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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

DATE: 17-MAY-2002

SUBJECT: PP#s 9F05066, 9F06023, and 7E04830. **Tetraconazole. Amendments Dated 19-NOV-2001, 27-DEC-2001, 08-FEB-2002, and 09-APR-2002. Additional Data to Amend HED's Residue Data and Analytical Methods Memorandum Concerning Sugar Beets, Bananas, and Peanuts (D278236, W. Donovan, 22-OCT-2001).**
PC Code: 120603. DP Barcodes: D282558, D281350, D280220, D280174, and D279986. Case#: 290888. Submission#s: S614374, S577243, S608564, and S607957. MRID#s: 455604-01 & -02; 455455-01; 455699-01; 456052-01; and 456510-01.

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THROUGH: G. Jeffrey Herndon, Chemist, Branch Senior Scientist G. Jeffrey Herndon
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TO: Lisa Jones/Mary Waller, PM Team 21
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Sipcam Agro USA, Inc. submitted additional data in response to a memo summarizing deficiencies in the tetraconazole residue chemistry database (D278236, W. Donovan, 22-OCT-2001). The initial review of the tetraconazole residue chemistry database was provided in three separate memos corresponding to the separate sugar beet, banana and peanut petitions (D254411, W. Donovan, 18-MAY-2000; D259205, W. Donovan, 18-MAY-2000; D259321, W. Donovan, 18-MAY-2000). Further, two amendment reviews updated the status of the tetraconazole residue chemistry database as additional residue chemistry data were submitted (D267481, W. Donovan, 12-OCT-2000 and D278236, W. Donovan, 22-OCT-2001). The following is HED's review of the most recent data submissions and summary of which deficiencies have been resolved and which remain outstanding.

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Executive Summary of Chemistry Deficiencies

- Final Metabolism Assessment Review Committee (MARC) determination of residues of concern for tolerance expression and risk assessment in plants, livestock, and rotational crops.
- Agency validation of the triazole method for livestock commodities.
- Multiresidue testing results for triazole.
- Poultry feeding study.

NOTE: The status of the tetraconazole residue chemistry database is likely to be affected by conclusions about how to regulate free triazole and/or its conjugates. The Agency is currently in the process of making this determination. Once this is accomplished, HED will provide an update about any additional data requirements.

Recommendations

The residue chemistry database does not presently support the establishment of tolerances for residues of tetraconazole *per se* in/on banana, the raw and processed commodities of sugar beets or peanuts, 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 5 and 8b. HED will initiate a human health risk assessment of the proposed uses of tetraconazole on sugar beets, peanuts, and bananas when the above deficiencies have been resolved and following determination of how to regulate triazole and its conjugates.

CONCLUSIONS

OPPTS GLN 860.1200: Proposed Uses and
OPPTS GLNs 860.1850 and 860.1900: Confined/Field Accumulation in Rotational Crops

1. The final report describing the results of the [^{14}C-phenyl] tetraconazole confined rotational crop study included residue levels following 30-, 120-, and 365-day PBIs for three dissimilar crops. In addition, the report provided identification/characterization of the species comprising the TRR for each RAC crop fraction (i.e., carrot root and top; wheat grain, forage and straw). Adequate field accumulation in rotational crop studies for parent tetraconazole were previously submitted. However, conclusions regarding the appropriate PBIs and residues of concern must await Agency determination of how to regulate free triazole and/or its conjugates. Depending on the outcome of the triazole and/or conjugate decision, additional rotational crop data may be requested. **This deficiency is now resolved.**

OPPTS GLN 860.1300: Nature of the Residue in Plants

2. The additional identification and characterization of sugar beet root metabolites appears to be satisfactory. Pending concurrence of the HED Metabolism Assessment Review Committee (MARC), the qualitative nature of the residue in sugar beet roots is understood. **This deficiency is now resolved.**

OPPTS GLN 860.1300: Nature of the Residue in Livestock

3. Storage stability data submitted as part of the radiovalidation study demonstrates that tetraconazole residues are stable in goat milk and muscle for at least seven years when stored frozen. **This deficiency is now resolved.**

OPPTS GLN 860.1340: Residue Analytical Method - Plant and Livestock Commodities

4a. The requested tetraconazole petition method validation (PMV) of the plant and livestock analytical methods was recently completed (D264936 & D264683, P.G. Schermerhorn, 18-DEC-2001) and determined to be acceptable (D280005, W. Donovan, 10-JAN-2002). **This deficiency is now resolved.**

4b. The results of the triazole/livestock commodity ILV appear to be acceptable. The triazole method and ILV will be forwarded to EPA's Analytical Chemistry Branch (ACB) for further evaluation. No further action by the petitioner is needed at this time. However, once regulation of triazole and/or its conjugates is determined, additional data may be needed. **Thus, this deficiency is now resolved.**

OPPTS GLN 860.1360: Multiresidue Method

5. The petitioner has agreed to test 1,2,4-triazole through the FDA multiresidue method. However, once regulation of triazole and/or its conjugates is determined, multiresidue testing of other compounds may be needed. **This deficiency remains unresolved.**

OPPTS GLN 860.1380: Storage Stability Data

6. The submitted studies adequately demonstrate the stability of 1,2,4-triazole in milk samples at -20 C for at least 18 months when stored in the dark, and in liver, muscle, and fat samples at -20 C for at least 12 months when stored in the dark. These intervals exceed the storage intervals involved in the cattle feeding studies. **Thus, this deficiency is now resolved.**

OPPTS GLN 860.1500 & 860.1520: Crop Field Trials & Processed Food/Feed

7a. In a meeting of the HED ChemSAC held on 15-MAY-2002, it was determined that, based on the consistency of the existing field trial data together with the results of the recently submitted side-by-side trial at the original and reduced application rates, no additional field trial data for sugar beets are required. Moreover, it was also determined that the appropriate tolerance levels are 3.5 and 0.10 ppm for sugar beet tops and roots, respectively. **This deficiency is now resolved.**

7b. Final recommendations concerning the Section F will be made following HED determination of how to regulate triazole and/or its conjugates. **This deficiency is now resolved.**

OPPTS GLN 860.1480: Meat, Milk, Poultry, & Eggs

8a. With the revised tolerance level for sugar beet tops of 3.5 ppm, HED agrees that the previously submitted bovine feeding study is adequate. **This deficiency is now resolved.**

8b. Although all extrapolated tetraconazole residue levels at 1x are calculated to be < LOQ, HED generally requires a feeding study unless metabolism data at a 10x dosing rate results in residues < LOQ for all commodities. This condition has not been met for tetraconazole: at 10x, residues in fat are extrapolated to be 0.041 ppm and those in liver are extrapolated to be 0.012 ppm, both exceeding the LOQ of 0.01 ppm. In addition, results from the poultry metabolism study indicate that TRR levels in egg white and yolk had not reached a plateau within the three days of dosing. Consequently, HED reiterates its request for a poultry feeding study, to be conducted at 1x, 3x, and 10x the anticipated dietary burden of 0.00375 ppm, dosing daily for a minimum of 28 days or until residues plateau in eggs. **This deficiency remains unresolved.**

DETAILED CONSIDERATIONS

1. Confined Rotational Crops.

Deficiency - Conclusion 1a from Memo, D278236, W. Donovan, 22-OCT-2001:

1a. Adequate field accumulation in rotational crop studies for parent tetraconazole have been submitted. However, conclusions regarding the appropriate plant-back intervals (PBIs) must await review of the completed [^{14}C-phenyl] tetraconazole confined rotational crop study and Agency determination of how to regulate free triazole and/or its conjugates. The final report describing the results of the [^{14}C-phenyl] tetraconazole confined rotational crop study should include residue levels following a 365-day PBI. In addition, the petitioner should provide identification/characterization of the species comprising the TRR as well as analysis of each RAC crop fraction (i.e., carrot root and top; sorghum grain, forage and stover). **This deficiency remains unresolved.**

Petitioner's Response

MRID# 456052-01, describing the results of confined rotational crop studies.

456052-01 F. Rizzo and G. Pizzingrilli (2002) Uptake, translocation and metabolism of [^{14}C-phenyl]Tetraconazole in rotated crops of cereals, carrots, and lettuce: Study Number: ABT.98.11. Unpublished study prepared by Isagro Ricerca Srl. 294 p.

MRID# 456052-01 details the final results of the interim confined rotational crop study described in MRID# 451550-05 and reviewed in the following memo: D267481, W. Donovan, 12-OCT-2000. In this study, treated soil was aged for four periods prior to the planting of each crop: 30, 120, 223, and 365 days. The earlier report provided TRR results for all aging periods except 365 days for selected crop fractions. The present submission provides results for all periods and includes identification/characterization of residues in each crop fraction.

The radioactive test substance, [^{14}C-phenyl] tetraconazole (specific activity 99.49 μCi/mg, radiochemical purity ≥97%), was mixed with nonlabeled tetraconazole in acetonitrile to yield a formulated test substance with a final specific activity of 21.341 μCi/mg. Following dilution of the formulated test substance with water, it was applied to twenty-two pots of soil by single dropwise application at approximately 500 g/ha (1.5X the maximum seasonal rate for sugar beets). To assist in the identification of metabolites found in the 1.5X treated samples, four additional pots received a single dropwise application of non labeled tetraconazole at 5000 g/ha (15X). Eleven pots were used for growing control crops, and 6 pots were used for sampling soil treated at a 1.5X rate with [^{14}C-phenyl] tetraconazole. All treated and control pots were maintained outdoors.

Six pots were aged for 30 days following [^{14}C-phenyl] tetraconazole application at 1.5x the maximum field rate and then seeded with carrot (1 pot), lettuce (1 pot) and winter wheat (4 pots). Six pots were aged for 120 days following [^{14}C-phenyl] tetraconazole application at 1.5x the maximum field rate and then seeded with carrot (1 pot), lettuce (1 pot) and winter wheat (4 pots).

Four pots were aged for 223 days following [^{14}C-phenyl] tetraconazole application at 1.5x the maximum field rate and then seeded with carrot (1 pot), lettuce (1 pot) and sorghum (2 pots). Six pots were aged for 365 days following [^{14}C-phenyl] tetraconazole application at 1.5x the maximum field rate and then seeded with carrot (1 pot), lettuce (1 pot) and winter wheat (4 pots).

Results

The total radioactive residue (TRR) was determined by combustion of finely ground samples. The samples of each crop were extracted with acetone-water: the extractable radioactivity was generally more than 80% of the TRR in all crop fractions. The extractable radioactivity was partitioned in n-hexane and in ethyl acetate. Table 1 summarizes the TRR and tetraconazole levels found in the crop matrices tested at the intervals indicated. Results from the 223 day sampling interval were omitted as these TRR results were presented in a previous review (D267481, W. Donovan, 12-OCT-2000), and are generally consistent with the values obtained at 120 and 365 DAT.

Table 1. Results of [^{14}C-phenyl] Tetraconazole Confined Rotational Crop Study.

RAC	Days from treatment to planting			Days from treatment to planting		
	30	120	365	30	120	365
	TRR (ppm)			Tetraconazole (ppm)		
Carrot, root	0.031	0.022	0.036	0.025	0.016	0.026
Carrot, top	0.033	0.051	0.117	0.019	0.023	0.059
Lettuce	0.025	0.043	0.018	0.010	0.020	0.012
Wheat, grain	0.026	0.025	0.005	0.004	0.004	0.004
Wheat, forage	0.152	0.089	0.024	0.118	0.045	0.016
Wheat, straw	1.389	0.855	0.269	0.667	0.372	0.126

The results of efforts to identify and/or characterize the TRR in each RAC at each interval are provided in Table 2.

Table 2. Summary of the characterization/identification of radioactive residues in rotational crop commodities grown in soil treated with [phenyl-^{14}C]tetraconazole at 0.446 lb ai/A (1.5x).

Metabolite [a]	30-DAT Carrot, root (TRR = 0.031 ppm)		120-DAT Carrot, root (TRR = 0.022 ppm)		365-DAT Carrot, root (TRR = 0.036 ppm)		30-DAT Carrot, tops (TRR = 0.033 ppm)		120-DAT Carrot, tops (TRR = 0.051 ppm)		365-DAT Carrot, tops (TRR = 0.117 ppm)	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	% TRR	ppm	%TRR	ppm	%TRR	ppm
Extractable	87.1	0.027	81.8	0.018	88.9	0.032	100.0	0.033	96.1	0.049	84.6	0.099
Tetraconazole	80.7	0.025	72.7	0.016	72.2	0.026	57.6	0.019	45.1	0.023	50.4	0.059
M14360-DFA	–	–	–	–	2.8	0.001	3.0	0.001	3.9	0.002	3.4	0.004
M14360-acid	–	–	–	–	2.8	0.001	9.1	0.003	11.8	0.006	14.5	0.017
M14360(C-1)alcohol-conjug	–	–	–	–	–	–	3.0	0.001	3.9	0.002	1.7	0.002
M14360-ketone	–	–	–	–	–	–	–	–	–	–	1.7	0.002
Water soluble	6.5	0.002	9.1	0.002	8.3	0.003	21.2	0.007	25.5	0.013	--	--
Bound	19.4	0.006	18.2	0.004	11.1	0.004	–	–	–	–	12.0	0.014
Cellulose	10	0.003	10.9	0.002	2.8	0.001	–	–	–	–	4.4	0.005
Lignin	3.9	0.001	--	--	--	--	–	–	–	–	2.0	0.002
Total characterized/ identified	**106**	**0.033**	**100**	**0.022**	**100**	**0.036**	**100**	**0.033**	**96.1**	**0.049**	**96.6**	**0.113**

Table 2. Continued

Metabolite [a]	30-DAT Lettuce (TRR = 0.025 ppm)		120-DAT Lettuce (TRR = 0.043 ppm)		365-DAT Lettuce (TRR = 0.018 ppm)		30-DAT Wheat, forage (TRR = 0.152 ppm)		120-DAT Wheat, forage (TRR = 0.089 ppm)		365-DAT Wheat, forage (TRR = 0.024 ppm)	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	% TRR	ppm	%TRR	ppm	%TRR	ppm
Extractable	96.0	0.024	97.7	0.042	100	0.018	90.8	0.138	86.5	0.077	87.5	0.021
Tetraconazole	40.0	0.010	46.5	0.020	66.7	0.012	77.6	0.118	50.6	0.045	66.7	0.016
M14360-ketone-conjug	8.0	0.002	7.0	0.003	5.6	0.001	–	–	2.3	0.002	–	–
M14360-DFA	–	–	–	–	–	–	4.0	0.006	6.7	0.006	–	–
M14360-acid	–	–	–	–	2.8	0.001	0.7	0.001	6.7	0.006	–	–
M14360(C-1)alcohol-conjug	16.0	0.004	23.3	0.010	22.2	0.004	1.3	0.002	4.5	0.004	–	–
M14360-alcohol-conjug	–	–	–	–	–	–	0.7	0.001	2.3	0.002	–	–
Water soluble	28.0	0.007	18.6	0.008	5.6	0.001	4.0	0.006	6.7	0.006	20.8	0.005
Bound	–	–	–	–	–	–	10.5	0.016	15.7	0.014	16.7	0.004
Cellulose	–	–	–	–	–	–	3.8	0.006	6.1	0.005	8.3	0.002
Lignin	–	–	–	–	–	–	1.8	0.003	2.7	0.002	2.9	0.001
Total characterized/ identified	**96.0**	**0.024**	**97.7**	**0.042**	**100**	**0.018**	**101**	**0.154**	**102**	**0.091**	**104**	**0.025**

Table 2. Continued

Metabolite [a]	30-DAT Wheat, straw (TRR = 1.389 ppm)		120-DAT Wheat, straw (TRR = 0.855 ppm)		365-DAT Wheat, straw (TRR = 0.269 ppm)		30-DAT Wheat, grain (TRR = 0.026 ppm)		120-DAT Wheat, grain (TRR = 0.025 ppm)		365-DAT Wheat, grain (TRR = 0.005 ppm)	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	% TRR	ppm	%TRR	ppm	%TRR	ppm
Extractable	82.4	1.144	81.9	0.700	82.2	0.221	80.8	0.021	76.0	0.019	80.0	0.004
Tetraconazole	48.0	0.667	43.5	0.372	46.8	0.126	15.4	0.004	16.0	0.004	80.0	0.004
M14360-ketone + conjug	5.9	0.082	5.7	0.049	-	-	-	-	-	-	-	-
M14360-DFA	11.0	0.153	9.9	0.085	7.1	0.019	-	-	-	-	-	-
M14360-acid	4.6	0.064	8.9	0.076	6.7	0.018	23.1	0.006	20.0	0.005	-	-
M14360(C-1)alcohol-conjug	6.3	0.088	3.3	0.028	11.5	0.031	-	-	-	-	-	-
M14360-alcohol-conjug	3.2	0.045	3.7	0.032	1.9	0.005	-	-	-	-	-	-
Water soluble*	3.2	0.045	6.8	0.058	8.2	0.022	42	0.011	40	0.010	-	-
Bound	15.1	0.210	15.8	0.135	16.0	0.043	23.1	0.006	24.0	0.006	-	-
Cellulose	5.8	0.080	7.0	0.060	7.4	0.020	3.8	0.006	6.1	0.005	-	-
Lignin	2.9	0.040	3.5	0.030	3.5	0.010	1.8	0.003	2.7	0.002	-	-
Total characterized/ identified	**97.5**	**1.354**	**97.7**	**0.835**	**98.1**	**0.263**	**104**	**0.027**	**100**	**0.025**	**80**	**0.004**

* For wheat straw, these values were expressed in terms of 5 compounds characterized as polar.

Adequate information regarding sample storage intervals for both the triazole- and phenyl-labeled confined rotational crop studies have been submitted. As all storage intervals were 7 months or less, no supporting storage stability data are required.

HED's Conclusion
The final report describing the results of the [^{14}C-phenyl] tetraconazole confined rotational crop study included residue levels following 30-, 120-, and 365-day PBIs for three dissimilar crops. In addition, the report provided identification/characterization of the species comprising the TRR for each RAC crop fraction (i.e., carrot root and top; wheat grain, forage and straw). Adequate field accumulation in rotational crop studies for parent tetraconazole were previously submitted. However, conclusions regarding the appropriate PBIs and residues of concern must await Agency determination of how to regulate free triazole and/or its conjugates. Depending on the outcome of the triazole and/or conjugate decision, additional rotational crop data may be requested. **This deficiency is now resolved.**

2. Identification/Characterization of Metabolites in Roots from the Sugar Beet Metabolism Study.

Deficiency - Conclusion 3b from Memo, D278236, W. Donovan, 22-OCT-2001:

3b. Based on the studies reported in MRID#s 447513-11 and 454194-01, tetraconazole metabolism in sugar beet leaves is now adequately understood. However, the primary purpose of conducting the phenyl-labeled sugar beet metabolism study at an exaggerated rate was to enable determination of the residues of concern in sugar beet roots. Thus, the petitioner should determine the TRR level of the sugar beet root samples treated at 4.5x and characterize/identify the compounds comprising the TRR. **This deficiency remains unresolved.**

Petitioner's Response
MRID# 455699-01, providing an addendum to the previous sugar beet metabolism study described in MRID# 454194-01.

455699-01 G. Pizzingrilli and F. Rizzo (2001) Metabolism of [^{14}C-phenyl] Tetraconazole in Sugar Beet: (experimental addendum to R/ABT.00.09 study): Study Number: addendum R/ABT.00.09. Addendum to MRID# 454194-01. Unpublished study prepared by Isagro Ricerca Srl. 60 p.

Further analysis of the 5x treated root samples showed that the TRR in these samples was 0.0421 ppm. Moreover, the main compound was parent tetraconazole (0.0298 ppm, 70.9% TRR). Three additional metabolites were present: M14360-acid (0.0023 ppm, 5.5% TRR), M14360-DFA (0.0027 ppm, 6.4% TRR), and a conjugate of M14360-alcohol, likely an O-glucoside (0.0028 ppm, 6.7% TRR). Thus, identified compounds accounted for 89.4% of the TRR. A summary of these findings is provided in Table 3, and the proposed metabolic pathway for tetraconazole in sugar beet roots is presented in Figure 1.

Table 3. Identification/characterization of TRR found in sugar beet root samples treated at 5x.

Metabolite [a]	Sugar beet root, 5x (TRR =0.0421 ppm)	
	%TRR	ppm
Extractable	92.9	0.0391
Tetraconazole	70.9	0.0298
M14360-DFA	6.4	0.0027
M14360-acid	5.5	0.0023
M14360-alcohol-conjug	6.7	0.0028
Bound	5.9	0.0025
Total characterized/ identified	**98.8**	**0.0416**

Extraction and analysis of the 5x root samples was performed about 14 months after the sugar beet harvesting. However, storage stability data showed no qualitative or quantitative changes in the root samples during this period.

HED's Conclusion
The additional identification and characterization of sugar beet root metabolites appears to be satisfactory. Pending concurrence of the HED MARC, the qualitative nature of the residue in sugar beet roots is understood. **This deficiency is now resolved.**

3. Goat Metabolism Study: Storage Stability in Goat Meat and Milk.

Deficiency - Conclusion 5 from Memo, D278236, W. Donovan, 22-OCT-2001:

5. The goat metabolism studies are acceptable provided the petitioner submits supporting sample storage intervals/storage stability data for the total toxic residues of tetraconazole in goat milk and tissues. This deficiency remains unresolved.

Petitioner' Response
Storage stability data for tetraconazole residues in goat tissues was included in the radiovalidation report (MRID 451550-04) and demonstrated stability for at least seven years.

HED's Conclusion
Storage stability data submitted as part of the radiovalidation study demonstrates that tetraconazole residues are stable in goat milk and muscle for at least seven years when stored frozen. **This deficiency is now resolved.**

4. Residue Analytical Method

Deficiency - Conclusions 6b and 8 from Memo, D278236, W. Donovan, 22-OCT-2001:

6b. A requested tetraconazole petition method validation (PMV) [D264681, W. Donovan, 07-APR-2000] of the plant and livestock analytical methods has yet to be completed. **Thus, this deficiency remains outstanding.**

8. Because the HED MARC tentatively determined that triazole is a residue of concern in livestock commodities, an enforcement method may be needed to detect triazole residues in livestock commodities. Accordingly, if the decision to regulate triazole is confirmed, 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 petition method validation (PMV). **This deficiency remains unresolved.**

Petitioner's Response

456510-01 K.H. Martin, R.L. van Hoven, and W.B. Nixon (2002). Independent Laboratory Validation of a Method for the Analysis of 1,2,4-Triazole in Milk, Eggs, and Tissue. Wildlife International, Ltd. Unpublished study submitted by Sipcam Agro USA, Inc. 51 p.

Wildlife International Ltd. conducted a successful independent laboratory validation study of the analytical method entitled "Analytical Method for the Determination of 1,2,4-Triazole Residues in Biological Substrates", developed by Isagro Ricera. Representative samples of milk, egg, and meat (london broil) were separately fortified with 1,2,4-triazole at 0.010 (1x LOQ), 0.10 (10x LOQ), and 0.50 ppm (50x LOQ). Samples were processed and analyzed for 1,2,4-triazole levels by gas chromatography equipped with a nitrogen/phosphorous detector (GC/NPD). The results are summarized in Table 4.

Table 4. 1,2,4-Triazole recovery results from ILV study of milk, egg, and meat.

Sample	Fortification Level (ppm)	Recovery
Milk	0.010	117 ± 1.4
	0.10	85.6 ± 6.2
	0.50	73.8 ± 1.8
Egg	0.010	80.8 ± 1.8
	0.10	79.7 ± 2.4
	0.50	82.3 ± 2.8
Meat	0.010	88.4 ± 6.9
	0.10	72.4 ± 2.8
	0.50	77.5 ± 0.9

Recoveries of 1,2,4-triazole were generally within the acceptable range of 70-120%. Thus, the ILV of the method for analysis of 1,2,4-triazole in milk, eggs, and meat may be considered successful. However, it was noted that the milk matrix requires additional clean-up steps or

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quantitation using peak area.

HED's Conclusion
The requested tetraconazole petition method validation (PMV) of the plant and livestock analytical methods was recently completed (D264936 & D264683, P.G. Schermerhorn, 18-DEC-2001) and determined to be acceptable (D280005, W. Donovan, 10-JAN-2002). **Thus, this deficiency is now resolved.**

The results of the triazole/livestock commodity ILV appear to be acceptable. The triazole method and ILV will be forwarded to EPA's Analytical Chemistry Branch (ACB) for further evaluation. No further action by the petitioner is needed at this time. However, once regulation of triazole and/or its conjugates is determined, additional data may be needed. **Thus, this deficiency is now resolved.**

5. Multiresidue Method.

Deficiency - Conclusion 9 from Memo, D278236, W. Donovan, 22-OCT-2001:

9. The petitioner has provided the requested multiresidue testing results for tetraconazole. However, multiresidue testing of triazole will also be required if triazole is determined to be a residue of concern. **This deficiency is partially resolved.**

Petitioner's Response
Efficacy of the FDA multiresidue method to detect 1,2,4-triazole will be determined.

HED's Conclusion
The petitioner has agreed to test 1,2,4-triazole through the FDA multiresidue method. However, once regulation of triazole and/or its conjugates is determined, multiresidue testing of other compounds may be needed. **This deficiency remains unresolved.**

6. Triazole storage stability data for livestock commodities.

Deficiency - Conclusion 10a from Memo, D278236, W. Donovan, 22-OCT-2001:

10a. 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. **This deficiency remains unresolved.**

Petitioner's Response

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455604-01 G. Zini (2001). Stability of 1,2,4-Triazole in Milk Stored at -20 C in the Dark. Isagro Ricerca. Unpublished study submitted by Sipcam Agro USA, Inc. 91 p.

455604-02 G. Zini (2001). Stability of 1,2,4-Triazole in Biological Substrates Stored at -20 C in the Dark. Isagro Ricerca. Unpublished study submitted by Sipcam Agro USA, Inc. 93 p.

The above studies report the results of storage stability studies conducted on 1,2,4-triazole in milk, liver, muscle, and fat. The results show that under frozen conditions in the dark, triazole is stable in these matrices for at least 12 months in liver, muscle, and fat; and at least 18 months in milk. The recoveries obtained from milk samples at the fortification levels indicated are shown in Table 5, while those obtained from liver, muscle, and fat are shown in Table 6.

Table 5. Storage Stability Results for Residues of Tetraconazole in Milk at -20 C.

Fortification Level (ppm)	Recovery at Time Interval Indicated (days)					
	0	35	91	234	368	560
0.0222	89.0	100.5	96.8	92.5	89.1	85.1
0.0444	99.4	101.9	98.9	88.7	85.0	90.4
0.444	95.5	96.1	95.0	87.9	88.2	90.4

Table 6. Storage Stability Results for Residues of Tetraconazole in Liver, Muscle, and Fat at -20 C.

Matrix	Fortification Level (ppm)	Recovery at Time Interval Indicated (days)				
		0	41	90	201	370
Liver	0.12	94.1	107.0	89.2	91.5	85.3
	1.2	107.6	96.4	91.5	94.7	105.7
Muscle	0.12	95.1	90.1	108.5	102.3	90.2
	1.2	95.0	81.8	90.0	89.8	95.7
Fat	0.12	94.6	81.1	82.1	89.3	100.0
	1.2	95.3	84.0	83.2	99.5	93.9

HED's Conclusion

The submitted studies adequately demonstrate the stability of 1,2,4-triazole in milk samples at -20C for at least 18 months when stored in the dark, and in liver, muscle, and fat samples at -20 C for at least 12 months when stored in the dark. These intervals exceed the storage intervals involved in the cattle feeding studies. **Thus, this deficiency is now resolved.**

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6. Crop Field Trials & Processed Food/Feed.

Deficiency - Conclusions 11 & 12 from Memo, D278236, W. Donovan, 22-OCT-2001:

11. Due to the new use rate proposed by the petitioner, a set of 12 field trials should be conducted for sugar beets based on the maximum proposed use rate of three applications at 0.1 lb ai/A with a 14 day pre-harvest interval (PHI) and a 14 day re-treatment interval (RTI). **This deficiency remains unresolved.**

12. The petitioner should submit a revised Section F to correct the following commodity definitions: sugar beet roots and tops to "beet, sugar, roots" and "beet, sugar, tops"; and sugar beet pulp and molasses to "beet, sugar, dried pulp" and "beet, sugar, molasses." Also, the petitioner should delete "sugar beet refined sugar" from the requested Section F revision. The appropriate tolerance levels for sugar beet and livestock RACs should be set according to the results of the requested sugar beet crop field trial data at the revised treatment rate. **This deficiency remains unresolved.**

Petitioner's Response

The petitioner submitted MRID# 455455-01, describing a side-by-side California sugar beet field trial using the original and revised use rates.

455455-01 S. Alcaraz. (2001) Magnitude of the Residue Study with Eminent 125 SL (tetraconazole) Applied to Sugar Beets in the Imperial Valley of Southern California. Research Designed for Agriculture and Wildlife International. Study Number: CA01-1909-01. Unpublished study submitted by Sipcam Agro USA, Inc. 141 p.

Samples of sugar beet roots and tops from one side-by-side field trial conducted using two different application patterns of Eminent® were analyzed for tetraconazole residue levels. Three separate plots were treated as follows: Treatment 1 was the untreated control. Treatment 2 received six applications of eminent at the rate of 13 fluid oz product/acre at each application on a 14-day spray schedule for a total rate of 0.65 lb ai/A for the season. Treatment 3 received three applications of Eminent® at the rate of 13 fluid oz product/acre at each application on a 28-day spray schedule for a total rate of 0.32 lb ai/A for the season. Sample harvest occurred at crop maturity and the PHI was 14 days. The results of the field trial are summarized in Table 7.

Table 7. Tetraconazole residues in sugar beets treated three or six times at 0.107 lb ai/A with a 14 day PHI.

Treatment No.	Matrix	Total Rate (lb ai/A)	RTI (days)	Tetraconazole (ppm)	Average Residue (ppm)
1	Untreated roots	0	NA	< 0.01	NA
2	Treated roots	0.646	14	0.0185	0.0385
2	Treated roots	0.646	14	0.0585	
3	Treated roots	0.320	28	< 0.01	0.0112
3	Treated roots	0.320	28	0.0123	

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1	Untreated tops	0	NA	< 0.10	NA
2	Treated tops	0.646	14	2.27	1.71
2	Treated tops	0.646	14	1.15	
3	Treated tops	0.320	28	0.842	0.790
3	Treated tops	0.320	28	0.737	

Based on the above results, the petitioner proposes that the tolerance level for sugar beet tops be reduced to 3.5 ppm and the tolerance for sugar beet root remain at 0.10 ppm.

The petitioner also submitted a revised Section F incorporating the requested commodity definitions.

HED's Conclusion

In a meeting of the HED ChemSAC held on 15-MAY-2002, it was determined that, based on the consistency of the existing field trial data together with the results of the recently submitted side-by-side trial at the original and reduced application rates, no additional field trial data for sugar beets are required. Moreover, it was also determined that the appropriate tolerance levels are 3.5 and 0.10 ppm for sugar beet tops and roots, respectively. **This deficiency is now resolved.**

Final recommendations concerning the Section F will be made following HED determination of how to regulate triazole and/or its conjugates. **This deficiency is now resolved.**

7. Meat, Milk, Poultry, and Eggs

Deficiency - Conclusions 13 & 15 from Memo, D278236, W. Donovan, 22-OCT-2001:

13. **The bovine feeding study deficiency remains unresolved.** HED notes that, depending on the results of the new sugar beet crop field trial data, a new bovine feeding study at 6.2 ppm tetraconazole might not be needed. This deficiency will be re-evaluated upon receipt of sugar beet crop field trial data reflecting residue levels at the new treatment rate.

15. The results of the submitted poultry metabolism study indicate that tetraconazole is the predominate residue in poultry tissue, and the highest levels are found in poultry fat. Further, this study indicates the possibility of finite residues of tetraconazole in poultry commodities and the potential for higher residues levels in poultry commodities following dosing at intervals longer than those used in the metabolism study. Therefore, HED cannot conclude that there is no expectation of finite residues of tetraconazole in poultry commodities based on the results of the poultry metabolism study. **The petitioner should submit a poultry feeding study. The need for tolerances for poultry commodities will be determined when an adequate poultry feeding study has been submitted. This is a new deficiency.**

Petitioner's Response

Beef/Dairy Cattle

Based on the newly proposed tolerance level of 3.5 ppm for sugar beet tops, the petitioner has updated the maximum theoretical dietary burden calculation, as shown in Table 8. With this modification, the MTDB is now 3.2 ppm for beef cattle. Thus, the maximum tetraconazole dosing level in the feeding study (3.4 ppm) is adequate to cover the MTDB and no new study is necessary.

Table 8. 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	3.5[b]	23	20	3.043	10	1.522
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	3.160	55	1.639

[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 (D259321, W. Donovan, 18-MAY-2000).

[b] Proposed tolerance level based on maximum seasonal rate of 3 x 0.107 lb ai/A = 0.321 lb ai/A.

Poultry

The petitioner does not agree that a poultry feeding study is needed. Rather than using the recommended tolerance level of 0.05 ppm for peanut nutmeat as the level in the poultry feed item peanut nutmeal, it is proposed to use the average residue level found in the field trials, namely, 0.015 ppm. Justification for this approach is based on the observation that peanut meal which is fed to poultry is not obtained from a single field location. Rather, it is sourced from centralized processing plants that bulk and blend peanuts from may grower's fields at one time in order to extract the oil, thus rendering the meal presscake as a fully blended by-product.

Accordingly, making use of a peanut nutmeal residue level of 0.015 ppm together with a maximum feeding rate of 25%, the petitioner calculates that the poultry metabolism study was conducted at an exaggerated rate of 2667x (i.e., anticipated dietary burden of 0.00375 ppm). Applying this value to the tetraconazole residue levels in poultry commodities gives anticipated tetraconazole residue levels all <0.01 ppm, the method LOQ. Therefore, on this basis, a poultry feeding study should not be necessary.

HED's Conclusion

With the revised tolerance level for sugar beet tops of 3.5 ppm, HED agrees that the previously submitted bovine feeding study is adequate. **This deficiency is now resolved.**

HED concurs with the revised anticipated dietary burden calculation to poultry based on feeding

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peanut nutmeal. Thus, the poultry metabolism study (MRID# 455044-01) was conducted at an exaggerated rate of 2667x. Extrapolating the results obtained at 10 ppm to 10x and 1x the anticipated dietary burden gives the calculated levels shown in Table 9.

Table 9. Extrapolated Tetraconazole Residue Levels in Poultry Commodities Based on Poultry Metabolism Study Results and Anticipated Dietary Burden Calculations.

Poultry Commodity	Tetraconazole Residues (ppm) At Dosing Level Indicated			LOQ (ppm)
	10 ppm (2667x)[a]	0.0375 ppm (10x)[b]	0.00375 ppm (1x)[b]	
Fat	11.031	0.0414	0.00414	0.01
Liver	3.214	0.0121	0.00121	0.01
Muscle	0.531	0.00199	0.000199	0.01
Egg Yolk	1.998	0.00749	0.000749	0.01
Egg White	1.187	0.00445	0.000445	0.01

[a] From Table 11 of Memo, D278236, W. Donovan, 22-OCT-2001.

[b] Calculated levels based on extrapolation of the metabolism results at 10 ppm to the level indicated.

Although all extrapolated tetraconazole residue levels at 1x are calculated to be < LOQ, HED generally requires a feeding study unless metabolism data at a 10x dosing rate results in residues < LOQ for all commodities. This condition has not been met for tetraconazole: at 10x, residues in fat are extrapolated to be 0.041 ppm and those in liver are extrapolated to be 0.012 ppm, both exceeding the LOQ of 0.01 ppm. In addition, results from the poultry metabolism study indicate that TRR levels in egg white and yolk had not reached a plateau within the three days of dosing. Consequently, HED reiterates its request for a poultry feeding study, to be conducted at 1x, 3x, and 10x the anticipated dietary burden of 0.00375 ppm, dosing daily for a minimum of 28 days or until residues plateau in eggs. **This deficiency remains unresolved.**

cc: W. Donovan, D. Vogel
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Figure 1. Proposed Metabolic Pathway of Tetraconazole in Sugar Beet Roots.