donovan, in preparation), a revised Section B is required to incorporate the following crop restrictions: "Peanuts and sugar beets may be rotated at any time. Rotation to all other crops is prohibited."

OPPTS GLN 860.1300: Nature of the Residue in Plants

Sugar beet

The petitioner has submitted the results of a sugar beet metabolism study (citation listed below) reflecting use of [^{14}C]tetraconazole labeled at the triazole ring. The in-life and analytical phases of the study were conducted at the ISAGRO s.r.l. Biochemistry Unit (Novara, Italy).

> 44751311 Pizzingrilli, G.; Rizzo, F. (1996) Metabolism of ((carbon-14)-triazole) Tetraconazole in the Sugar Beet: Lab Project Number: R/ABT.95.06. Unpublished study prepared by Isagro Ricerca S.R.L. 210 p.

The test substance, [triazole-^{14}C]tetraconazole (specific activity 136.63 μCi/mg and radiochemical purity >98%), was mixed with nonlabeled tetraconazole and water to obtain a formulated test substance with a specific activity of 13.6 μCi/mg. The formulated test substance was applied as three foliar spray applications at 0.089 lb ai/A/application (equivalent to ~0.44x the maximum proposed seasonal application rate) to sugar beet plants planted in pots and maintained outdoors. Applications were made at 21-day retreatment intervals using a nitrogen-assisted Devilbliss atomizer. The first application was made when plants were 10 weeks old and the last application was made 35 days prior to maturity. Control plants in separate pots received applications of a blank formulation. Duplicate samples of sugar beet plants (leaves and roots) were collected two hours following each application, and four replicate samples were collected at maturity (35 days following the last application); a single control sample was collected at each

interval. Leaves were separated from the root by hand using scissors. The root was washed with running water and wiped dry. Fresh weights were determined, and all samples were stored at -20 C until analysis.

Total radioactive residues (TRR)

Sugar beet roots and leaves were sequentially homogenized with acetone:water (70:30, v:v) and acetone, then centrifuged. Radioactivity in the acetone:water and acetone extracts was determined by LSC; radioactivity in the nonextractable residues was determined by combustion/LSC. Duplicate samples were analyzed for all immature harvest intervals, and four replicate samples were analyzed for the mature (35-Day) samples. The petitioner did not explain how TRR values were determined; the study reviewer assumes that the petitioner summed the radioactivity in extracts and nonextractable residues. The TRR in sugar beets are presented in Table 1. The reported limits of quantitation (LOQs) for TRR determinations were 0.0002-0.0048 ppm tetraconazole equivalents.

Table 1. Total radioactive residues in sugar beet commodities following administration of three spray applications of [^{14}C]tetraconazole at 0.089 lb ai/A/application (0.27 lb ai/A, 0.44x).

Harvest interval	TRR (ppm), tetraconazole equivalents [a]	
	Sugar beet roots	Sugar beet leaves
Two hours following first application	<0.003, <0.005 (<0.004)	1.499, 1.658 (1.579)
Following second application	0.005, 0.007 (0.006)	1.511, 2.216 (1.864)
Immediately following third application (0-Day)	0.007, 0.009 (0.008)	2.633, 3.580 (3.107)
35 days following third application (35-Day)	0.006, 0.006, 0.008, 0.009 (0.007)	1.240, 1.267, 1.268, 1.567 (1.336)

[a] Average residues are in parenthesis.

Extraction and hydrolysis of residues

Because the TRR in sugar beet roots from all sampling intervals were below 0.01 ppm, no additional extraction or analysis was conducted on this matrix. The acetone:water and acetone extracts of sugar beet leaves were combined, and the combined extracts were subjected to further extraction procedures. The petitioner provided adequate descriptions of the fractionation schemes used for the analysis of tetraconazole residues. During the extraction and fractionation procedures, aliquots of extracts, hydrolysates, and nonextractable residues were analyzed for radioactivity by LSC or combustion/LSC.

The combined acetone:water extracts were concentrated to aqueous by rotary evaporation. The remaining aqueous extract was sequentially partitioned with n-hexane (2x; Extract D), ethyl acetate (2x; Extract E), and water-saturated n-butanol (2x; Extract F), then acidified to pH 3 with

12 N HCl and extracted again with water-saturated –butanol (2x; Extract G). Each extract was reserved for TLC analysis.

The remaining aqueous phase of the 35-Day leaf sample was then subjected to enzymatic hydrolysis (β-glucosidase in 0.1 M acetate buffer, pH 4.8, at 37 C for 72 hours). The enzyme hydrolysate was partitioned with water-saturated n-butanol (2x). A separate subsample of the aqueous phase of the 35-Day leaf sample and a subsample of the 0-day leaf aqueous phase was subjected to acid hydrolysis (6 N HCl at 60 C for 5 hours). The acid hydrolysate was partitioned with water-saturated n-butanol (2x). The n-butanol phases following enzymatic and acid hydrolysis were reserved for TLC analysis. No conjugates were cleaved by enzyme hydrolysis, while acid hydrolysis decreased the polar components, giving rise to two new compounds.

The distribution of ^{14}C-activity in the extracts of triazole-label sugar beet matrices is presented in Table 2.

Characterization and identification of residues

The organic extracts of the leaf samples from each sampling interval were concentrated to dryness and redissolved in methanol for analysis by TLC to determine the metabolic profile. Extract F was not completely dissolved in methanol; therefore, the solid phase was dissolved in water and both phases (Extracts F_m and F_w) were analyzed by TLC. TLC analyses were conducted on reverse-phase 60 F_{254} silica gel plates (Merck) using solvent systems of ethyl acetate, chloroform:methanol (7:3, v:v), or chloroform:methanol:water (55:40:5, v:v:v). Radioactivity was detected and quantified using radiographic imaging. ^{14}C-Labeled reference standards of tetraconazole and the following metabolites were used for TLC co-chromatography: triazolyl acetic acid (TAA), M14360-acid, M14360-DFA, M14360-difluoroacetic acid, triazole, and M14360-alcohol. Refer to Figure 1 (Attachment II) for full chemical names and structures of identified metabolites.

Extracts E, F, and G required additional cleanup by silica gel column chromatography prior to analysis by TLC. Residues were eluted with ethyl acetate:methanol (gradient from 0-100% methanol) or methanol:water (80:20, v:v). The collected fractions containing significant radioactivity were combined, evaporated to dryness, and redissolved in methanol for TLC analysis.

Metabolite 3, collected from Extract F_m, was evaporated to dryness and redissolved in isobutanol. Gaseous HCl was bubbled through the mixture at 65 C for 30 minutes until esterification was complete. The residue was then evaporated to dryness, redissolved in water, and partitioned with ethyl acetate (3x; Extract S). Extract S was analyzed by TLC, and then residues were acetylated with 2.5 M triethylamine and acetic acid (for one hour at 40 C, and overnight at room temperature). Residues were evaporated to dryness, redissolved in water, and partitioned with n-hexane (3x; Extract T). Extract T was analyzed by TLC. Metabolite 3 in Extract T was identified by GC/MS as triazolyl-hydroxyproprionic acid (THP); identification was confirmed by co-chromatography with ^{14}C-labeled reference standard.

Nonextractable residues of 0- and 35-Day sugar beet leaves following acetone:water extraction were subjected to further extractions at reflux. A subsample was sequentially refluxed for 3 hours with acetone (80 C; Extract M), methanol (80 C; Extract N), and with water (100 C; Extract O). The nonextractable residues following the reflux extractions were refluxed at 110 C with 5% NaOH for 3 hours, and centrifuged to separate the cellulose fraction from the soluble fraction. The soluble fraction was then acidified with hydrochloric acid to pH 2 and centrifuged again to separate the lignin fraction from the soluble fraction. Small amounts of radioactivity were released from nonextractable residues by the refluxing extractions. TLC analysis of the pooled acetone and methanol extracts (Extracts M and N) identified the parent as the major metabolite and eight minor metabolites observed in other leaf extracts. Nonextractable residues of the 35-Day leaves were primarily incorporated in the cellulose fraction (1.85% TRR, 0.035 ppm), with smaller amounts associated with the lignin fraction (0.08% TRR, 0.002 ppm).

The identified ^{14}C-residues in 0- and 35-Day triazole-label sugar beet leaves are presented in Table 3. The chemical names and structures of tetraconazole and its metabolites identified from the sugar beet metabolism study are depicted in Figure 1 (Attachment II).

Storage stability

Information pertaining to sample storage intervals (from harvest to final TLC analyses of extracts) was not provided. The petitioner is required to provide this information and determine from calculated intervals whether storage stability data are required. The Agency does not require supporting storage stability for plant metabolism studies for samples analyzed within 4-6 months of collection, provided evidence is given that attempts were made to limit degradation of residues by appropriate storage of matrices and extracts during the analytical portion of the study.

Proposed metabolic pathway of tetraconazole in sugar beets

Based on the results of the sugar beet metabolism studies, the petitioner proposes that tetraconazole is slowly metabolized in succession to M14360-DFA, M14360-alcohol, M14360-acid, triazole, TAA, THP, and more polar metabolites. Eventually, residues are incorporated into the natural matrix as bound residues, such as the support structure (primarily cellulose) of the plant.

Summary

Following three foliar applications, with 21-day retreatment intervals of [triazole-^{14}C]tetraconazole at 0.089 lb ai/A/application (equivalent to ~0.44x the maximum proposed seasonal rate), the total radioactive residues (TRR), expressed as tetraconazole equivalents, in sugar beets harvested immediately after each application and 35 days after the final application (at maturity) were <0.01 ppm in all root samples and 1.336-3.107 ppm in leaves.

The petitioner did not conduct further analyses of residues in sugar beet roots because the TRR were <0.01 ppm. In sugar beet leaves, ~93-98% TRR were identified/characterized. Tetraconazole was the major residue component identified in sugar beet leaves harvested 0 days (82.14% TRR, 2.550 ppm) and 35 days (48.43% TRR, 0.652 ppm) following the final application. The following additional minor metabolites (≤10% TRR) were tentatively identified in both the 0- and 35-Day leaf samples: triazolyl acetic acid, triazolyl-hydroxypropionic acid, M14360-acid, M14360-DFA, M14360-alcohol, and triazole. Nonextractable residues in sugar beet leaves were characterized as being mostly associated with lignin.

Table 2. Distribution of total radioactive residues in sugar beet leaves following up to three spray applications of triazole-labeled [^{14}C]tetraconazole at 0.089 lb ai/A/application.

Fraction	% TRR	ppm [a]	Characterization/Identification [b]
Sugar beet leaves - following first application (TRR = 1.579 ppm)			
Combined acetone:water	99.72	**1.575**	Sequentially extracted with n-hexane, EtOAc, –butanol, acid n-butanol, and water.
n-Hexane (Extract D)	97.34	**1.537**	TLC analysis resolved: Tetraconazole 97.34% TRR 1.537 ppm
EtOAc (Extract E)	1.95	**0.031**	
n-Butanol (Extract F)	NR [c]	NR	
Acid n-butanol (Extract G)	NR	NR	
Aqueous (Extract H)	0.44	**0.007**	TLC analysis resolved one or more unknown compounds present at 2.39 % TRR (0.038 ppm).
Nonextractable	0.28	**0.004**	N/A.
Sugar beet leaves - following second application (TRR = 1.864 ppm)			
Combined acetone:water	98.05	**1.828**	Sequentially extracted with n-hexane, EtOAc, –butanol, acid n-butanol, and water.
n-Hexane (Extract D)	96.31	**1.795**	TLC analysis resolved: Tetraconazole 96.31% TRR 1.796 ppm
EtOAc (Extract E)	1.44	**0.027**	
n-Butanol (Extract F)	NR	NR	
Acid n-butanol (Extract G)	NR	NR	
Aqueous (Extract H)	0.30	**0.006**	TLC analysis resolved one or more unknown compounds present at 1.74% TRR (0.031 ppm).
Nonextractable	1.95	**0.036**	N/A.
Sugar beet leaves - immediately following third application (0-Day; TRR = 3.107 ppm)			
Combined acetone:water	96.05	**2.984**	Sequentially extracted with n-hexane, EtOAc, –butanol, acid n-butanol, and water.
n-Hexane (Extract D)	80.97	**2.516**	TLC analysis resolved: Tetraconazole 82.14% TRR 2.550 ppm TAA 1.34% TRR 0.042 ppm THP 2.42% TRR 0.077 ppm M14360-acid 1.38% TRR 0.043 ppm M14360-DFA 2.79% TRR 0.090 ppm Triazole 2.42% TRR 0.071 ppm M14360-alcohol 0.93% TRR 0.029 ppm Unknown, Metab 7 0.43% TRR 0.014 ppm Polar Unknowns 0.57% TRR 0.017 ppm
EtOAc (Extract E)	3.33	**0.103**	
n-Butanol (Extract F)	6.19	**0.192**	
Acid n-butanol (Extract G)	2.47	**0.077**	
Aqueous (Extract H)	3.10	**0.096**	TLC analysis resolved one or more unknown compounds present at 2.19% TRR (0.066 ppm). Subjected to acid hydrolysis (12 N HCl), and partitioned with n-butanol.
HCl hydrolysate (n-butanol)	NR	NR	TLC analysis resolved at least two unknown compounds present at 0.91% TRR (0.030 ppm).

(*continued; footnotes follow*)

Fraction	% TRR	ppm [a]	Characterization/Identification [b]
Nonextractable	3.95	0.123	Sequentially refluxed with acetone, methanol, and water; remaining residues were refluxed with NaOH (cellulose fraction) and HCl (lignin fraction), and centrifuged.
Acetone/methanol (Extracts M and N)	1.47	0.044	N/A.
Cellulose	0.91	0.029	N/A.
Lignin	0.02	0.001	N/A.
Bound	1.55	0.049	N/A.
Sugar beet leaves - 35 days following third application (35-Day; TRR = 1.336 ppm)			
Combined acetone:water	91.30	**1.220**	Sequentially extracted with n-hexane, EtOAc, n-butanol, acid n-butanol, and water.
n-Hexane (Extract D)	46.95	**0.627**	TLC analysis resolved: Tetraconazole 48.43% TRR 0.652 ppm TAA 5.55% TRR 0.073 ppm THP 7.06% TRR 0.094 ppm M14360-acid 4.78% TRR 0.063 ppm M14360-DFA 9.73% TRR 0.128 ppm Triazole 5.57% TRR 0.074 ppm M14360-alcohol 1.11% TRR 0.015 ppm Unknown, Metab 7 0.88% TRR 0.011 ppm Polar Unknowns 1.26% TRR 0.017 ppm
EtOAc (Extract E)	4.12	**0.055**	
n-Butanol (Extract F)	19.88	**0.266**	
Acid n-butanol (Extract G)	10.83	**0.145**	
Aqueous (Extract H)	9.53	**0.127**	TLC analysis resolved one or more unknown compounds present at 5.62% TRR (0.074 ppm). Separate subsamples subjected to enzyme (β-glucosidase) and acid hydrolysis (12 N HCl), and partitioned with n-butanol; enzyme hydrolysis was unsuccessful.
HCl hydrolysate (n-butanol)	NR	NR	TLC analysis resolved at least two unknown compounds present at 3.91% TRR (0.054 ppm).
Nonextractable	8.70	**0.116**	Sequentially refluxed with acetone, methanol, and water; remaining residues were refluxed with NaOH (cellulose fraction) and HCl (lignin fraction), and centrifuged.
Acetone/methanol (Extracts M and N)	1.47	0.044	N/A.
Cellulose	0.91	0.029	N/A.
Lignin	0.02	0.001	N/A.
Bound	1.55	0.049	N/A.

[a] **Bolded** ppm were calculated by the study reviewer based on the reported % TRR.

[b] Metabolites were determined for each extract; however, total quantitative values were only reported for the organic extracts as a group. Metabolites were tentatively identified using TLC and coelution with ^{14}C-labeled reference standards; THP was tentatively identified by GC/MS.

[c] NR = Not reported.

Table 3. Summary of radioactive residues identified in sugar beet leaves following three spray applications of triazole-label [^{14}C]tetraconazole at 0.089 lb ai/A/application.

Fraction	Sugar Beet Leaves; 0-Day [a] (TRR = 3.107)		Sugar Beet Leaves; 35-Day [a] (TRR = 1.336 ppm)	
	% TRR	ppm	% TRR	ppm
Identified [b]				
Tetraconazole	82.14	2.550	48.43	0.652
TAA	1.34	0.042	5.55	0.073
THP	2.42	0.077	7.06	0.094
M14360-acid	1.38	0.043	4.78	0.063
M14360-DFA	2.79	0.090	9.73	0.128
Triazole	2.42	0.071	5.57	0.074
M14360-alcohol	0.93	0.029	1.11	0.015
Total identified	**93.42**	**2.902**	**82.23**	**1.099**
Characterized				
Unknown, Metabolite 7	0.43	0.014	0.88	0.011
Polar unknowns	0.57	0.017	1.26	0.017
Unknowns (acid hydrolysate)	0.91	0.030	3.91	0.054
Acetone/methanol reflux	1.47	0.044	2.59	0.035
Cellulose	0.91	0.029	1.85	0.025
Lignin	0.02	0.001	0.08	0.002
Total characterized/identified	**97.73**	**3.037**	**92.8**	**1.243**
Nonextractable	1.55	0.049	4.18	0.054

[a] Days after final treatment.

[b] Tetraconazole and its metabolites were tentatively identified by TLC and/or confirmed by GC/MS; refer to Figure 1 (Attachment II) for full chemical names and structures.

Conclusions: The submitted sugar beet metabolism study is inadequate. The study was conducted using only triazole-labeled tetraconazole and did not include information pertaining to sample storage intervals (from harvest to the final TLC analyses of extracts). The HED MARC determined that data from sugar beet studies using phenyl-labeled tetraconazole and tetrafluoroethyl-labeled tetraconazole is needed to fully assess the nature of the residue in sugar beets (D264157, W. Donovan and D. Nixon, 19-APR-2000). Moreover, the submitted study used an application rate of approximately 0.4x the maximum seasonal rate, resulting in no detectable residues in sugar beet roots. The additional studies should be conducted at exaggerated rates (see discussion in OPPTS 860.1300) so that tetraconazole and metabolites can be adequately identified/characterized in sugar beet roots and tops.

The HED MARC tentatively determined that the residue of concern in sugar beets, peanuts, and bananas is tetraconazole *per se*. However, this conclusion cannot be finalized until the MARC considers the results of additional data as specified in the MARC results memo (D264157, W. Donovan and D. Nixon, 19-APR-2000).

OPPTS GLN 860.1300: Nature of the Residue in Livestock

Ruminants

The petitioner submitted the results of two goat metabolism studies (citations listed below). In one study; tetraconazole was uniformly labeled in the phenyl ring. In another study, tetraconazole was labeled at carbon positions 3 and 5 of the triazole ring. The biological and analytical phase of the studies were conducted by Huntingdon Life Sciences Ltd. (Cambridgeshire, England). The experimental parameters were similar for both studies; therefore, the results are combined in this review.

> 44751312 Elsom, L. (1994) (Carbon-14)-Tetraconazole: The Metabolism in the Lactating Goat: Lab Project Number: AGR87/942017.Unpublished study prepared by Huntingdon Life Sciences, Ltd.185 p.
>
> 44751313 Elsom, L. (1998) (Dichlorophenyl-(carbon-14))-Tetraconazole: The Metabolism in the Lactating Goat: Lab Project Number:AGR96/971551. Unpublished study prepared by Huntingdon Life Sciences, Ltd. 114 p.

The test substances, [phenyl-^{14}C]tetraconazole (specific activity 101.88 μCi/mg, radiochemical purity >97%) or [triazole-^{14}C]tetraconazole (specific activity 136.63 μCi/mg, radiochemical purity >97%) were separately mixed with nonlabeled tetraconazole to obtain an active ingredient with a specific activity of 21.13 μCi/mg (phenyl label) or 28.136 μCi/mg (triazole label). The active ingredient was then diluted with either methanol or acetonitrile, and dispensed into gelatin capsules containing dextran. The capsules were administered orally by balling gun once daily for five consecutive days. One goat received 20 mg/day of [triazole-^{14}C]tetraconazole and another

goat received 19.2 mg/day of [phenyl-^{14}C]tetraconazole. The dose rates were equivalent to ~0.45 ppm of [^{14}C]tetraconazole per day based on daily food consumption for each goat. It could not be determined from the study reports whether or not a control goat was utilized. The goats were fed a concentrate ration of commercial goat feed twice daily, and meadow hay and water were provided *ad libitum*. The petitioner provided sufficient descriptions of preparation of dose capsules and livestock husbandry practices as well as data concerning daily feed intake, body weights, and milk production.

Milk was collected twice daily (in the morning and afternoon). The goats were sacrificed ~23 hours after the final dose, and the following samples were collected: liver, kidneys, muscle (foreleg and rump), and fat (subcutaneous, omental, and perirenal). All milk and tissue samples were stored frozen (<-15 C) until analysis.

Total radioactive residues (TRR)

Triplicate aliquots of milk were analyzed directly by LSC. Liver, kidney, muscle, and fat were homogenized, and triplicate aliquots were subjected to combustion/LSC. The TRR in goat milk and tissues are presented in Table 4. The limit of quantitation (LOQ) for TRR determinations was not reported.

Table 4. Total radioactive residues in milk and edible tissues from goats following administration of [^{14}C]tetraconazole at ~0.45 ppm in the diet for 5 consecutive days.

Matrix	TRR, ppm [^{14}C]tetraconazole equivalents	
	Phenyl Label	Triazole Label
Milk		
Day 1 PM	0.061	0.12
Day 2 AM	0.036	0.20
Day 2 PM	0.099	0.36
Day 3 AM	0.045	0.35
Day 3 PM	0.113	0.44
Day 4 AM	0.053	0.43
Day 4 PM	0.113	0.54
Day 5 AM	0.052	0.49
Day 5 PM	0.118	0.59
Day 6 AM	0.063	0.51
Liver	3.440	3.21
Kidney	0.872	0.82
Muscle		
Foreleg	0.069	0.34
Rump	0.068	0.33

Matrix	TRR, ppm [^{14}C]tetraconazole equivalents	
	Phenyl Label	Triazole Label
Fat		
Subcutaneous	0.791	0.65
Omental	0.814	0.84
Perirenal	0.807	0.76

Samples of urine, feces, and cage washings were collected and were analyzed for TRR. The data indicated that most of the radioactivity was excreted: ~41-49%, ~23-27%, and ~1% of the administered dose was eliminated in urine, feces, and cage washings, respectively. The petitioner submitted data indicating that ~82%-87% of the administered dose was recovered from milk, tissues, blood, urine, and feces.

Extraction and hydrolysis of residues

The petitioner provided adequate descriptions of the fractionation schemes used for the analysis of tetraconazole residues in/on goat milk and tissues. During the extraction and fractionation procedures, aliquots of extracts, hydrolysates, and nonextractable residues were analyzed for radioactivity by LSC or combustion/LSC. Fractions containing significant residues were concentrated and reserved for chromatographic analysis. The general extraction and fractionation procedures are summarized below; the same procedures were used for both labels unless otherwise specified.

Milk: Residues in milk were extracted twice with acetonitrile (ACN) and then centrifuged. The ACN extracts were combined, evaporated to dryness, and subjected to further extraction with methanol (2-3x), then reserved for TLC and HPLC analysis.

Liver, kidney, and muscle: Residues in liver, kidney, and muscle were sequentially extracted with ACN (2-3x) and/or ACN:water (2-3x; 1:1, v:v), and centrifuged. The extracts were pooled, and residues were concentrated and redissolved in methanol for analysis by TLC and HPLC.

Nonextractable residues of liver following ACN and ACN:water extraction were suspended in a buffer solution (0.1 M, pH 7.5-10) and subjected to protease digestion (at 37 C for 2 days). The protease hydrolysate was partitioned with ACN or dichloromethane. The triazole-label ACN phase was concentrated and dissolved in methanol for TLC and HPLC analysis. Nonextractable residues following protease hydrolysis of the phenyl-label liver were refluxed with 6 N HCl for 6 hours.

Subsamples of triazole-label liver, kidney, and muscle, and phenyl-label liver and kidney ACN and ACN:water extracts were concentrated to dryness, redissolved in sodium acetate buffer (0.1 M, pH 5), and subjected to enzyme incubation with β-glucuronidase (at 37 C for 12-16 hours). The enzyme hydrolysate was concentrated to dryness and redissolved in ACN or methanol for

TLC and HPLC analysis. Enzyme treatment released glucuronide conjugates of tetraconazole and M14360-ketone in both kidney and liver.

Fat: Residues in fat were sequentially extracted with hexane (2x) and ACN (2x), and centrifuged. The phenyl-label hexane extracts were combined and partitioned with ACN (2x), and the ACN phase was collected, concentrated, and redissolved in ACN for TLC and HPLC analysis. The triazole-label hexane extract was partitioned with ACN, and the hexane layer was re-partitioned with ACN:water (9:1, v:v); the ACN and ACN:water phase was collected, concentrated, and redissolved in methanol for TLC and HPLC analysis. Nonextractable residues remaining following the initial hexane and ACN extractions were subjected to protease enzyme digestion as described for liver.

The distribution of ^{14}C-activity in the extracts and hydrolysates of phenyl- and triazole-label goat milk and tissues is presented in Tables 5a and 5b, respectively.

Characterization and identification of residues

Both TLC and HPLC systems were utilized to analyze the phenyl- and triazole-label liver extracts and hydrolysates. For HPLC analysis, extracts were injected onto an HPLC system using an HiRPB column, a gradient mobile phase of water and ACN or ACN and 0.1% trifluoroacetic acid, and a UV detector at 254 nm; radioactivity was detected using a radioactivity flow-through monitor. TLC analysis was conducted on normal-phase 60 F254 silica gel plates using solvent systems of dichloromethane:methanol (95:5, v:v), ACN:ethyl acetate:water (65:30:5, v:v:v), chloroform:methanol:water (55:40:5, v:v:v), or chloroform:methanol:water:formic acid (75:25:3:3, v:v:v:v). Radioactivity was detected and quantified using a radioanalytic imaging system. Metabolite peaks were identified by co-chromatography with ^{14}C-labeled reference standards. The following reference standards were used for co-chromatography: tetraconazole, triazole, M14360-acid, M14360-alcohol, M14360-DFA, M14360-ketone, M14360-M(C-1) alcohol, and M14360-benzylic alcohol (see Figure 1, Attachment II, for chemical names and structures of identified metabolites).

Aliquots of the triazole-label ACN and ACN:water extracts of liver after re-suspension in methanol were co-chromatographed with the corresponding phenyl-label ACN extracts using three different TLC systems. The major unknown peak from the triazole-label extract co-chromatographed with a component in the phenyl-label extract; the unknown was identified as M14360-ketone using two different TLC systems and reference standard co-elution.

A summary of the characterized and identified ^{14}C-residues in phenyl- and triazole-label goat matrices is presented in Tables 6a and 6b, respectively.

Table 5a. Distribution of total radioactive residues in milk and tissues from a lactating goat dosed with [phenyl-^{14}C]tetraconazole at 0.45 ppm in the diet for 5 consecutive days.

Fraction	% TRR [a]	ppm	Characterization/Identification [b]
Milk, Day 1 PM (TRR = 0.061 ppm)			
ACN	98.5	0.060	TLC analysis resolved: Tetraconazole 75.0% TRR 0.046 ppm M14360-DFA 9.8% TRR 0.006 ppm Other 6.6% TRR 0.004 ppm Plus two unknowns present at 0.003 ppm (each ≤3.3% TRR; ≤0.002 ppm).
Nonextractable	1.5	0.001	Not further analyzed (N/A).
Milk, Day 2 AM (TRR = 0.036 ppm)			
ACN	98.2	0.035	TLC analysis resolved: Tetraconazole 66.7% TRR 0.024 ppm M14360-DFA 14.3% TRR 0.005 ppm Other 5.6% TRR 0.002 ppm Plus four unknowns present at <0.005 ppm (each ≤5.6% TRR; ≤0.002 ppm).
Nonextractable	1.8	0.001	N/A.
Milk, Day 2 PM (TRR = 0.099 ppm)			
ACN	98.3	0.097	TLC analysis resolved: Tetraconazole 79.8% TRR 0.079 ppm M14360-DFA 6.1% TRR 0.006 ppm Other 5.1% TRR 0.005 ppm Plus four unknowns present at 0.007 ppm (each ≤3.0% TRR; ≤0.003 ppm).
Nonextractable	1.7	0.002	N/A.
Milk, Day 3 AM (TRR = 0.045 ppm)			
ACN	95.4	0.043	TLC analysis resolved: Tetraconazole 71.1% TRR 0.032 ppm M14360-DFA 8.9% TRR 0.004 ppm Other 4.4% TRR 0.002 ppm Plus four unknowns present at 0.004 ppm (each ≤6.7% TRR; ≤0.003 ppm).
Nonextractable	4.6	0.002	N/A.
Milk, Day 3 PM (TRR = 0.113 ppm)			
ACN	98.1	0.111	TLC analysis resolved: Tetraconazole 81.4% TRR 0.092 ppm M14360-DFA 7.1% TRR 0.008 ppm Other 3.5% TRR 0.004 ppm Plus five unknowns present at 0.008 ppm (each ≤2.7% TRR; ≤0.003 ppm).
Nonextractable	1.9	0.002	N/A.

(continued; footnotes follow)

Table 5a (*phenyl label, continued*).

Fraction	% TRR [a]	ppm	Characterization/Identification [b]
Milk, Day 4 AM (TRR = 0.053 ppm)			
ACN	95.1	0.050	TLC analysis resolved: Tetraconazole 66.0% TRR 0.035 ppm M14360-DFA 11.3% TRR 0.006 ppm Other 5.7% TRR 0.003 ppm Plus four unknowns present at 0.006 ppm (each ≤5.7% TRR; ≤0.003 ppm).
Nonextractable	4.9	0.003	N/A.
Milk, Day 4 PM (TRR = 0.113 ppm)			
ACN	98.5	0.111	TLC analysis resolved: Tetraconazole 70.8% TRR 0.080 ppm M14360-DFA 8.8% TRR 0.010 ppm Other 4.4% TRR 0.005 ppm Plus five unknowns present at 0.015 ppm (each ≤5.3% TRR; ≤0.006 ppm).
Nonextractable	1.5	0.002	N/A.
Milk, Day 5 AM (TRR = 0.052 ppm)			
ACN	98.5	0.051	TLC analysis resolved: Tetraconazole 69.2% TRR 0.036 ppm M14360-DFA 7.7% TRR 0.004 ppm Other 3.8% TRR 0.002 ppm Plus five unknowns present at <0.010 ppm (each ≤4.4% TRR; ≤0.005 ppm).
Nonextractable	1.5	0.001	N/A.
Milk, Day 5 PM (TRR = 0.118 ppm)			
ACN	98.6	0.116	TLC analysis resolved: Tetraconazole 78.8% TRR 0.093 ppm M14360-DFA 5.1% TRR 0.006 ppm Other 3.4% TRR 0.004 ppm Plus five unknowns present at 0.013 ppm (each ≤6.8% TRR; ≤0.008 ppm).
Nonextractable	1.4	0.002	N/A.
Milk, Day 6 AM (TRR = 0.063 ppm)			
ACN	98.1	0.062	TLC analysis resolved: Tetraconazole 76.2% TRR 0.048 ppm M14360-DFA 4.8% TRR 0.003 ppm Other 4.8% TRR 0.003 ppm Plus four unknowns present at <0.008 ppm (each ≤7.9% TRR; ≤0.005 ppm).
Nonextractable	1.9	0.001	N/A.

(*continued; footnotes follow*)

Table 5a (*phenyl label, continued*).

Fraction	% TRR [a]	ppm	Characterization/Identification [b]		
Fat, Omental (TRR = 0.814 ppm)					
Hexane	79.7	0.649	TLC analysis resolved:		
			Tetraconazole	77.4% TRR	0.630 ppm
			M14360-ketone	1.2% TRR	0.010 ppm
			Other	0.5% TRR	0.004 ppm
ACN	19.8	0.161	TLC analysis resolved:		
			Tetraconazole	18.8% TRR	0.153 ppm
			M14360-ketone	0.5% TRR	0.004 ppm
			Other	0.5% TRR	0.004 ppm
Nonextractable	NR [c]	NR	Subjected to protease digestion.		
Protease hydrolysate	NR	NR	N/A.		
Nonextractable	0.5	0.004	N/A.		
Fat, Perirenal (TRR = 0.807 ppm)					
Hexane	73.1	0.590	TLC analysis resolved:		
			Tetraconazole	71.1% TRR	0.574 ppm
			M14360-ketone	1.1% TRR	0.009 ppm
			Other	7.4% TRR	0.006 ppm
ACN	25.4	0.205	TLC analysis resolved:		
			Tetraconazole	24.2% TRR	0.195 ppm
			M14360-ketone	0.6% TRR	0.005 ppm
			Other	6.2% TRR	0.005 ppm
Nonextractable	NR	NR	Subjected to protease digestion.		
Protease supernatant	0.5	0.004	N/A.		
Nonextractable	1.0	0.010	N/A.		
Fat, Subcutaneous (TRR = 0.791 ppm)					
Hexane	90.6	0.717	TLC analysis resolved:		
			Tetraconazole	78.4% TRR	0.620 ppm
			M14360-ketone	4.2% TRR	0.033 ppm
			Other	7.0% TRR	0.055 ppm
			Plus one unknown present at 1.1% TRR (0.009 ppm).		
ACN	7.5	0.059	TLC analysis resolved:		
			Tetraconazole	7.2% TRR	0.057 ppm
			M14360-ketone	0.1% TRR	0.001 ppm
			Other	0.1% TRR	0.001 ppm
Nonextractable	NR	NR	Subjected to protease digestion.		
Protease supernatant	0.8	0.006	N/A.		
Nonextractable	1.2	0.009	N/A.		

(*continued; footnotes follow*)

Table 5a (*phenyl label, continued*).

Fraction	% TRR [a]	ppm	Characterization/Identification [b]
Kidney (TRR = 0.872 ppm)			
ACN	64.2	0.560	TLC analysis resolved: Tetraconazole 22.4% TRR 0.195 ppm M14360-ketone 3.9% TRR 0.034 ppm Tetraconazole conjugate 31.3% TRR 0.273 ppm Other 3.4% TRR 0.030 ppm Plus four unknowns present at 0.028 ppm (each ≤1.3% TRR; ≤0.011 ppm) Following enzyme hydrolysis (β-glucuronidase) TLC analysis resolved: Tetraconazole 48.7% TRR 0.425 ppm M14360-ketone 3.8% TRR 0.033 ppm Tetraconazole conjugate 3.4% TRR 0.030 ppm Other 3.6% TRR 0.031 ppm Plus five unknowns present at 0.042 ppm (each ≤1.1% TRR; ≤0.010 ppm)
ACN:Water	6.5	0.057	TLC analysis resolved: Tetraconazole 0.5% TRR 0.004 ppm M14360-ketone 0.1% TRR <0.001 ppm Tetraconazole conjugate 3.0% TRR 0.026 ppm Other 1.8% TRR 0.016 ppm Plus four unknowns present at 0.011 ppm (each ≤0.7% TRR; ≤0.006 ppm) Following enzyme hydrolysis (β-glucuronidase) TLC analysis resolved: Tetraconazole 3.8% TRR 0.033 ppm M14360-ketone 0.5% TRR 0.004 ppm Tetraconazole conjugate 0.2% TRR 0.002 ppm Other 1.0% TRR 0.009 ppm Plus five unknowns present at 0.009 ppm (each ≤0.6% TRR; ≤0.005 ppm)
Nonextractable	NR	NR	Subjected to protease digestion.
Protease supernatant	11.0	0.096	N/A.
Nonextractable	18.3	0.160	N/A.

(*continued; footnotes follow*)

Table 5a (*phenyl label, continued*).

Fraction	% TRR [a]	ppm	Characterization/Identification [b]		
Liver (TRR = 3.440 ppm)					
ACN	89.2	3.068	TLC analysis resolved:		
			Tetraconazole	78.1% TRR	2.685 ppm
			M14360-ketone	3.3% TRR	0.114 ppm
			Tetraconazole conjugate	6.8% TRR	0.233 ppm
			Other	1.1% TRR	0.037 ppm
			Following enzyme hydrolysis (β-glucuronidase)		
			TLC analysis resolved:		
			Tetraconazole	83.3% TRR	2.866 ppm
			M14360-ketone	4.3% TRR	0.147 ppm
			Tetraconazole conjugate	0.5% TRR	0.018 ppm
			Other	1.1% TRR	0.037 ppm
ACN:Water	2.5	0.086	TLC analysis resolved:		
			Tetraconazole	0.4% TRR	0.015 ppm
			M14360-ketone	0.1% TRR	0.002 ppm
			M14360-alcohol	0.1% TRR	0.003 ppm
			Tetraconazole conjugate	1.3% TRR	0.046 ppm
			Other	0.5% TRR	0.016 ppm
			Plus one unknown present at ≤0.1% TRR (≤0.004 ppm)		
			Following enzyme hydrolysis (β-glucuronidase)		
			TLC analysis resolved:		
			Tetraconazole	1.8% TRR	0.063 ppm
			M14360-ketone	0.3% TRR	0.009 ppm
			M14360-alcohol	<0.1% TRR	0.001 ppm
			Tetraconazole conjugate	0.1% TRR	0.003 ppm
			Other	0.3% TRR	0.010 ppm
Nonextractable	NR	NR	Subjected to protease digestion.		
Protease supernatant	1.2	0.041	N/A.		
Nonextractable	7.1	0.244	N/A.		
Muscle, Foreleg (TRR = 0.069 ppm)					
ACN	86.5	0.060	TLC analysis resolved:		
			Tetraconazole	79.7% TRR	0.055 ppm
			M14360-ketone	2.9% TRR	0.002 ppm
Nonextractable	13.5	0.009	N/A.		

(*continued; footnotes follow*)

Table 5a (*phenyl label, continued*).

Fraction	% TRR [a]	ppm	Characterization/Identification [b]		
Muscle, Rump (TRR = 0.068 ppm)					
ACN	87.3	0.059	TLC analysis resolved:		
			Tetraconazole	61.8% TRR	0.042 ppm
			M14360-ketone	2.9% TRR	0.002 ppm
			Other	<1.5% TRR	<0.001 ppm
ACN/Water	NR	NR	TLC analysis resolved:		
			Tetraconazole	13.2% TRR	0.009 ppm
			M14360-ketone	1.5% TRR	0.001 ppm
			Other	<1.5% TRR	<0.001 ppm
			Plus one unknown present at <1.5% TRR (<0.001 ppm)		
Nonextractable	12.7	0.009	N/A.		

[a] %TRR values were normalized by the petitioner.

[b] Initial identification of metabolites by TLC was confirmed using HPLC; percent TRR values of metabolites were calculated by the study reviewer; "Other" refers to radioactivity in the TLC/HPLC analyses not associated with specific components.

[c] NR = Not reported.

Table 5b. Distribution of total radioactive residues in milk and tissues from a lactating goat dosed with [triazole-^{14}C]tetraconazole at 0.45 ppm in the diet for 5 consecutive days.

Fraction	% TRR [a]	ppm	Characterization/Identification [b]		
Milk, Day 1 (TRR = 0.17 ppm)					
ACN	97.36	0.17	TLC/HPLC analysis resolved: Tetraconazole Triazole Plus up to two unknowns present at ≤0.01 ppm.	23.5% TRR 70.6% TRR	0.04 ppm 0.12 ppm
Nonextractable	2.64	<0.01	Not further analyzed (N/A).		
Milk, Day 2 (TRR = 0.35 ppm)					
ACN	97.82	0.34	TLC/HPLC analysis resolved: Tetraconazole Triazole Plus up to two unknowns present at ≤0.01 ppm.	17.1% TRR 74.3% TRR	0.06 ppm 0.26 ppm
Nonextractable	2.18	<0.01	N/A.		
Milk, Day 3 (TRR = 0.43 ppm)					
ACN	98.16	0.42	TLC/HPLC analysis resolved: Tetraconazole Triazole Plus up to two unknowns present at ≤0.01 ppm.	20.9% TRR 72.1% TRR	0.09 ppm 0.31 ppm
Nonextractable	1.84	0.01	N/A.		
Milk, Day 4 (TRR = 0.50 ppm)					
ACN	98.00	0.49	TLC/HPLC analysis resolved: Tetraconazole Triazole Plus up to two unknowns present at ≤0.01 ppm.	16.0% TRR 78.0% TRR	0.08 ppm 0.39 ppm
Nonextractable	2.00	0.01	N/A.		
Milk, Day 5 (TRR = 0.53 ppm)					
ACN	97.97	0.52	TLC/HPLC analysis resolved: Tetraconazole Triazole Plus up to two unknowns present at ≤0.01 ppm.	13.2% TRR 79.2% TRR	0.07 ppm 0.42 ppm
Nonextractable	2.03	0.01	N/A.		
Fat, Omental (TRR = 0.84 ppm)					
Hexane/ACN extract: ACN partition	97.94	0.82	TLC/HPLC analysis resolved: Tetraconazole Triazole Polar material	81.0% TRR 13.1% TRR 1.2% TRR	0.68 ppm 0.11 ppm 0.01 ppm
Nonextractable	NR [c]	NR	Subjected to protease digestion and ACN extraction.		
Protease hydrolysate	1.49	0.01	N/A.		
Nonextractable	0.57	<0.01	N/A.		

(continued; footnotes follow)

Table 5b (*triazole label, continued*).

Fraction	% TRR [a]	ppm	Characterization/Identification [b]		
Fat, Perirenal (TRR = 0.75 ppm)					
Hexane/ACN extract: ACN partition	89.72	0.68	TLC/HPLC analysis resolved: Tetraconazole Triazole Polar material	 72.0% TRR 13.3% TRR 8.0% TRR	 0.54 ppm 0.10 ppm 0.06 ppm
Nonextractable	NR	NR	Subjected to protease digestion and ACN extraction.		
Protease supernatant	7.88	0.06	TLC/HPLC analysis resolved: Tetraconazole Triazole Polar material	 1.3% TRR 5.3% TRR 1.3% TRR	 0.01 ppm 0.04 ppm 0.01 ppm
Nonextractable	2.41	0.02	N/A.		
Fat, Subcutaneous (TRR = 0.65 ppm)					
Hexane/ACN extracts: ACN partition	98.31	0.64	TLC/HPLC analysis resolved: Tetraconazole Triazole	 66.2% TRR 27.7% TRR	 0.43 ppm 0.18 ppm
Nonextractable	NR	NR	Subjected to protease digestion and ACN extraction.		
Protease supernatant	1.21	0.01	N/A.		
Nonextractable	0.48	<0.01	N/A.		
Kidney (TRR = 0.82 ppm)					
ACN	98.47	0.81	TLC/HPLC analysis resolved: Tetraconazole Triazole Polar material Following enzyme hydrolysis (β-glucuronidase) TLC/HPLC analysis resolved: Tetraconazole Triazole	 13.4% TRR 57.3% TRR 24.4% TRR 42.7% TRR 48.8% TRR	 0.11 ppm 0.47 ppm 0.20 ppm 0.35 ppm 0.40 ppm
Nonextractable	1.53	0.01	N/A.		
Liver (TRR = 3.21 ppm)					
ACN	94.48	3.03	TLC/HPLC analysis resolved: Tetraconazole Triazole Polar material Following enzyme hydrolysis (β-glucuronidase) TLC/HPLC analysis resolved: Tetraconazole Triazole	 73.5% TRR 8.4% TRR 8.7% TRR 82.2% TRR 7.8% TRR	 2.36 ppm 0.27 ppm 0.28 ppm 2.64 ppm 0.25 ppm
Nonextractable	NR	NR	Subjected to protease digestion and ACN extraction.		
Protease supernatant	2.36	0.08	N/A.		
Nonextractable	3.16	0.10	N/A.		

(*continued; footnotes follow*)

Table 5b (*triazole label, continued*).

Fraction	% TRR [a]	ppm	Characterization/Identification [b]		
Muscle, Foreleg (TRR = 0.34 ppm)					
ACN	98.43	0.33	TLC/HPLC analysis resolved:		
			Tetraconazole	11.8% TRR	0.04 ppm
			Triazole	79.4% TRR	0.27 ppm
			Polar material	0.5% TRR	0.01 ppm
			Following enzyme hydrolysis (β-glucuronidase)		
			TLC/HPLC analysis resolved:		
			Tetraconazole	11.8% TRR	0.04 ppm
			Triazole	76.5% TRR	0.26 ppm
			Polar material	0.5% TRR	0.01 ppm
Nonextractable	1.57	0.01	N/A.		
Muscle, Rump (TRR = 0.33 ppm)					
ACN	98.41	0.32	TLC/HPLC analysis resolved:		
			Tetraconazole	9.1% TRR	0.03 ppm
			Triazole	84.8% TRR	0.28 ppm
			Polar material	3.0% TRR	0.01 ppm
			Following enzyme hydrolysis (β-glucuronidase)		
			TLC/HPLC analysis resolved:		
			Tetraconazole	9.1% TRR	0.03 ppm
			Triazole	81.8% TRR	0.27 ppm
			Polar material	3.0% TRR	0.01 ppm
Nonextractable	1.59	0.01	N/A.		

[a] %TRR values were normalized by the petitioner.

[b] Metabolites were identified by two TLC systems and by HPLC; ppm values are averages of the three analyses, and the percent TRR values were calculated by the study reviewer.

[c] NR = Not reported.

Table 6a. Summary of radioactive residues characterized/identified in milk and tissues of a lactating goat dosed with [phenyl-^{14}C]tetraconazole at 0.45 ppm in the diet for 5 consecutive days.

Fraction	Milk, Day 5 PM (TRR = 0.118 ppm)		Fat, Omental (TRR = 0.814 ppm)		Fat, Perirenal (TRR = 0.807 ppm)		Fat, Subcutaneous (TRR = 0.791 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]								
Tetraconazole	78.8	0.093	96.2	0.783	95.3	0.769	85.6	0.677
M14360-DFA	5.1	0.006	--	--	--	--	--	--
M14360-ketone	--	--	1.7	0.014	1.7	0.014	4.3	0.034
M14360-alcohol	--	--	--	--	--	--	--	--
Total identified	**83.9**	**0.099**	**97.9**	**0.797**	**97.0**	**0.783**	**89.9**	**0.711**
Characterized								
Tetraconazole conjugates	--	--	--	--	--	--	--	--
Unknowns	11.0	0.013	--	--	--	--	1.1	0.009
Other [b]	3.4	0.004	1.6	0.013	1.4	0.011	7.1	0.056
Protease hydrolysate	--	--	NR	NR	0.5	0.004	0.8	0.006
Total identified/characterized	**98.3**	**0.116**	**99.5**	**0.810**	**98.9**	**0.798**	**98.9**	**0.782**
Nonextractable	1.4	0.002	0.5	0.004	1.0	0.010	1.2	0.009

(continued; footnotes follow)

Table 6a *(phenyl label, continued).*

Fraction	Kidney (TRR = 0.872 ppm)		Liver (TRR = 3.440 ppm)		Muscle, foreleg (TRR = 0.069 ppm)		Muscle, rump (TRR = 0.068 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]								
Tetraconazole	52.5	0.458	85.1	2.929	79.7	0.055	75.0	0.051
M14360-DFA	–	--	–	–	–	–	–	–
M14360-ketone	4.2	0.037	4.5	0.156	2.9	0.002	4.4	0.003
M14360-alcohol	--	--	<0.1	0.001	--	--	--	--
Total identified	**56.7**	**0.495**	**<89.7**	**3.086**	**82.6**	**0.057**	**79.4**	**0.054**
Characterized								
Tetraconazole conjugates	3.7	0.032	0.6	0.021	--	--	--	--
Unknowns	5.8	0.051	--	--	--	--	<1.5	<0.001
Other [b]	4.6	0.040	1.4	0.047	--	--	<1.5	<0.001
Protease hydrolysate	11.0	0.096	1.2	0.041	--	--	--	--
Total identified/characterized	**81.8**	**0.714**	**<92.9**	**3.195**	**82.6**	**0.057**	**<82.4**	**<0.056**
Nonextractable	18.3	0.160	7.1	0.244	13.5	0.009	12.7	0.009

[a] Metabolites were identified by TLC and confirmed by HPLC

[b] Other refers to radioactivity in the TLC/HPLC analysis not associated with specific compounds.

Table 6b. Summary of radioactive residues characterized/identified in milk and tissues of a lactating goat dosed with [triazole-^{14}C]tetraconazole at 0.45 ppm in the diet for 5 consecutive days.

Fraction	Milk, Day 5 (TRR = 0.53 ppm)		Fat, Omental (TRR = 0.84 ppm)		Fat, Perirenal (TRR = 0.75 ppm)		Fat, Subcutaneous (TRR = 0.65 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]								
Tetraconazole	13.2	0.07	81.0	0.68	73.3	0.55	66.2	0.43
Triazole	79.2	0.42	13.1	0.11	18.6	0.14	27.7	0.18
Total identified	**92.4**	**0.49**	**94.1**	**0.79**	**91.9**	**0.69**	**93.9**	**0.61**
Characterized								
Unknowns	<1.9	<0.01	--	--	--	--	--	--
Polar material	--	--	1.2	0.01	9.3	0.07	--	--
Protease hydrolysate	–	--	1.49	0.01	--	--	1.21	0.01
Total identified/characterized	**<94.3**	**<0.50**	**96.79**	**0.81**	**101.2**	**0.76**	**95.11**	**0.62**
Nonextractable	2.03	0.01	0.57	<0.01	2.41	0.02	0.48	<0.01

Fraction	Kidney (TRR = 0.82 ppm)		Liver (TRR = 3.21 ppm)		Muscle, foreleg (TRR = 0.34 ppm)		Muscle, rump (TRR = 0.33 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]								
Tetraconazole	42.7	0.35	82.2	2.64	11.8	0.04	9.1	0.03
Triazole	48.8	0.40	7.8	0.25	76.5	0.26	81.8	0.27
Total identified	**91.5**	**0.75**	**90.0**	**2.89**	**88.3**	**0.30**	**90.9**	**0.30**
Characterized								
Unknowns	--	--	--	--	--	--	--	--
Polar material	--	--	--	--	0.5	0.01	3.0	0.01
Protease hydrolysate	–	--	2.36	0.08	--	--	--	--
Total identified/characterized	**91.5**	**0.75**	**92.36**	**2.97**	**88.8**	**0.31**	**93.9**	**0.31**
Nonextractable	1.53	0.01	3.16	0.10	1.57	0.01	1.59	0.01

[a] Metabolites were identified by two TLC systems and HPLC; reported residue values are averages of the three analyses.

Storage stability

Based on the dates of study initiation and completion, samples may have been stored frozen for up to 351 days for the triazole-label study and 255 days for the phenyl-label study. The actual dates of dosing, sampling, extraction, and HPLC/TLC analysis were not provided. The storage stability of tetraconazole residues in goat matrices was not addressed by the petitioner. Storage stability data are required to support the storage conditions and intervals of samples collected from the current goat metabolism studies.

Proposed metabolic pathway of tetraconazole in ruminants

Based on the results of the goat metabolism studies, the petitioner proposes that tetraconazole is metabolized to form triazole. The metabolic pathway may be through initial oxidation of tetraconazole to tetraconazole-difluoroacetate (DFA), followed by ether displacement to form tetraconazole-alcohol. The glutathione conjugation of tetraconazole-alcohol and/or -acid and subsequent triazole cleavage may also occur.

Study summary

Following oral administration of [phenyl-^{14}C]tetraconazole or [triazole-^{14}C]tetraconazole to lactating goats for 5 consecutive days at 0.45 ppm, the TRR, expressed as tetraconazole equivalents, ranged from 0.036 ppm to 0.59 ppm in milk. In tissues, the respective TRR were 3.21 and 3.44 ppm in liver, 0.87 and 0.82 ppm in kidney, 0.069 and 0.34 ppm in leg muscle, 0.068 and 0.33 ppm in rump muscle, 0.79 and 0.65 ppm in subcutaneous fat, 0.81 and 0.84 ppm in omental fat, and 0.81 and 0.76 ppm in perirenal fat of goats dosed with the phenyl and triazole labels. In general, residues were higher in triazole-label milk and muscle samples; otherwise, residue levels were similar between the two labels.

The studies adequately characterized/identified radioactive residues in goat milk and tissues. The parent, tetraconazole, was identified in all goat matrices and was the principal residue component in phenyl-label milk (78.8% TRR, 0.093 ppm), phenyl- and triazole-label fat (66.2-96.2% TRR, 0.43-0.78 ppm), phenyl- and triazole-label kidney (52.5% and 42.7% TRR, 0.46 and 0.35 ppm), phenyl- and triazole-label liver (85.1% and 82.2% TRR, 2.93-2.64 ppm), and phenyl-label muscle (75.0-79.7% TRR, 0.051-0.055 ppm). The parent, however, was a minor residue component in triazole-label milk (13.2% TRR, 0.53 ppm), and triazole-label muscle (9.1-11.8% TRR, 0.03-0.04 ppm).

Triazole was identified as the major residue component in triazole-label milk (79.2% TRR, 0.42 ppm), triazole-label kidney (48.8% TRR, 0.40 ppm), triazole-label muscle (76.5-81.8% TRR, 0.26-0.27 ppm), but was a minor metabolite in fat (13.1-27.7% TRR, 0.11-0.18 ppm), and triazole-label liver (7.8% TRR, 0.25 ppm). Three additional minor metabolites were identified in phenyl-label tissues and milk only: M14360-ketone in fat, kidney, liver, and muscle (1.7-4.5% TRR, 0.002-0.16 ppm), M14360-DFA in milk (5.1% TRR, 0.006 ppm), and M14360-alcohol in liver (<0.1% TRR, 0.001 ppm).

Conclusions: The goat metabolism studies are acceptable provided the petitioner submits supporting storage stability data for the total toxic residues of tetraconazole in goat milk and tissues. It appears that milk and tissues samples may have been stored frozen for up to 351 days prior to study completion. The petitioner is required to provide evidence that the identity of residues did not change during the period between collection and final chromatographic analysis. Typically, this can be achieved by analyses of a representative substrate early in the study and at its completion. Such analyses should show that the basic profile of radiolabeled residues has not changed during that time.

The HED MARC tentatively determined that the residues of concern in livestock commodities are tetraconazole and triazole. However, before this conclusion can be finalized, the Committee must consider the findings from a scheduled Hazard Identification Assessment Review Committee (HIARC) meeting on triazole and evaluate data from a poultry nature of the residue study.

OPPTS GLN 860.1340: Residue Analytical Method - Plant Commodities

Residue data-collection method

Samples of sugar beet commodities collected from the field, processing, and storage stability studies were analyzed by Wildlife International, Ltd. (Easton, MD) for residues of tetraconazole using a GC/ECD. A brief description of the method follows.

Residues in sugar beet matrices (except molasses) were repeatedly homogenized and extracted with acetone, then centrifuged and filtered. Samples of molasses were initially shaken with aqueous acetone (50%) and transferred to a separatory funnel; the bottle was repeatedly rinsed with acetone, and the acetone phases were combined in a separatory funnel. A saturated solution of sodium chloride was added to the combined acetone extracts, and residues were partitioned into dichloromethane. The organic phase was filtered and concentrated by rotary evaporation, and residues were re-dissolved in hexane:acetone (9:1, v:v). Residues were further purified by alumina column chromatography; residues were eluted with hexane:acetone (1:1, v:v for sugar beet roots; 7:3, v:v for sugar beet tops and processed commodities). The solvent was evaporated, and residues were re-dissolved in ethyl acetate for quantitation by GC/ECD.

To assess the suitability of the GC/ECD method for data collection, the petitioner provided method validation data. Method validation and concurrent method recoveries were generated by fortifying untreated sugar beet commodities with tetraconazole and then analyzing the spiked samples with the data-collection method. The results of the method validation are presented in Table 7. The reported LOQs were 0.010 ppm for sugar beet roots, molasses, and refined sugar, 0.10 ppm for sugar beet tops, and 0.20 ppm for sugar beet dry pulp. Sample calculations and representative chromatograms were provided.

Table 7. Method validation and concurrent method recoveries of tetraconazole from fortified untreated samples of sugar beet commodities analyzed using GC/ECD.

Commodity	Fortification Level, ppm	% Recovery [a]	Mean ± s.d. [b]
Method Validation Data			
Sugar beet, roots	0.010	101-111 (3)	104 ± 5.8
	1.00	90.3-94.9 (3)	93.3 ± 2.6
Sugar beet, tops	0.10	105-112 (3)	109 ± 3.8
	1.00	96.2-99.9 (3)	97.7 ± 1.9
Sugar beet, dry pulp	0.20	91.5-94.3 (3)	93.2 ± 1.5
	1.00	91.3-94.8 (3)	92.7 ± 1.9
Sugar beet, molasses	0.010	90.3-108 (3)	100 ± 9.0
	1.00	95.3-103 (3)	99.8 ± 4.0
Sugar beet, refined sugar	0.010	109; 130, 143	127 ± 17.2
	1.00	100-102 (3)	101 ± 1.0
Concurrent Method Recovery Data			
Sugar beet, roots	0.010	102-116 (5)	108 ± 6.3
	0.10	89.3-107 (5)	98.3 ± 7.1
	0.50	91.8	91.8
	1.00	86.7-96.6 (9)	92.3 ± 3.0
Sugar beet, tops	0.10	91.2-99.3 (5); 128	103 ± 14.3
	0.50	94.9-107 (4)	99.5 ± 5.4
	1.00	92.0-96.8 (4)	95.1 ± 2.2
	3.00	94.4, 97.3	95.9 ± 2.1
	6.00	96.0, 98.2	97.1 ± 1.6
Sugar beet, roots (processing)	0.010-1.00	88.2-117 (3)	99.0 ± 15.7
- dry pulp	0.20-1.00	99.7-107 (3)	102.6 ± 3.9
- molasses	0.010-1.00	93.2-102 (3)	98.0 ± 4.5
- refined sugar	0.010-0.10	84.2-99.4 (3)	93.9 ± 8.5

[a] Each recovery value represents one sample unless otherwise indicated in parentheses. Recovery values outside the acceptable 70-120% range are listed separately.

[b] The mean and standard deviation were calculated by the petitioner, except for processing samples which were calculated by the study reviewer.

Independent Laboratory Validation

The petitioner submitted an ILV (citation listed below) to support the proposed enforcement method for plants. The ILV was performed by Midwest Research Institute, Kansas City, MO.

450684-04 Seymour, D. (2000) Validation of a Method for the Determination of Tetraconazole in Agricultural Commodities. Laboratory Project ID: 310031.1.001. Unpublished study prepared by Midwest Research Institute, 41 p.

Six samples each of peanuts, peanut oil, bananas, and refined sugar were prepared for analysis. Duplicate samples at each of two fortification levels (0.01 and 1.0 ppm) and two control blanks were analyzed for recovery. Adequate method recoveries were obtained for tetraconazole (Table 8). The analytical laboratory communicated with the sponsor regarding concerns about the formation of a hard cake layer following a centrifugation step while processing the first sample set of bananas. The sponsor provided confirmation that the cake formation should not impact recovery results and the ILV continued with no modifications to the method.

Tetraconazole residues were less than the limit of quantitation of 0.01 ppm in all control samples. The analytical laboratory reported that the total time required for extraction, clean up, and analysis of one set of samples ranged from 15-23 person-hours depending on the commodity analyzed (3-4 calendar days). Adequate sample calculations and representative chromatograms were provided.

Table 8. Independent method validation recoveries of tetraconazole from commodities using a GC/ECD method.

Matrix	Fortification Level, ppm	% Recovery [a]	Mean ± s.d. [b]
Peanut	0.01	80.9, 80.1	80.5 ± 0.6
	1.0	79.6, 69.8	74.7 ± 6.9
Peanut Oil	0.01	97.8, 102.5	100.2 ± 3.3
	1.0	97.2, 100.9	99.1 ± 2.6
Banana	0.01	96.7, 98.7	97.7 ± 1.4
	1.0	89.6, 95.7	92.6 ± 4.3
Refined Sugar	0.01	100.7, 89.2	95.0 ± 8.1
	1.0	88.7, 98.9	93.8 ± 7.2

[a] Each recovery value represents one sample.
[b] The mean and standard deviation were calculated by the study reviewer.

Conclusions: The petitioner utilized a GC/ECD method for the determination of tetraconazole residues in/on samples of sugar beet commodities that were collected from the field, processing, and storage stability studies. The validated method LOQs were 0.010 ppm for sugar beet roots, molasses, and refined sugar, 0.10 ppm for sugar beet tops, and 0.20 ppm for sugar beet dry pulp. The method validation and concurrent method recovery data indicate that this method is adequate for data collection. In addition, the petitioner submitted an ILV of the GC/ECD method,

demonstrating adequate recoveries from fortified samples of peanut, peanut oil, banana, and refined sugar.

The registration requirements for residue analytical methods in plants remains unfulfilled. The GC/ECD method should be subjected to radiovalidation using samples from the plant metabolism studies to determine whether the method recovers total toxic residues of tetraconazole from weathered plant matrices. The GC/ECD plant method has been forwarded to the Agency laboratories for PMV (D264681, W. Donovan, 07-APR-2000). Conclusions about the adequacy of the analytical method for enforcement purposes will be deferred until completion of the PMV.

The GC/ECD method should be supplemented by a confirmatory method that is significantly different (such as mass spectrometry). Provided that a satisfactory confirmatory method is provided, HED will not require an interference study.

OPPTS GLN 860.1340: Residue Analytical Methods - Livestock Commodities

Samples of cattle milk and tissues from the submitted feeding study were analyzed by Huntingdon Life Sciences Ltd. (Cambridgeshire, England) for residues of tetraconazole using a GC/ECD method similar to that described for plant commodities. A description of the data-collection method including concurrent method recovery data were submitted in conjunction with the feeding study data. A brief description of the method follows.

Milk (whole and skimmed) samples were shaken with acetone for 15 minutes, Celite 521 was added, and the solution was centrifuged. Sodium chloride was added to the supernatant, and residues were partitioned into dichloromethane. The organic phase was filtered, concentrated by rotary evaporation, and residues were re-dissolved in acetone. The acetone was evaporated off, and residues were re-dissolved in hexane:acetone (6:4, v:v). Residues were then purified further by alumina column chromatography; residues were eluted with hexane:acetone (6:4, v:v). The solvent was evaporated, and residues were re-dissolved in ethyl acetate for quantitation by GC/ECD.

Samples of cream were initially shaken with acetone and Celite 521, samples of fat were homogenized with Celite 521 and hexane:acetone (75:25, v:v) in a blender, and samples of kidney, liver, and muscle were homogenized with acetone in a blender. Cream, fat, kidney, liver, and muscle samples were then centrifuged and filtered. Silicone anti-foaming agent was added, and the solvent was allowed to evaporate. Residues were re-dissolved in water, a saturated sodium chloride solution was added, and residues were partitioned into dichloromethane (4x) and filtered. If the phases failed to separate at any point, the emulsion was phase separated by centrifugation. The dichloromethane phases were combined, and the solvent was removed by evaporation. Residues were re-dissolved in hexane and partitioned into acetonitrile (2x). The acetonitrile phase was collected, the solvent was allowed to evaporate, and residues were re-

dissolved in hexane:acetone (6:4, v:v) for alumina column chromatography (as described above for milk).

The petitioner submitted concurrent method recoveries in conjunction with the feeding study, generated by fortifying untreated cattle milk and tissue samples with tetraconazole. Concurrent recoveries are presented in Table 9. The respective limit of detection (LOD) was 0.003 ppm and the LOQ was 0.01 ppm for all cattle matrices. Sample calculations and representative chromatograms were provided.

Table 9. Concurrent method recoveries of tetraconazole from fortified untreated samples of cattle milk and tissues analyzed using GC/ECD.

Livestock Commodity	Fortification Level, ppm	% Recovery [a]	Mean ± s.d. [b]
Concurrent Method Recovery Data			
Cattle, whole milk	0.01	70-90 (13)	88 ± 8.4
	0.02	81-97 (5)	
	0.05	88-104 (8)	
	0.10	88-102 (4)	
Cattle, skimmed milk	0.01	69; 86	83 ± 10.8
	0.05	82, 95	
Cattle, milk cream	0.01	77, 79	90 ± 7.6
	0.02	94	
	0.03	95	
	0.05	90	
	0.13	91, 96	
	0.20	96	
Cattle, fat	0.01	84, 85	88 ± 11.5
	0.02	82	
	0.10	85-96 (3)	
	0.50	92	
Cattle, kidney	0.01	76, 126	
	0.10	92, 95	
Cattle, liver	0.01	83	
	0.10	85, 88	
	2.50	88	
Cattle, muscle	0.01	82	
	0.02	91	
	0.10	66, 90	

[a] Each recovery value represents one sample unless otherwise indicated in parentheses.
[b] The mean and standard deviation were calculated by the petitioner.

Samples of cattle milk and tissues from the feeding study were also analyzed by Isagro Ricerca (Novara, Italy) for residues of **triazole** using a GC method similar to the GC/ECD method used for the detection of tetraconazole residues. A description of the data collection method including concurrent method recovery data were submitted in conjunction with the feeding study data. A brief description of the method follows.

Milk (whole and skimmed) samples were shaken with acetone, and Celite 521 and activated charcoal were added, and the solution was shaken for 30 minutes. The solution was filtered, sodium chloride was added to the filtrate, and the filtrate was concentrated to aqueous.

Initially, the moisture content of samples of cream and tissues (minced) was determined by weighing before and after 23 hours of drying in an oven at 105 C. Water, acetone, and Celite were added to the substrate and homogenized. NaCl was added, and the mixture was filtered. The solid was washed with acetone; the combined aqueous/acetone extract was evaporated to aqueous and shaken with hexane. The lower salted aqueous phase was percolated, collected, and concentrated.

The concentrated aqueous phase of milk, cream, and tissues was then purified by Extrelut® column chromatography. Triazole residues were eluted with propan-2-ol alcohol:dichloromethane (1:1, v:v), and the eluate was filtered and concentrated. Residues were redissolved in ethyl acetate and methyl alcohol in an ultrasonic bath and filtered again prior to GC analysis using an alkali flame ionization detection (FID) or thermionic detection (NPD).

Concurrent method recovery values were generated using untreated cattle milk and tissue samples fortified with triazole. Concurrent method recoveries were adequate and within the acceptable range of 70-120%. The reported LODs were 0.010 ppm in milk and 0.015 ppm in fat, liver, and muscle, and LOQs were 0.015 ppm in milk and 0.020 ppm in fat, liver, and muscle. Sample calculations and representative chromatograms were provided.

Independent Laboratory Validation

The petitioner submitted an ILV (citation listed below) to support the proposed enforcement method for livestock. The ILV was performed by LARPEST Piacenza Italy (LAR).

450684-05 Isagro Ricera. (09-DEC-1998) Validation of a Method for Residues of Tetraconazole in Drinking Water, Vegetal Crops, and Food of Animal Origin. Laboratory Identification Code: 2258. Unpublished study prepared by Isagro Ricera, Milano, Italy 199 p.

Twelve samples each of barley grain, barley straw, grapes, apples, tomatoes, milk, fat, muscle, and eggs were prepared for analysis. Five samples at each of two fortification levels (approximately LOQ and 10 x LOQ, except for barley straw where the higher level was 100 x LOQ) and two control blanks were analyzed for recovery. The method is based on organic solvent extraction followed by a column chromatography clean up and gas chromatography using

a nitrogen phosphorous detector for vegetable substrates and water and an electron capture detector for biological substrates. Adequate method recoveries were obtained for tetraconazole (Table 10). No communication between the independent laboratory and the sponsor was reported, and no changes to the analytical method were recommended. Also, no information was provided regarding the time required for sample analysis. Tetraconazole residues were less than the limit of quantitation of 0.01 ppm in all control samples. Adequate sample calculations and representative chromatograms were provided.

Table 10. Independent method validation recoveries of tetraconazole from commodities using GC/NPD and GC/ECD methods.

Matrix	Fortification Level (ppm)	% Recovery [a]	Mean ± s.d. [b]
Barley grain	0.0107	77.8, 104.0, 81.5, 105.9, 89.0	91.6 ± 12.8
	0.107	94.2, 83.5, 79.8, 83.8, 97.0	87.7 ± 7.5
Barley straw	0.020	90.1, 101.5, 93.8, 90.9, 102.9	95.8 ± 6.0
	2.0	111.4, 103.0, 97.3, 104.5, 104.4	104.1 ± 5.0
Grape	0.0175	93.1, 93.7, 100.6, 90.8, 89.7	93.6 ± 4.3
	0.175	89.7, 100.0, 88.6, 81.7, 88.6	89.7 ± 6.6
Apples	0.016	100.0, 95.2, 76.2, 101.8, 106.3	95.9 ± 11.7
	0.533	108.0, 107.2, 100.2, 97.3, 97.4	102.0 ± 5.2
Tomatoes	0.016	88.8, 114.5, 75.6, 88.1, 105.7	94.5 ± 15.5
	0.16	81.3, 97.5, 94.0, 87.9, 91.9	90.5 ± 6.2
Milk	0.0096	97.9, 97.7, 95.8, 89.6, 103.1	96.8 ± 4.9
	0.096	96.8, 96.8, 79.2, 92.7, 99.0	92.9 ± 8.0
Muscle	0.020	84.1, 85.0, 82.7, 97.2, 98.8	89.6 ± 7.8
	0.20	80.0, 87.9, 86.2, 80.7, 101.6	87.3 ± 8.7
Fat	0.020	104.7, 91.7, 92.5, 105.0, 102.0	99.2 ± 6.6
	0.20	102.7, 104.3, 92.0, 101.0, 98.0	99.6 ± 4.8
Eggs	0.01	95.4, 93.9, 83.6, 93.6, 97.5	92.8 ± 5.4
	0.10	81.3, 91.2, 89.9, 89.4, 87.2	87.8 ± 3.9

[a] Each recovery value represents one sample.
[b] The mean and standard deviation were calculated by the study reviewer.

Conclusions: The petitioner utilized a GC/ECD method for the determination of tetraconazole residues in samples of cattle milk and tissues that were collected from the feeding study. The validated LOQ of the GC/ECD method was 0.01 ppm for all cattle matrices. A GC/FID method

was used for the determination of triazole residues in/on the same samples; the validated LOQs of the GC/FID method were 0.015 ppm in milk and 0.020 ppm in fat, liver, and muscle. The concurrent method recovery data indicate that these methods are adequate for data collection. In addition, the petitioner submitted an ILV of the GC/ECD method, demonstrating adequate recoveries from fortified samples of milk, eggs, muscle, fat.

The petitioner has indicated that the GC/ECD method may also be used for enforcement of tetraconazole tolerance levels in livestock commodities. The method should be subjected to radiovalidation using samples from the ruminant metabolism studies to determine whether the method recovers total toxic residues of tetraconazole from weathered livestock matrices. The GC/ECD livestock method has been forwarded to the Agency laboratories for petition method validation (D264681, W. Donovan, 07-APR-2000). Conclusions about the adequacy of the analytical method for enforcement purposes will be deferred until completion of the PMV.

Because the HED MARC tentatively determined that triazole is a residue of concern in livestock commodities, an enforcement method is needed to detect triazole residues in livestock commodities. Accordingly, if the HIARC confirms the decision to regulate triazole, the petitioner should have an ILV study conducted on the GC/FID method for determination of triazole residues in livestock commodities. If the results of the ILV are acceptable, the method will be forwarded to the Agency laboratory for PMV.

OPPTS GLN 860.1360: Multiresidue Method

Data concerning the recovery of tetraconazole residues of concern in plants and livestock using FDA's multiresidue method protocols (PAM Vol. I) have not been submitted and are required for this tolerance petition request.

OPPTS GLN 860.1380: Storage Stability Data

Sample storage conditions and intervals - plant commodities

The petitioner maintained the integrity of sugar beet root and top samples collected from the field trials with adequate sample-handling procedures. Within two hours of harvest, samples were bagged and placed into coolers containing blue or dry ice. Samples were shipped frozen by FedEx to Wildlife International Ltd. (Easton, MD) where they were stored frozen (-28 to -1 C) until residue analysis. Total storage intervals were 12-70 days (~0.5-2.5 months) for roots, and 17-78 days (~0.5-2.5 months) for tops.

Untreated and treated samples of sugar beet roots from the processing study were harvested by hand (dug up with a spade and tops cut off with a machete) and transported under ambient conditions on the day of harvest to Englar Food Laboratories, Inc. (Moses Lake, WA) for processing. Separate RAC samples were shipped frozen directly to the analytical laboratory (Wildlife International, Ltd., Easton, MD). Samples at Englar Food Laboratories were stored refrigerated (~4 C) upon arrival at the facility and then stored frozen following processing. Sugar beet root samples were processed into dry pulp, molasses, and refined sugar. The processing was completed within 10 days of harvest, and total storage intervals were 28 days for

sugar beet roots, 35 days for dry pulp and molasses, and 30 days for refined sugar.

Sample storage conditions and intervals - livestock commodities

In the ruminant feeding study, milk samples were stored at 4 C immediately following collection, and tissue samples were stored frozen (-20 C) shortly after necropsy. Milk and tissue samples were submitted to the Huntingdon Life Sciences Department of Environmental Analysis for analysis of tetraconazole residues. All matrices were stored for less than 37 days (~1 month). Data to support the storage intervals and conditions for milk and tissue samples from the feeding study are not required because samples were analyzed for tetraconazole within approximately one month.

Separate subsamples of milk and tissues were shipped frozen from Huntingdon Life Sciences to the Residue Analysis Unit of Isagro Ricerca (Novara, Italy) for analysis of triazole residue. Upon arrival samples were stored frozen (-20 C); samples were stored for up to 101 days (3.5 months). The petitioner indicated that a storage stability study of triazole residues in livestock commodities is ongoing at Isagro Ricerca, but that preliminary data demonstrate that residues of triazole are stable in cattle milk for up to 1 year and in cattle tissues for up to 3 months.

Storage stability data - plant commodities

A freezer storage stability study was conducted concurrently with the sugar beet field trials and processing study. Samples of untreated sugar beet commodities were fortified with tetraconazole and stored under frozen conditions. Samples were analyzed for residues of tetraconazole at 0-, 34- to 36-, and/or 70-day storage intervals using the GC/ECD method described above. Unfortified samples were fortified with tetraconazole at the time of analysis for fresh fortification recoveries. The reported LOQs were 0.010 for roots, molasses, and refined sugar, 0.10 ppm for tops, and 0.20 ppm for dry pulp. Apparent residues of tetraconazole were less than the respective LOQs in/on three samples each of untreated sugar beet roots and tops, and two samples each of untreated dry pulp, molasses, and refined sugar. The results of the storage stability study are presented in Table 11.

Table 11. Stability of tetraconazole in/on sugar beet matrices fortified with tetraconazole and stored frozen for up to 70 days.

Crop/Matrix	Storage Interval (days)	Fresh Fortification %Recovery [a]	Stored Sample % Recovery	Stored Sample Corrected % Recovery [b]
Beet, sugar, roots	0	90.2, 93.2, 93.3, 95.7	--	--
	36	101, 103 (102)	81.5, 96.9	79.9, 95.0
	70	96.9, 97.4 (97.2)	87.4, 95.5	89.9, 98.3
Beet, sugar, tops	0	96.2, 97.0, 99.9	--	--
	36	92.4, 98.3 (95.4)	87.4, 90.5	91.6, 94.9
	70	95.2, 95.7 (95.5)	84.3, 84.4	88.3, 88.4
Beet, sugar, dried pulp	0	95.3, 96.2	--	--
	34	99.9, 103 (101.5)	93.8, 94.6	92.4, 93.2
Beet, sugar, molasses	0	95.0, 98.3	--	--
	34	99.9, 105 (102.5)	101, 102	98.5, 99.5
Beet, sugar, refined sugar	0	97.2, 97.8	--	--
	34	99.3, 99.7 (99.5)	94.1, 96.0	94.6, 96.5

[a] Average value is reported in parentheses.

[b] Corrected percent recovery was calculated by the study reviewer by dividing each stored sample recovery by the average of the fresh fortification recoveries.

Conclusions: The storage intervals and conditions for sugar beet commodities collected from the field and processing studies are supported by adequate storage stability data. Residues of tetraconazole are stable under frozen storage conditions (-20 C) in/on sugar beet roots and tops for up to 70 days (~2.5 months) and in the processed commodities of sugar beet (dry pulp, molasses, and refined sugar) for up to 34 days (~1 month). Samples from the field and processing studies were stored frozen for up to 78 and 35 days, respectively.

All livestock matrices collected from the dairy cattle feeding study were stored frozen for less than 37 days (~1 month) prior to analysis for residues of tetraconazole. Data to support the storage intervals and conditions for milk and tissue samples from the feeding study are not required because samples were analyzed for tetraconazole residues within approximately one month. Separate subsamples of milk and tissues, stored for up to 101 days (3.5 months), were also analyzed for triazole residues. The petitioner indicated that a storage stability study of triazole residues in livestock commodities is ongoing at Isagro Ricerca; the petitioner reported that preliminary data suggest that residues of triazole are stable in cattle milk for up to 1 year and in cattle tissues for up to 3 months. HED will verify these statements when the petitioner submits the final storage stability report for triazole.

OPPTS GLN.860.1500: Crop Field Trials

Beet, sugar

The petitioner submitted sugar beet field trial data (citation listed below) to support the establishment of the proposed tolerances for residues of tetraconazole *per se* in/on sugar beet roots at 0.1 ppm and sugar beet tops at 7.0 ppm.

> 44751314 Hattermann, D. (1999) Raw Agricultural Commodity (RAC) Residue and Residue Decline Evaluation of Tetraconazole Applied to Sugar Beets: Lab Project Number: 38008A015: 190C-101: 1714-98-380-01-08B47. Unpublished study prepared by Landis International, Inc. 419 p.

A total of eleven sugar beet field trials were conducted during the 1998 growing season in CA(2), CO(1), ID(1), MI(1), MN(2), MT(1), ND(2), and WY(1). Mature sugar beet plants (tops with roots) were harvested 14 days following the last of six sequential broadcast spray applications, with a 12- to 16-day retreatment interval, of the 1 lb/gal SC formulation at **0.107 lb** ai/A/application (1x the proposed single application rate). The total applied rate was 0.638-0.649 lb ai/A (1x the proposed maximum seasonal application rate). Applications were made in 10.1-30.4 gal of water/A using a tractor-mounted, CO_2 backpack, or hand-held boom sprayer. To generate residue decline data, additional samples were collected from the ND trial site at PHIs of 0, 3, 7, 30, and 60 days following the last application. Each test site consisted of one control and one treatment plot.

Duplicate untreated and treated samples of sugar beet plants were collected at the specified PHI, except in the decline study where only a single untreated and treated sample was collected at each PHI except the 14-day PHI. Tops with the crowns attached were separated from the roots using a knife; excess soil was removed by a brush or clean gloved hand. Samples were bagged, placed into coolers containing blue or dry ice or into field freezers within 2 hours of sampling. Samples were shipped frozen by FedEx to Wildlife International Ltd. (Easton, MD) for residue analysis. Samples were stored frozen (-28 to -1 C) until analysis.

Samples were analyzed for residues of tetraconazole using the GC/ECD method described under the "Residue Analytical Methods" section. This method is adequate for data collection based on adequate concurrent method recovery data (see Table 7). Apparent residues of tetraconazole were less than the LOQ (0.010 ppm for sugar beet roots and 0.10 ppm for sugar beet tops) in/on 17 samples each of untreated sugar beet roots and tops. Residues of tetraconazole in/on treated samples of sugar beet matrices are presented in Table 12.

Table 12. Residues of tetraconazole in/on sugar beets following six applications of the 1 lb/gal SC formulation at 0.107 lb ai/A/application (1x the maximum proposed single and seasonal rates).

Trial location (EPA Region)	PHI [a] (days)	Tetraconazole Residues (ppm) [b]	
		Sugar beet roots	Sugar beet tops
Tulare, CA (Region 10)	14	0.0280, 0.0394 [c]	3.24, 3.37 [c]
Tulare, CA (Region 10)	14	0.0592, 0.0626	1.13, 2.22
Weld, CO (Region 8)	14	0.0698, **0.103** (0.0864 HAFT)	3.05, 3.31
Power, ID (Region 11)	14	0.0162, 0.0224 [c]	2.01, 2.58 [c]
Ingham, MI (Region 5)	14	0.0194, 0.0315	1.34, 1.57
Wilkin, MN (Region 5)	14	0.0196, 0.0388	2.16, 2.70
Wilkin, MN (Region 5)	14	0.0375, 0.0498	3.89, **5.90**
Yellowstone, MT (Region 7)	14	0.0290, 0.0293	1.47, 1.84
Grand Forks, ND (Region 5)	0	0.0221	3.21
	3	0.0149	2.17
	7	0.0089	1.96
	14	0.0144, 0.0178	1.27, 1.60
	30	0.0114	1.49
	60	0.0141	0.869
Steele, ND (Region 5)	14	0.0132, 0.0136	1.65, 1.92
Park, WY (Region 9)	14	0.0820, 0.0904	2.11, 3.09

[a] PHI= preharvest interval

[b] Residues were reported as not corrected for concurrent recoveries.

[c] The highest residue value of duplicate analyses is reported.

Conclusions: In support of this petition, 11 trials reflecting the maximum proposed use pattern for sugar beets were conducted. For the establishment of tolerances on sugar beet commodities, Tables 1 and 5 of OPPTS GLN 860.1500 specify that 12 field trials should be conducted in Regions 5 (5 trials), 7 (1 trial), 8 (1 trial), 9 (1 trial), 10 (2 trials), and 11 (2 trials). HED will not require the petitioner to conduct an additional field trial in Region 11 because there does not appear to be wide variability in residues obtained in the current submission.

The submitted field trial data indicate that residues of tetraconazole will not exceed the proposed tolerance of 7 ppm in/on sugar beet tops, when the 1 lb/gal SC formulation of tetraconazole is applied according to the maximum proposed use pattern. However, the field trial data indicate that the proposed tolerance of 0.1 ppm for tetraconazole residues in/on sugar beet roots should be increased to 0.15 ppm. Residues of tetraconazole were 0.0132-0.103 ppm and 1.13-5.90 ppm, respectively, in/on sugar beet roots and tops harvested 14 days following the last of six sequential broadcast applications of the 1 lb/gal SC formulation at 0.107 lb ai/A/application (1x the maximum proposed single and seasonal application rates).

The residue decline data suggest that residues of tetraconazole dissipated from 3.21 ppm (0-Day PHI) to 0.869 ppm (60-day PHI) in/on sugar beet tops. A meaningful decline trend was not observed in sugar beet roots.

The petitioner should submit a revised Section F to correct the commodity definitions for tetraconazole tolerances for sugar beet roots and tops to "beet, sugar, roots" and "beet, sugar, tops".

OPPTS GLN 860.1520: Processed Food/Feed

Beet, sugar

The petitioner submitted one volume of data depicting the potential for concentration of residues of tetraconazole in the processed commodities of sugar beets. The citation is listed below.

> 44751315 Hattermann, D. (1999) Processed Commodity (PC) Residue Evaluation of Tetraconazole Applied to Sugar Beets: Lab Project Number: 38008A016: 1714-98-380-01-08B54. Unpublished study prepared by Landis International, Inc. 180 p.

One sugar beet field trial was conducted during the 1998 growing season in WA. Mature sugar beet roots were harvested 14 days following the last of six sequential broadcast foliar applications, with 12- to 15-day retreatment intervals, of the 1 lb/gal SC formulation at 0.533 lb ai/A/application. The total applied rate was 3.2 lb ai/A (~5x the maximum proposed seasonal application rate). Applications were made in 17.33-17.97 gal of water/A using a CO_2 backpack sprayer. A separate plot was left untreated and served as a control. Untreated and treated samples of sugar beet roots were harvested by hand (dug up with a spade and tops cut off with a machete) and transported under ambient conditions on the day of harvest to Englar Food Laboratories, Inc. (Moses Lake, WA) for processing. Separate RAC samples were shipped frozen directly to the analytical laboratory (Wildlife International, Ltd., Easton, MD). Samples received at Englar Food Laboratories were stored refrigerated (~4 C) upon arrival, and stored frozen following processing.

Sugar beet root samples were processed into dry pulp, molasses, and refined sugar within 10 days of harvest. The processing was performed according to simulated commercial procedures. A brief description of the processing procedures follows. The roots were washed in water and sliced into cossettes. The sliced cossettes were placed in a counter current diffuser with a mixture of fresh water and pulp press water. Sugar from cossettes was extracted into water, and the extract (raw juice) was purified by the addition of lime and carbon dioxide and heated at 80-85 C. Impurities in the extract were precipitated by the addition of a settling aid. The clear liquid was filtered, carbonated with carbon dioxide gas, heated at 90-95 C, and filtered again. The thin juice was concentrated by evaporation to produce thick juice and frozen until further processing. Once thawed, the thick juice was heated at 60-65 C in a vacuum pan and centrifuged to separate the sugar from the molasses. The sugar was then washed with hot water and dried with hot air to produce refined sugar. The beet pulp left over from the sugar extraction was dried

to produce dry pulp. The petitioner submitted adequate descriptions and material balance sheets for the processing procedures. The processed samples were shipped frozen to Wildlife International, Ltd. (Easton, MD), where samples were stored frozen until residue analysis.

Samples of processed sugar beets were analyzed for residues of tetraconazole using the GC/ECD method previously described under the "Residue Analytical Methods" section. Apparent residues of tetraconazole were less than the respective LOQ (<0.010 ppm in roots, molasses, and refined sugar; <0.20 ppm in dry pulp) in/on two samples of untreated sugar beet roots, and one sample each of dry pulp, molasses, and refined sugar processed from untreated sugar beet roots. Residues of tetraconazole in/on treated samples are presented in Table 13.

Table 13. Residues of tetraconazole in the processed commodities of sugar beet roots harvested 14 days following six foliar applications of the 1 lb/gal SC formulation at 0.533 lb ai/A (5x the maximum proposed seasonal rate).

Substrate	Tetraconazole Residues (ppm) [a]	Concentration/Reduction Factor [b]
Sugar beet roots	0.262, 0.397 (0.330)	--
- Dry pulp	0.609, 0.788 (0.699)	2.1x
- Molasses	0.817, 1.01 (0.914)	2.8x
- Refined sugar	0.0346, 0.0451 (0.0399)	0.1x

[a] Averages are reported in parentheses.

[b] Concentration/reduction factors were calculated using the average values and the residues in the sugar beet roots from the processor.

Conclusions: The submitted sugar beet processing data are adequate for the purposes of this petition. Residues of tetraconazole did not concentrate in refined sugar but concentrated 2.1x in dry pulp and 2.8x in molasses processed from sugar beet roots bearing detectable residues.

The maximum expected residue of tetraconazole in sugar beet dry pulp and molasses are 0.181 and 0.242 ppm, calculated by multiplying the HAFT residue (0.0864 ppm; see sugar beet field trial) and the observed concentration factor (2.1x dry pulp and 2.8x molasses). Based on this calculation, the proposed tolerances of 0.3 ppm for residues of tetraconazole in sugar beet dry pulp and molasses are appropriate. The petitioner is, however, requested to submit a revised Section F to correct the commodity definitions for tetraconazole tolerances for dry pulp and molasses to "beet, sugar, dried pulp" and "beet, sugar, molasses."

The proposed tolerance for residues of tetraconazole in sugar beet refined sugar is not required. Expected tetraconazole residues in refined sugar do not exceed the proposed tolerance for the RAC. The petitioner should delete this commodity (sugar beet refined sugar) from the requested Section F revision.

OPPTS GLN 860.1480: Meat, Milk, Poultry, Eggs

Dairy Cattle Feeding Studies

The petitioner submitted three volumes of data (citations listed below) depicting the magnitude of tetraconazole and triazole residues in the milk and tissues of dairy cattle.

> 44751316 Redgrave, V. (1997) Tetraconazole: Residues in Milk and Tissues of Dairy Cows: Lab Project Number: AGR 97/963664. Unpublished study prepared by Huntingdon Life Sciences, Ltd. 109 p.
>
> 44751317 Redgrave, V. (1998) Tetraconazole: Residues in Milk and Tissues of Dairy Cows: Lab Project Number: AGR 99/972293. Unpublished study prepared by Huntingdon Life Sciences, Ltd. 219 p.
>
> 44751318 Zini, G. (1997) Analysis of 1.2.4-Triazole Residues in Bovine Biological Substrates (Milk, Cream, Skimmed Milk, and Tissues): Lab Project ID Study No. 2221. Unpublished study performed by Isagro Ricerca (Novara, Italy) and submitted by Sostram Corp. (Roswell, GA). 233 p.

The study described in MRID 44751316 was a pilot designed to provide information for determining base dose levels for a subsequent definitive transfer study. The study was designed to quantify residues of tetraconazole found in milk and tissues from dairy cows following oral administration of tetraconazole for 28 days at a dosage of 50 mg ai per cow per day (nominally equivalent to 2.5 ppm in the diet assuming a daily feed intake of 20 kg). Details and results from this pilot study are not presented in this document because the basic objective of the study was to determine appropriate feeding levels for subsequent dairy cattle feeding studies.

The study described in MRIDs 44751317 and 44751318 is the definitive cattle feeding study. The results from this study were used by the petitioner as the basis for tolerance establishment in milk and ruminant tissues. MRID 44751317 reports residues of tetraconazole whereas MRID 44751318 reports residues of triazole. These cattle feeding data are presented and evaluated herein for their adequacy in fulfilling registration requirements under OPPTS GLN 860.1480.

Anticipated maximum dietary burden of tetraconazole for beef and dairy cattle

The commodities of sugar beet which may be used by beef and dairy cattle as feed items include tops, dried pulp, and molasses. HED also notes that in conjunction with the proposed uses of tetraconazole on peanuts, peanut meal may be used by livestock as a feed item. The anticipated maximum dietary burden, based on ingestion of these tetraconazole-treated feed items by beef and dairy cattle and the tolerances currently being proposed, is presented below in Table 14.

Table 14. Estimation (based on U.S. feeding practices as reflected in Table 1 of OPPTS 860.1000) of the maximum theoretical dietary burden of tetraconazole to beef and dairy cattle.

Feed Commodity	Proposed Tolerance, ppm	% Dry Matter	Beef Cattle		Dairy Cattle	
			% of Diet	Burden, ppm	% of Diet	Burden, ppm
Peanut, meal	0.05 [a]	85	15	0.009	15	0.009
Sugar beet, tops	7	23	20	6.087	10	3.043
Sugar beet, dried pulp	0.3	88	20	0.068	20	0.068
Sugar beet, molasses	0.3	75	10	0.040	10	0.040
		TOTAL	65	6.204	55	3.160

[a] A tolerance for peanut meal is not needed. Tetraconazole residues expected in peanut meal will not exceed the recommended tolerance of 0.05 ppm for the RAC.

Discussion of data

The in-life and analytical phases of the study were conducted by Huntingdon Life Sciences (Cambridgeshire, England). Friesian dairy cows were orally dosed twice daily for 28-30 consecutive days with tetraconazole in corn oil added to the concentrate feed. The daily target dose levels were equivalent to 0.34 ppm (Treatment 1 or low dose), 1.02 ppm (Treatment 2 or mid-level dose), and 3.4 ppm (Treatment 3 or high dose) based on an assumed feed intake of 20 kg/cow/day. The administered doses of 0.34, 1.02, and 3.4 ppm are approximately equivalent to 0.1x, 0.3x, and 1x, respectively, the anticipated maximum dietary burden for dairy cattle of 3.160 ppm and approximately 0.05x, 0.2x, and 0.5x the anticipated maximum dietary burden for beef cattle of 6.204 ppm.

A total of 14 dairy cows were used in the study: three cows each for the low and mid dosing levels, five cows for the high dosing level, and three cows for control. Control livestock received concentrate feed with corn oil without tetraconazole. The cows were milked twice daily (a.m. and p.m.) and were supplied with a feed concentrate at each milking. In addition, hay was made available daily, and water was provided *ad libitum* throughout the study. The petitioner submitted adequate information pertaining to daily food consumption, milk production, and general health of the test livestock.

The morning and evening milk collections were weighed individually and composited daily for each cow. Milk was collected on Days -7, -1, 1, 3, 5, 7, 10, 14, 18, 21, 24, 26, 27, 28, 31, 33, 35, 37, 40, and 42. Subsamples of milk collected on Days 14 and 28 were separated into cream and skim milk. Milk subsamples were stored at 4 C immediately following collection and delivered

to the analytical division for residue analysis. A separate subsample of milk was stored frozen (-20 C) within 45 minutes of sampling in case of loss or reassay. Cattle were sacrificed within 24 hours of the final dose, except for two cows from the highest dose group which were maintained on a "no-treatment diet" after the 28th day of dosing and were sacrificed after 7 and 14 days of withdrawal (36 and 43 days after initiation of dosing). Samples of liver, kidney, fat (pooled perirenal and omental), subcutaneous fat, and skeletal muscle (pooled pectoralis and adductor muscle of the thigh) were collected after sacrifice. Tissue samples were coarsely chopped and mixed before division into three subsamples which were stored frozen (-20 C).

One set of subsamples was shipped to the Huntingdon Life Sciences Department of Environmental Analysis (Cambridgeshire, England) for determination of tetraconazole residues in milk and tissues. All samples shipped to Huntingdon were stored for less than 37 days (~1 month) prior to analysis for tetraconazole residues. The collected samples were analyzed for residues of tetraconazole using the previously described GC/ECD method with validated LOQ and LOD of 0.01 and 0.003 ppm, respectively. Residues of tetraconazole were below the LOD (<0.003 ppm) in each milk sample from Treatment 1 (low dose), and skimmed milk samples (Day 14 and 28) from all dosing levels. Residues of tetraconazole in milk and tissue samples from all dosing levels are presented in Table 15; residue values are not corrected or adjusted for method recoveries. Apparent residues of tetraconazole were less than the LOQ (<0.01 ppm) in untreated milk (n=25 samples), skim milk (n=6), cream (n=6), subcutaneous fat (n=3), peritoneal fat (n=3), liver (n=3), kidney (n=3), and muscle (n=3).

Separate subsamples of milk and tissues were shipped frozen from Huntingdon Life Sciences to the Residue Analysis Unit of Isagro Ricerca (Novara, Italy) for determination of triazole residues. Subsamples were analyzed for residues of triazole using the GC/FID method described under the "Residue Analytical Methods" section. The reported LOQs were 0.015 in milk and 0.020 ppm in fat, liver, and muscle. The reported LODs were 0.010 ppm in milk and 0.015 ppm in fat, liver, and muscle. Residues of triazole were below the LOQ in each milk (whole and skimmed), cream, fat, and muscle samples from Treatments 1 and 2 (low and mid doses). Residues of triazole in milk and tissue samples from all dosing levels are presented in Table 16; residue values are not corrected or adjusted for method recoveries. Apparent residues of triazole were less than the LOQ (<0.01, <0.015, or <0.020 ppm) in untreated milk (n=15 samples), skim milk (n=6), cream (n=6), subcutaneous fat (n=3), peritoneal fat (n=3), liver (n=3), kidney (n=3), and muscle (n=3).

Table 15. Residues of **tetraconazole** in dairy cattle matrices following oral administration of tetraconazole at target feeding levels of 0.34 ppm, 1.02 ppm, and 3.4 ppm for 28-30 consecutive days.

Dosing or Sampling Day [a]	Tetraconazole Uncorrected Residues (ppm)		
	Low Dose (0.34 ppm)	Mid Dose (1.02 ppm)	High Dose (3.4 ppm)
Milk			
1	N/A [b]	N/A	<0.003, <0.003, <0.003, <0.003, <0.003
3	N/A	N/A	0.013, 0.014, 0.016, 0.016, 0.018
5	N/A	N/A	0.013, 0.015, 0.015, 0.021, 0.022
7	<0.003, <0.003, <0.003	0.003, 0.005, 0.006	0.013, 0.015, 0.017, 0.023, 0.027
10	N/A	N/A	0.012, 0.013, 0.014, 0.020, 0.025
14	<0.003, <0.003, <0.003	0.004, 0.004, 0.005	0.013, 0.013, 0.017, 0.019, 0.029
18	N/A	N/A	0.013, 0.016, 0.023, 0.024, **0.048**
21	<0.003, <0.003, <0.003	0.004, 0.005, 0.006	0.014, 0.015, 0.017, 0.023, 0.023
24	N/A	N/A	0.015, 0.016, 0.016, 0.021, 0.025
26	N/A	0.013	N/A
27	N/A	0.014	N/A
28	<0.003, <0.003, <0.003	0.005, 0.006, 0.016	0.016, 0.019, 0.021, 0.024, 0.029
31	--	--	<0.003, 0.004
33	--	--	<0.003, <0.003
35	--	--	<0.003, <0.003
37	--	--	<0.003
40	--	--	<0.003
42	--	--	<0.003
Skimmed Milk			
14	<0.003, <0.003, <0.003	<0.003, <0.003, <0.003	<0.003, <0.003, <0.003, <0.003, <0.003
28	<0.003, <0.003, <0.003	<0.003, <0.003, <0.003	<0.003, <0.003, <0.003, <0.003, **0.003**
Cream			
14	0.020, 0.021, 0.022	0.046, 0.047, 0.068	0.194, 0.244, 0.248, 0.306, 0.340
28	0.017, 0.020, 0.023	0.068, 0.084, 0.125	0.224, 0.243, 0.275, 0.367, **0.391**
Fat, subcutaneous			
30	<0.003, <0.003	0.033	0.159
31	0.003	0.025, 0.030	0.011, 0.061

(continued; footnotes follow)

Table 15 *(continued)*.

Dosing or Sampling Day [a]	Tetraconazole Uncorrected Residues (ppm)		
	Low Dose (0.34 ppm)	Mid Dose (1.02 ppm)	High Dose (3.4 ppm)
36	--	--	**0.205**
43	--	--	<0.003
Fat, peritoneal			
30	0.007, 0.029	0.052	**0.199**
31	0.011	0.031, 0.069	0.041, 0.116
36	--	--	0.099
43	--	--	<0.003
Kidney			
30	0.004, 0.005	0.020	**0.057**
31	0.007	0.014, 0.039	0.040, **0.067**
36	--	--	0.006
43	--	--	<0.003
Liver			
30	0.144, 0.371	0.392	1.386
31	0.290	0.073, 0.662	1.012, **1.636**
36	--	--	0.245
43	--	--	0.022
Muscle, skeletal			
30	<0.003, <0.003	0.006	**0.015**
31	<0.003	0.004, 0.004	0.007, 0.011
36	--	--	0.010
43	--	--	<0.003

[a] Tissues sampled at 30 days were from cows treated for 29 consecutive days; tissues samples at 31 days were from cows treated for 30 consecutive days; and tissues sampled at 36 and 43 days were from cows treated for 28 consecutive days.

[b] N/A = Not analyzed.

Table 16. Residues of **triazole** in dairy cattle matrices following oral administration of tetraconazole at target feeding levels of 0.34 ppm, 1.02 ppm, and 3.4 ppm for 28-30 consecutive days.

Dosing or Sampling Day [a]	Triazole Uncorrected Residues (ppm)		
	Low Dose (0.34 ppm)	Mid Dose (1.02 ppm)	High Dose (3.4 ppm)
Milk			
1	N/A [b]	N/A	<0.01, <0.01, <0.01, <0.01, <0.01
3	N/A	N/A	<0.01, <0.01, <0.01, <0.01, <0.01
5	N/A	N/A	<0.01, <0.01, <0.01, <0.01, <0.01
7	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01, <0.01, 0.019
10	N/A	N/A	<0.01, 0.017, 0.022, 0.022, **0.023**
14	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, 0.016, 0.016, 0.021
18	N/A	N/A	<0.01, <0.01, <0.015, <0.015, **0.019**
21	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.015, <0.015, <0.015
24	N/A	N/A	<0.01, <0.015, <0.015, <0.015, 0.016
28	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.015, <0.015, <0.015
31	--	--	<0.01, <0.01
33	--	--	<0.01, <0.01
35	--	--	<0.01, <0.01
37	--	--	<0.01
40	--	--	<0.01
42	--	--	<0.01
Skimmed Milk			
14	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.015, <0.015, <0.015, **0.021**
28	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.015, <0.015, <0.015
Cream			
14	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.015, 0.019, 0.022, 0.020, 0.024
28	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.015, 0.017, 0.020, 0.022, **0.029**

Table 16 (*continued*).

Dosing or Sampling Day [a]	Triazole Uncorrected Residues (ppm)		
	Low Dose (0.34 ppm)	Mid Dose (1.02 ppm)	High Dose (3.4 ppm)
Fat, subcutaneous			
30	<0.015, <0.015	<0.015	<0.015
31	<0.015	<0.015, <0.015	<0.015, **0.022**
36	--	--	<0.015
43	--	--	<0.015
Fat, peritoneal			
30	<0.015, <0.015	<0.015	<0.020
31	<0.015	<0.015, <0.020	<0.015, <0.020
36	--	--	<0.015
43	--	--	<0.015
Kidney			
30	<0.015, <0.015	0.033	**0.034**
31	<0.015	<0.015, 0.026	<0.020, 0.033
36	--	--	<0.015
43	--	--	<0.015
Liver			
30	0.041, 0.050	0.083	0.211
31	0.060	0.070, 0.101	0.193, **0.243**
36	--	--	0.179
43	--	–	0.032
Muscle, skeletal			
30	<0.015, <0.015	<0.015, <0.015	<0.015
31	<0.015	<0.015	<0.015, <0.015, <0.015
36	--	--	<0.015
43	--	--	<0.015

[a] Tissues sampled at 30 days were from cows treated for 29 consecutive days; tissues samples at 31 days were from cows treated for 30 consecutive days; and tissues sampled at 36 and 43 days were from cows treated for 28 consecutive days.

[b] N/A = Not analyzed.

Conclusions: The submitted dairy cattle feeding data are adequate for the purpose of establishing a tolerance for secondary transfer of tetraconazole and triazole residues in dairy cattle milk, but not in tissues. The submitted feeding study had a maximum feed rate equivalent to 3.4 ppm tetraconazole, which covers the MTDB for dairy cattle. However, the MTDB of beef cattle is 6.2 ppm. Thus, in order to determine the appropriate tolerance levels in cattle tissues, a feeding study with a feed rate equivalent to at least 6.2 ppm tetraconazole is needed. With the data presently available and applying a multiplication factor of (6.2/3.4) = 1.82, the tolerance levels that follow may be derived. However, these levels are subject to change once the data from the requested feeding study are submitted and once the HIARC has issued recommendations about triazole.

The combined maximum residues of tetraconazole and triazole were 0.071 ppm in whole milk, 0.024 ppm in skimmed milk, and 0.420 ppm in cream. These data suggest that the proposed tolerance of 0.02 ppm for milk is inadequate and that a specific tolerance value should be established for milk fat. The petitioner should submit a revised Section F to propose a tolerance for residues of tetraconazole and triazole in "milk, fat (0.08 ppm in whole milk) at 2.5 ppm."

The combined maximum residues of tetraconazole and triazole were 0.227 ppm in subcutaneous fat and 0.219 ppm in peritoneal fat. These data suggest that the proposed tolerance of 0.1 ppm for cattle fat is inadequate. For the purposes of conditional registration/temporary tolerances, the petitioner should submit a revised Section F to propose tolerances for residues of tetraconazole and triazole in the "fat of cattle, goats, hogs, horses, and sheep at 0.50 ppm".

The combined maximum residues of tetraconazole and triazole were 0.101 ppm in kidney and 1.879 ppm in liver. These data suggest that separate tolerances should be established for kidney and liver because of the 18x difference in the magnitude of the expected residues. For the purposes of conditional registration/temporary tolerances, the petitioner should submit a revised Section F to propose tolerances for residues of tetraconazole and triazole in the "meat byproducts (except liver) of cattle, goats, hogs, horses, and sheep at 0.20 ppm" and in the "liver of cattle, goats, hogs, horses, and sheep at 3.5 ppm".

The combined maximum residues of tetraconazole and triazole were <0.030 ppm in muscle. These data suggest that the proposed tolerance of 0.01 ppm for cattle meat is inadequate. For the purposes of a conditional registration/temporary tolerances, the petitioner should submit a revised Section F to propose a tolerance for residues of tetraconazole and triazole in the "meat of cattle, goats, hogs, horses, and sheep at 0.060 ppm.

OPPTS GLNs 860.1850 and 860.1900: Confined/Field Accumulation in Rotational Crops

The petitioner submitted a confined rotational crop study in conjunction with the peanut petition (PP#9F06023, D259321, W. Donovan, in preparation). Pending submission of storage stability data to validate the storage conditions and intervals of rotational crop commodities, the submitted confined rotational crop study for triazole-labeled tetraconazole is adequate. However,

as the triazole-labeled study showed evidence for cleavage of tetraconazole occurring between the phenyl and triazole rings, a rotational crop study using phenyl-labeled tetraconazole is needed to determine whether this moiety is transported into the rotational crops.

Although the petitioner has not proposed plantback restrictions for rotational crops on the product label, rotational restrictions are required. Subject to change based on the results of the requested phenyl-labeled tetraconazole rotational crop study, the rotational restrictions are specified in the "OPPTS GLN 860.1200: Proposed Uses" section of this document. If the petitioner wishes to have rotational restrictions other than those specified in this document, then the petitioner should submit limited field trial data depicting tetraconazole residues of concern in/on rotational crops at the plantback interval(s) the petitioner wants to support.

The HED MARC tentatively determined that the residue of concern in rotational crops is tetraconazole *per se*. However, before this conclusion can be finalized, the MARC requires review of the requested rotational crop study using phenyl-labeled tetraconazole, and consideration of the HIARC deliberations on triazole (D264157, W. Donovan and D. Nixon, 19-APR-2000).

Codex Issues

There are no established Codex, Canadian, or Mexican limits for residues of tetraconazole in/on plant or livestock commodities (see Attachment I). Therefore, no compatibility issues exist with regards to the proposed tolerances discussed in this petition review.

List of Attachments

I. International Residue Limit Status Sheet
II. Figure 1

AGENCY MEMORANDA CITED IN THIS DOCUMENT

CBTS No(s):	16886
DP Barcode:	D222979
Subject:	Tetraconazole - Review of 8/16/95 Meeting Landis - Field Trial Requirements for Imported Coffee and Bananas.
From:	G.F. Kramer
To:	S. Robbins
Date:	2/14/96
MRIDs:	None

DP Barcode:	D252214 and D252213
Subject:	ID#99ND0005. Section 18 Exemption for the Use of Tetraconazole on Sugarbeets in North Dakota and Minnesota.
From:	W. Dykstra and L. Cheng
To:	D. Deegan/M. Laws
Date:	3/18/99
MRIDs:	None

DP Barcode:	D259321
Subject:	PP#9F06023; Petition For Permanent Tolerances For Use Of Tetraconazole On Peanuts
From:	W. Donovan
To:	M. Waller/L. Jones
Date:	Currently Under Review
MRIDs:	44865403, 44865407, and 44900501

DP Barcode:	D259205
Subject:	PP#7E04830; Petition For Import Tolerances For Use Of Tetraconazole On Bananas
From:	W. Donovan
To:	M. Waller/L. Jones
Date:	Currently Under Review
MRIDs:	44268106-44268111

DP Barcode:	D264157
Subject:	Tetraconazole. Results of the HED Metabolism Assessment Review Committee (MARC) Meetings Held on 07- and 14-MAR-2000.
From:	W. Donovan and D. Nixon
To:	G.F. Kramer
Date:	19-APR-2000

DP Barcode:	D264681
Subject:	Tetraconazole in/on Bananas, Peanuts, and Sugar Beets. Request for Petition Method Validation (PMV).
From:	W. Donovan
To:	F.D. Griffith, Jr.
Date:	07-APR-2000

ATTACHMENT I

INTERNATIONAL RESIDUE LIMIT STATUS

CHEMICAL: Tetraconazole

CODEX NO. N/A

CODEX STATUS:

✓ No Codex Proposal
Step 6 or above

PROPOSED U.S. TOLERANCES:

Petition No: PP#9F05066

Agency Reviewer: W. Donovan

Residue (if Step 8):

Residues Proposed For Inclusion in the Tolerance Expression: Tetraconazole [(±)-2-(2,4-dichlorophenyl)-3-(1H-1,2,4-triazol-1-yl)propyl 1,1,2,2-tetrafluoroethyl ether]

Crop(s)	Limit (mg/kg)	Crop(s)	Limit (mg/kg)
		Sugarbeet roots	0.1 ppm
		Sugarbeet tops	7 ppm
		Sugarbeet pulp (dried)	0.3 ppm
		Sugarbeet molasses	0.3 ppm
		Sugarbeet refined sugar	0.01 ppm
		Milk	0.02 ppm
		Cattle meat	0.01 ppm
		Cattle meat byproducts	2 ppm
		Cattle fat	0.1 ppm

CANADIAN LIMITS:

✓ No Canadian limit

Residue:

MEXICAN LIMITS:

✓ No Mexican limit

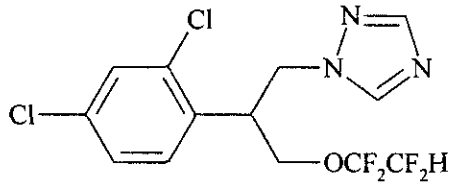
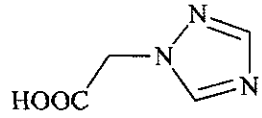
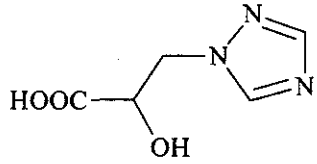
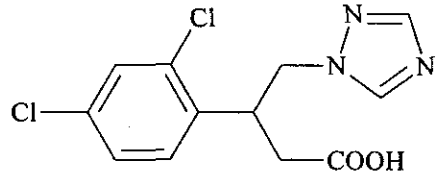
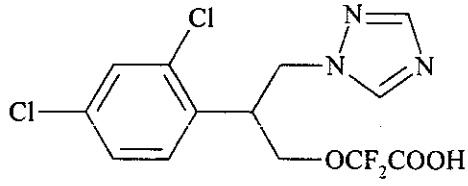
Residue:

Crop(s)	Limit (mg/kg)	Crop(s)	Limit (mg/kg)

NOTES:

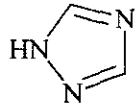
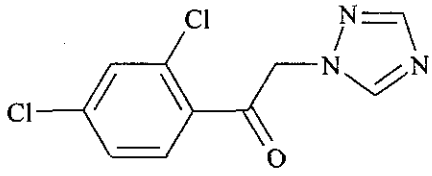
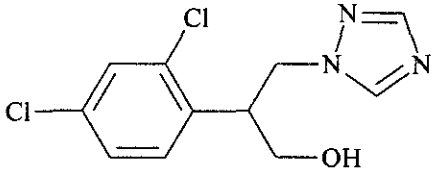
ATTACHMENT II

Figure 1. Tetraconazole and its metabolites in plant (sugar beet) and livestock (ruminant) commodities.

Common Name Chemical Name	Structure	Substrate
Tetraconazole (±)-2-(2,4-dichlorophenyl)-3-(1H-1,2,4-triazol-1-yl)propyl 1,1,2,2-tetrafluoroethyl ether		Sugar beet leaves Phenyl- and triazole-label goat milk, fat, kidney, liver, and muscle
Triazolyl acetic acid (TAA) (1H-1,2,4-triazol-1-yl)acetic acid		Sugar beet leaves
Triazolyl-hydroxypropionic acid (THP) (2-hydroxy-3-[1H-1,2,4-triazol-1-y]propionic acid		Sugar beet leaves
M14360-acid 2-(2,4-dichlorophenyl)-3-(1H-1,2,4-triazol-1-yl)-propionic acid		Sugar beet leaves
M14360-difluoroacetic acid (M14360-DFA) 5-(2,4-dichlorophenyl)-2,2-difluoro-6-(1H-1,2,4-triazol-1-yl)-oxahexanoic acid		Sugar beet leaves Phenyl-label goat milk

(*continued next page*)

Figure 1 (*continued*).

Common Name Chemical Name	Structure	Substrate
Triazole 1,2,4-triazole		Sugar beet leaves Triazole-label goat milk, fat, kidney, liver, and muscle
M14360-ketone 1-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-yl)acetophenone		Phenyl-label goat fat, kidney, liver, and muscle
M14360-alcohol 2-(2,4-dichlorophenyl)-3-(1H-1,2,4-triazol-1-yl)-1-propanol		Sugar beet leaves Phenyl-label goat liver