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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

DATE: 22-OCT-2001

SUBJECT: PP#s 9F05066, 9F06023, and 7E04830. **Tetraconazole. Additional Data to Amend HED's Residue Data and Analytical Methods Memorandum Concerning Sugar Beets, Bananas, and Peanuts (D267481, W. Donovan, 12-OCT-2000).**
PC Code: 120603. DP Barcodes: D278236, D275535, D272577, D272566, and D270913. Case#s: 292134, 290888. Submission#s: S603683, S597946, S592350, S592248, S588931. MRID#s: 452261-01; 452642-02 thru -05, 452642-07; 452992-01 thru -03; 453188-01 thru -03; 453209-01 & -02; 454194-01; and 455044-01.

FROM: William H. Donovan, Ph.D., Chemist William H. Donovan
Registration Action Branch 1/Health Effects Division (RAB1/HED) (7509C)

THROUGH: G. Jeffrey Herndon, Chemist, Branch Senior Scientist
RAB1/HED (7509C)

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

Sipcam Agro USA, Inc. submitted additional data in response to a memo summarizing deficiencies in the tetraconazole residue chemistry database (D267481, W. Donovan, 12-OCT-2000). 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). The following is HED's review of the additional data and summary of which deficiencies have been resolved and which remain outstanding.

Executive Summary of Chemistry Deficiencies

- Completion of [^{14}C-phenyl] tetraconazole confined rotational crop study (including 365-DAT results and analysis of each RAC crop fraction).
- Analysis of 4.5x treated root samples in [^{14}C-phenyl] tetraconazole sugar beet metabolism study.
- Final Metabolism Assessment Review Committee (MARC) determination of residues of concern in plants, livestock, and rotational crops*.
- Sample storage intervals/storage stability data supporting the goat metabolism study.
- Agency validation of the plant and livestock analytical methods*.
- Independent laboratory validation (ILV) of triazole method for livestock commodities*.
- Multiresidue testing results for triazole*.
- Triazole storage stability data supporting storage intervals of cattle milk and tissue samples*.
- 12 sugar beet crop field trials conducted at new use rate.
- Revised Section F.
- Bovine feeding study with a minimum dose rate equivalent to 6.2 ppm tetraconazole (appropriate dose rate/need for study depends on results of requested sugar beet crop field trial data at new tetraconazole application rate).
- Poultry feeding study.

* Likely to be affected by conclusions about how to regulate free triazole and/or its conjugates. HED is currently in the process of making this determination.

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 1a, 3b, 5, 6b, 8, 9, 10a, 11, 13, and 15, and submit a revised Section F to correct commodity definitions and/or adjust tolerance levels as appropriate (Conclusion 12). 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.

CONCLUSIONS

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

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.**

1b. 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. **This deficiency is now resolved.**

OPPTS GLN 860.1300: Nature of the Residue in Plants

2. The requested information pertaining to sample storage intervals in the [^{14}C-triazole] tetraconazole sugar beet metabolism study has been provided. As the longest sample storage interval was less than 6 months, no supporting storage stability data are required. Thus, the triazole-labeled tetraconazole sugar beet study is considered to be acceptable. **This deficiency is now resolved.**

3a. In view of the fact that short-chain fluorinated alkane compounds are gases and thus not likely to be detected in plant and livestock tissues by conventional analytical techniques, HED retracts its request for the petitioner to re-analyze samples for these chemicals. **This deficiency is now resolved.**

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.**

4. The requested information concerning sample storage intervals in the grape metabolism study has been provided. No grape storage stability data are necessary. **This deficiency**

is now resolved.

OPPTS GLN 860.1300: Nature of the Residue in Livestock

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.**

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

6a. The results of the submitted radiovalidation study are adequate to demonstrate the extraction efficiency of the analytical method for weathered plant commodities. **This deficiency is now resolved.**

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.**

7. Adequate confirmatory methods have been provided for plant and livestock matrices. **This deficiency is now resolved.**

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.**

OPPTS GLN 860.1360: Multiresidue Method

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.**

OPPTS GLN 860.1380: Storage Stability Data

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.**

10b. The petitioner submitted data depicting the stability of tetraconazole residues in/on frozen sugar beet root samples. These results demonstrate that tetraconazole residues are stable for at least three years in/on sugar beet root samples held in frozen storage.

10c. The petitioner submitted storage stability data demonstrating that tetraconazole residues are stable in/on wheat straw and grain samples held in frozen storage for at least three years. In separate studies, the petitioner also demonstrated that the metabolic profile of [^{14}C-phenyl] tetraconazole treated wheat samples changed only slightly over of a period of three years in frozen storage.

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

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.**

OPPTS GLN 860.1480: Meat, Milk, Poultry, Eggs

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.

14. The petitioner has submitted the previously requested poultry metabolism study, including results for ^{14}C labeling in the triazole and phenyl rings. The results will be presented to the HED MARC for determination of the residues of concern in poultry-derived commodities. **This deficiency is now resolved.**

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.**

16. Typically, tolerances are required on all animal commodities having detectable residue levels at a 10x dosing rate or below. The MTDB to poultry based on peanut, meal (0.05 ppm (proposed tolerance level)) contributing a maximum of 25% of the diet is 0.0125 ppm. Based on this, the poultry metabolism study was conducted at an exaggerated rate of 800x. The stated LOQ for the GC/ECD method used in the bovine feeding study was 0.01 ppm for livestock commodities. Assuming that this LOQ also applies to poultry commodities, tolerance levels should be proposed for all poultry commodities having tetraconazole residues above 0.01 ppm from a poultry feeding study at a dosing rate of 10x or below. Thus, the petitioner should conduct the requested poultry feeding study at 1x, 3x, and 10x, indicating the LOD and LOQ of the analytical method employed in the analysis.

DETAILED CONSIDERATIONS

1. Proposed Uses and Rotational Crops.

Deficiency - Conclusion 1 from Memo, D267481, W. Donovan, 12-OCT-2000:

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, 18-MAY-2000), a revised Section B is required to incorporate the following restriction: "Sugar beets may be rotated at any time. Rotation to all other crops is prohibited." Rotation to peanuts may be allowed once the database supporting peanut use is complete and found satisfactory. **This deficiency remains unresolved.**

Deficiency - Conclusion 15 from Memo, D267481, W. Donovan, 12-OCT-2000:

15. The submitted interim report demonstrates that tetraconazole residue levels in carrot and lettuce are in excess of 0.01 ppm following a 223-day plant-back interval (PBI). Conclusions regarding the appropriate PBIs will be made upon review of the completed [^{14}C-phenyl] tetraconazole confined rotational crop study, which should include residue levels following a 365-day PBI and appropriate information concerning the storage intervals of analyzed samples. 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). Storage stability data to validate the storage condition and intervals of samples from the triazole-labeled rotational crop study are still required. **This deficiency remains unresolved.**

Petitioner's Response

MRID#s 453209-01 & -02, describing the results of field rotational crop studies.

453209-01 G. Zini (2000) Tetraconazole residues in rotational crops after treatment of bare soil: Study Number: GZ0100. Unpublished study prepared by Isagro Ricerca Srl. 661 p.

453209-02 G. Zini (2000) Tetraconazole residues in rotational crops following three years of consecutive applications to primary treated crops: Study Number: GZ0200. Unpublished study prepared by Isagro Ricerca Srl. 1161 p.

MRID# 453209-01 details the results of a conventional field rotational crop study, where direct applications of tetraconazole were made to bare soil, at application rates of 0.112, 0.223, 0.669, and 1.34 lb ai/A in separate plots. The field phase of the study was conducted at three different sites in England. Rotational crops (spring wheat, peas, potatoes, oilseed rape and sugar beets) were sown into the treated soil 7-9 days following tetraconazole application. The rotational crops were harvested at maturity and then analyzed for tetraconazole residues.

The three field trial locations were identified as Spalding in Lincolnshire, Hemington, and Melbourne, both in Derbyshire. The PBI at Spalding was 7 days, while the PBIs at Hemington and Melbourne were 9 days. The applied formulation was Domark® 10EC, containing tetraconazole at a nominal concentration of 100 grams per liter. The test substance was diluted

into water, then applied in a broadcast pattern over the soil in a spray volume of 250 L/ha.

Crops were harvested between September and October 1990. All sample analyses were completed by May 1992. Thus, the longest sample storage interval was 21 months.

Tetraconazole residues obtained from the different rotational crop RACs are summarized in Table 1. The results from the lowest two application rates are not included as residues were mostly not detected.

Table 1. Tetraconazole residue levels found from field rotational crop studies conducted in England.

RAC	Spalding		Hemington		Melbourne	
	0.67 lb ai/A (2x)	1.34 lb ai/A (4x)	0.67 lb ai/A (2x)	1.34 lb ai/A (4x)	0.67 lb ai/A (2x)	1.34 lb ai/A (4x)
Spring wheat, grain	ND	ND	ND	ND	ND	ND
Spring wheat, straw	<0.02	<0.02	0.02	0.03	0.05	<0.02
Peas, seeds	ND	ND	ND	ND	ND	ND
Potatoes	ND	ND	ND	ND	ND	ND
Oilseed rape	ND	ND	<0.02	ND	ND	ND
Sugar beet, roots	ND	ND	ND	ND	ND	ND
Sugar beet, leaves	ND	<0.02	<0.02	ND	ND	<0.02

Each value represents the mean of the samples obtained from two replicate treated plots, with the exception of spring wheat from Hemington, where only one treated plot was prepared.
ND = not detected, i.e., below the limit of detection (LOD) of 0.01 ppm.
Values indicated as <0.02 indicates that tetraconazole was detected but below the LOQ of 0.02 ppm.

Residues of tetraconazole are not likely to be present in rotated crops planted into treated bare soil even approximately one week following normal rates of application, with the possible exception of wheat straw. Furthermore, tetraconazole residues will not likely be present in the edible portion of rotated crops following 2x to 4x higher than maximum label application rates onto bare soil. The small amounts of tetraconazole found occasionally in some samples (spring wheat straw, sugar beet leaves) are probably due to contamination of soil particles on the vegetal substrates.

MRID# 453209-02 details a supplementary rotational crop study, which presents the results of tetraconazole residue levels found in rotational crops following three years of consecutive applications to primary treated crops. This study was also carried out in England. In this study,

the PBIs ranged from 76 - 328 days. In all rotational crop samples analyzed, tetraconazole residue levels were below the LOD of 0.01 ppm.

In addition, the petitioner also submitted information pertaining to sample storage intervals in the triazole- and phenyl-labeled confined rotational crop studies in MRID#s 452642-04 and -05, respectively. This information is summarized in Table 2.

> 452642-04 G. Pizzingrilli and F. Rizzo (2000) Uptake, Translocation and Metabolism of [^{14}C-triazole] Tetraconazole in Rotated Crops of Winter Wheat, Carrots, and Lettuce: Study Number: R/ABT.96.05 Addendum to MRID# 448654-07. Unpublished study prepared by Isagro Ricerca Srl. 4 p.

> 452642-05 G. Pizzingrilli and F. Rizzo (2000) Uptake, Translocation and Metabolism of [^{14}C-phenyl] Tetraconazole in Rotated Crops of Cereals, Carrots, and Lettuce: Study Number: R/ABT.98.11 Addendum to MRID# 451550-05. Unpublished study prepared by Isagro Ricerca Srl. 4 p.

Table 2. Sample storage intervals involved in the triazole- and phenyl-labeled confined rotational crop studies.

Matrix	Triazole-label study			Phenyl-label study		
	Harvest	Last analysis	Max. storage interval	Harvest	Last analysis	Max storage interval
Lettuce (30, 120 days)	8/21/97	3/31/98	7 months	7/12/99	9/30/99	3 months
Lettuce (223 days)	NA	NA	NA	7/20/99	9/30/99	2 months
Lettuce (365 days)	7/22/98	8/31/98	1 month	6/6/00	7/31/00	2 months
Carrots (30, 120 days)	8/21/97	3/31/98	7 months	7/30/99	11/30/99	4 months
Carrots (223 days)	NA	NA	NA	9/1/99	9/30/99	1 month
Carrots (365 days)	7/22/98	8/31/98	1 month	7/25/00	9/30/00	2 months
Wheat grain/straw (30, 120, 365 days)	4/30/98	6/30/98	2 months	NA	NA	NA

Matrix	Triazole-label study			Phenyl-label study		
	Harvest	Last analysis	Max. storage interval	Harvest	Last analysis	Max storage interval
Wheat grain/straw (30, 120 days)	NA	NA	NA	7/3/00	9/30/00	3 months
Wheat forage (30, 120, 365 days)	4/30/98	6/30/98	2 months	NA	NA	NA
Wheat forage 30, 120 days)	NA	NA	NA	5/15/00	9/30/00	5 months
Sorghum (223 days)	NA	NA	NA	10/27/99	11/30/99	1 month

Thus, the longest sample storage interval was approximately 7 months.

HED's Conclusion

Adequate field accumulation in rotational crop studies for parent tetraconazole have been submitted. However, conclusions regarding the appropriate 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.**

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. **This deficiency is now resolved.**

2. Sample Storage Intervals in the Sugar Beet Metabolism Study.

Deficiency - Conclusion 2a from Memo, D267481, W. Donovan, 12-OCT-2000:

2a. The original sugar beet metabolism study involved three applications of triazole-labeled tetraconazole at a rate of 0.089 lb ai/A, for a total rate of 0.27 lb ai/A (0.9X the revised label rate). Pending receipt of information pertaining to sample storage intervals (from harvest to the final TLC analyses of extracts), HED considers the triazole-labeled tetraconazole sugar beet study to be acceptable. **This deficiency remains unresolved.**

Petitioner's Response

MRID# 452642-01, providing information about sample storage intervals in the [^{14}C-triazole] tetraconazole sugar beet metabolism study.

> 452642-01 G. Pizzingrilli and F. Rizzo (2000) Metabolism of [^{14}C-triazole] Tetraconazole in Sugar Beet: Supplemental Information on Sample Storage Intervals: Study Number: R/ABT.95.06 Addendum to MRID# 447513-11. Unpublished study prepared by Isagro Ricerca Srl. 4 p.

Sugar beet roots and leaves were harvested in September, 1995. No TLC analysis was performed for sugar beet roots as the TRR level was <0.01 ppm. Sugar beet leaves were analyzed between September and December 1995. Thus, the longest storage interval was 4 months (9/1/1995 - 12/31/1995).

The petitioner also submitted additional information pertaining to the stability of tetraconazole residues on sugar beet roots in frozen storage in the following document:

> 452992-01 G. Zini (2000) Stability of tetraconazole in sugar beet roots stored frozen for three years: Study Number: 1998. Unpublished study prepared by Isagro Ricerca Srl. 48 p.

In this study, samples of sugar beet roots were fortified separately with either 0.103 or 0.310 ppm tetraconazole and maintained in frozen storage at approximately -20°C. At various time intervals, up to approximately three years, samples were analyzed for tetraconazole residue levels. The results are summarized in Table 3.

Table 3. Storage stability results for sugar beet roots fortified with tetraconazole and stored at -20°C.

Fortification Level (ppm)	Days Stored	% Recovery
0.103	0	101.6
0.310	0	99.7
0.103	32	98.6
0.310	32	103.7
0.103	60	117.8
0.310	60	102.7
0.103	94	103.7
0.310	94	99.2
0.103	181	102.0
0.310	181	95.7

Fortification Level (ppm)	Days Stored	% Recovery
0.103	387	119.4
0.310	387	109.8
0.103	849	87.4
0.310	849	93.4
0.103	1192	103.0
0.310	1192	100.3

These results demonstrate that tetraconazole residues are stable on sugar beet root samples for at least three years in frozen storage.

HED's Conclusion

The requested information pertaining to sample storage intervals in the [^{14}C-triazole] tetraconazole sugar beet metabolism study has been provided. As the longest sample storage interval was less than 6 months, no supporting storage stability data are required. Thus, the triazole-labeled tetraconazole sugar beet study is considered to be acceptable. **This deficiency is now resolved.**

The petitioner submitted data depicting the stability of tetraconazole residues in/on frozen sugar beet root samples. These results demonstrate that tetraconazole residues are stable for at least three years in/on sugar beet root samples held in frozen storage.

3. Analysis of the Tetrafluoroethyl Group in Plant and Livestock Commodities.

Deficiency - Conclusion 2b from Memo, D267481, W. Donovan, 12-OCT-2000:

2b. The HED Metabolism Assessment Review Committee (MARC) previously expressed concern about the fate of the tetrafluoroethyl group because of the possibility that fluorinated alkane compounds may contribute to the kidney histopathological changes observed in the dog chronic oral feeding study that was used to establish a chronic reference dose for tetraconazole. In light of the synthetic challenges involved with the preparation of [^{14}C-tetrafluoroethyl] tetraconazole, HED will not require completion of this aspect of the study. Instead, HED recommends that the petitioner attempt to analyze plant and livestock commodity samples from the metabolism studies for the presence of short-chain fluorinated alkane compounds using a suitably sensitive analytical method such as mass spectrometry. HED seeks data that would establish an upper limit to the quantity of fluorinated alkanes formed during tetraconazole metabolism in plant (primary and rotational crops) and livestock commodities. Thus, analysis of samples containing the highest levels of radioactivity would be appropriate. Conclusions regarding the nature of the residue in sugar beets will be deferred until completion of the phenyl-labeled sugar beet metabolism study, and submission of information regarding the levels of fluorinated alkane compounds. **This deficiency is partially resolved.**

Petitioner's Response

452642-06 G. Camaggi (2000) Comments on the Likely Metabolic Fate of the Tetrafluoroethyl Group of Tetraconazole: Document Number: SA-JRF-00-03. Unpublished study prepared by Isagro Ricerca Srl. 7 p.

This document provides information supporting the view that formation of short-chain alkanes during tetraconazole metabolism is not expected based on what is known about tetraconazole chemistry. Further, even if such compounds were formed, their detection would be nearly impossible based on the fact that, at room temperatures, fluoroethanes are gases whose boiling points range between -50° and +10°C.

In order to address the deficiency regarding the phenyl-labeled sugar beet metabolism study, the petitioner submitted the following document:

454194-01 F. Rizzo and G. Pizzingrilli (2001) Metabolism of [^{14}C-phenyl]Tetraconazole in Sugar Beet: Study Number ABT.00.09. Unpublished study prepared by Isagro Ricerca Srl. 198 p.

This report described the results of a sugar beet metabolism study when treating sugar beets three times with a 28-day retreatment interval (RTI) at a rate of 100 g/ha (seasonal rate of 0.267 lb ai/A, 0.9x) or three times at 500 g/ha (1.34 lb ai/A, 4.5x), with the final application being made on 8/22/00. The preharvest interval (PHI) was 23 days as samples were harvested on 9/14/00. No sample analysis dates were provided. However, the experimental end date was given as 4/3/01. Thus, the maximum sample storage interval was approximately 6 months. LSC analysis after combustion of sugar beet samples gave the TRR values given in Table 4.

Table 4. TRR results for sugar beet RACs.

RAC	Treatment Rate	TRR (ppm)
Sugar beet leaves	0.9x	5.034
	4.5x	26.910
Sugar beet roots	0.9x	0.0073
	4.5x	Not analyzed

Identification/characterization was carried out on the sugar beet leaf samples that were treated at 0.9x. The results of this process are summarized in Table 5.

Table 5. Identification of residues in sugar beet leaf samples treated at 0.9x.

Identified Compound	ppm	% TRR
Tetraconazole	3.567	70.86
M14360(C-1)-alcohol	0.005	0.10

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Identified Compound	ppm	% TRR
M14360-alcohol	0.021	0.42
M14360-acid	0.013	0.26
M14360-DFA	0.180	3.58
M14360-alcohol-O-glucoside	0.187	3.71
M14360-alcohol-O-diglucoside	0.081	1.61
M14360-alcohol-O-malonyldiglucoside	0.514	10.21
M14360-hydroxydetriazolyl-O-malonyldiglucoside	0.175	3.48
Total identified	4.743	94.23

HED's Conclusion

In view of the fact that short-chain fluorinated alkane compounds are gases and thus not likely to be detected in plant and livestock tissues by conventional analytical techniques, HED retracts its request for the petitioner to re-analyze samples for these chemicals. **This deficiency is now resolved.**

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.**

4. Sample storage information/storage stability data supporting the grape metabolism study.

Deficiency - Conclusion 4 from Memo, D267481, W. Donovan, 12-OCT-2000:

4. Pending submission of information pertaining to sample storage intervals (from harvest to the final TLC analyses of extracts) and, if necessary, storage stability data, the grape metabolism studies are adequate. **This deficiency remains unresolved.**

Petitioner's Response

452642-02 F. G. Pizzingrilli and Rizzo (2000) [^{14}C-triazole] M 14360 Metabolism in Grapes and Wine (Main Study): Supplemental Information on Sample Storage Intervals: Document Number R/ABT.91.05 Addendum to MRID# 442681-07. Unpublished study prepared by Isagro Ricerca Srl. 4 p.

(14)

452642-02 F. G. Pizzingrilli and Rizzo (2000) [^{14}C-phenyl] M 14360 Metabolism in Grapes and Wine (Main Study): Supplemental Information on Sample Storage Intervals: Document Number R/ABT.91.06 Addendum to MRID# 442681-08. Unpublished study prepared by Isagro Ricerca Srl. 4 p.

MRID#s 452642-02 & 452642-03 provided the harvest and analysis dates for the grape metabolism studies conducted with triazole- and phenyl-labeled tetraconazole, respectively. The maximum sample storage interval involved in the both studies was 1 month.

HED's Conclusion
The requested information concerning sample storage intervals in the grape metabolism study has been provided. No grape storage stability data are necessary. **This deficiency is now resolved.**

5. Sample storage information/storage stability data supporting the goat metabolism study.

Deficiency - Conclusion 5 from Memo, D267481, W. Donovan, 12-OCT-2000:

5. 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. **This deficiency remains unresolved.**

Petitioner's Response
None.

HED's Conclusion
This deficiency remains unresolved.

6. Residue Methods Radiovalidation and Agency Validation of Analytical Methods.

Deficiency - Conclusion 6 from Memo, D267481, W. Donovan, 12-OCT-2000:

6. The results of the submitted radiovalidation study are adequate to demonstrate the extraction efficiency of the analytical method for weathered livestock commodities. Radiovalidation of the proposed analytical enforcement method for plant matrices is still required. Also, Agency validation of the analytical methods has not been completed yet. **Thus, this deficiency is partially resolved.**

Petitioner's Response
The petitioner submitted MRID# 452261-01, describing a radiovalidation study of tetraconazole residues in wheat.

452261-01 F. Rizzo and G. Pizzingrilli. (2000) Tetraconazole: Radiovalidation of Residue Analytical Method in Wheat: Study Number ABT.00.10. Unpublished study prepared by Isagro Ricerca, 84 p.

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In addition, the petitioner submitted MRID# 452642-07, an addendum to the wheat radiovalidation study, reporting storage stability information.

> 452642-07 F. Rizzo and G. Pizzingrilli. (2000) Addendum to the study "Tetraconazole: Radiovalidation of Residue Analytical Method in Wheat" [Storage Stability of Extractable TRR in Wheat Straw]: Study Number Addendum R/ABT.00.10. Unpublished study prepared by Isagro Ricerca, 26 p.

In these studies, the extraction efficiency of the analytical method was demonstrated by comparing the amount of tetraconazole found in wheat grain and straw samples that had been treated with labeled tetraconazole in the wheat metabolism study reported in MRID# 451550-01 to the tetraconazole residue levels found by the analytical method. Table 6 summarizes the results obtained.

Table 6. Radiovalidation results obtained using samples from the wheat metabolism study.

Sample	Tetraconazole label position	Analysis Method	Tetraconazole (ppm)	% recovery (residue method/metabolism)
Wheat grain	triazole	metabolism	0.105	69.5
		residue	0.073	
Wheat grain	phenyl	metabolism	0.088	69.3
		residue	0.061	
Wheat straw	triazole	metabolism	6.756	75.6
		residue	5.104	
Wheat straw	phenyl	metabolism	6.971	82.7
		residue	5.763	

To demonstrate the stability of tetraconazole residues on wheat straw samples in frozen storage, the petitioner re-analyzed in November, 2000 a sample originally analyzed in October, 1997. This exercise demonstrated that the profile of extractable TRR of wheat straw samples changed only slightly over a period of three years. Table 7 provides a comparison of the re-analyzed samples.

Table 7. Storage stability demonstration of [^{14}C-phenyl] tetraconazole treated wheat straw samples.

Main Species Identified	10/1997 Analysis (ppm)	11/2000 Analysis (ppm)
Tetraconazole, S-4	7.849	7.635
M14360-DCP-3OH, S-5	0.124	0.128
M14360-DCP-5OH, S-6	0.102	0.099
M14360-alcohol, S-8	0.056	0.048
unknown, S-27	0.123	0.098
M14360-DFA, S-29	0.033	0.026
M14360-acid, S-30	0.024	0.019
unknown, S-38	0.094	0.099
M14360-CP-(C-1)alcohol, S-45	0.322	0.468
M14360-ketone, S-53	0.043	0.010
M14360(C-1)-alcohol, S-55	0.015	0.017

Further information regarding the stability of tetraconazole residues in wheat grain and straw in frozen storage was provided in the following two volumes:

452992-02 G. Zini (2000) Stability of tetraconazole in wheat grain stored frozen for three years: Study Number: 2022. Unpublished study prepared by Isagro Ricerca Srl. 50 p.

452992-03 G. Zini (2000) Stability of tetraconazole in wheat straw stored frozen for three years: Study Number: 2023. Unpublished study prepared by Isagro Ricerca Srl. 52 p.

The results of these studies are summarized in Table 8.

Table 8. Tetraconazole recoveries from wheat grain and straw storage stability samples at various storage intervals.

RAC	Recoveries (%) at Indicated Storage Interval (days)							
	0	28	71	103	242	389	733	1076
0.070 ppm wheat grain	99.3	105.0	95.2	98.6	97.1	94.1	99.3	108.6
0.697 ppm wheat grain	100.4	96.1	95.0	100.0	98.1	93.5	93.3	104.2
0.070 ppm wheat straw	100.0	148.1	111.5	125.0	154.8	98.1	96.1	183.3

RAC	Recoveries (%) at Indicated Storage Interval (days)							
	0	28	71	103	242	389	733	1076
0.697 ppm wheat straw	100.5	103.2	96.9	93.9	101.7	103.5	97.7	97.2

The high recoveries found in the 0.070 ppm fortified wheat straw samples was attributed to an interfering peak from the sample matrix. This peak was relatively small and did not affect the results for the wheat straw samples fortified at 0.7 ppm. The results demonstrate stability of tetraconazole residues in wheat grain and straw samples for at least three years in frozen storage.

HED's Conclusion
The results of the submitted radiovalidation study are adequate to demonstrate the extraction efficiency of the analytical method for weathered plant commodities.. **This deficiency is now resolved.**

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.**

The petitioner submitted storage stability data demonstrating that tetraconazole residues are stable in/on wheat straw and grain samples held in frozen storage for at least three years. In separate studies. the petitioner also demonstrated that the metabolic profile of [^{14}C-phenyl] tetraconazole treated wheat samples changed only slightly over of a period of three years in frozen storage.

7. Confirmatory Method

Deficiency - Conclusion 7 from Memo, D267481, W. Donovan, 12-OCT-2000:

7. The proposed analytical enforcement methods should be supplemented by confirmatory methods for plants and livestock that are significantly different (such as mass spectrometry (MS)). If the petitioner proposes confirmatory methods which employ MS, then an interference study is not necessary (chromatograms and spectra of fortified samples should be submitted along with the limit of quantitation (LOQ)). **This deficiency remains unresolved.**

Petitioner's Response
MRID#s 453188-01 & -02.

453188-01 G. Zini. (2001) Confirmatory method for residue of tetraconazole in sugar beet (roots and leaves): Study Identification Code: 2347. Unpublished study prepared by Isagro Ricerca, 84 p.

In this study, five samples each of sugar beet roots and leaves were separately fortified with tetraconazole at 0.02 and 0.20 ppm. Recoveries were determined using the proposed analytical method (gas chromatography with electron capture detection (GC/ECD)), and confirmed using gas chromatography with mass spectrometry (GC/MS). Mean recoveries ranged from 91 - 103% in all fortified samples analyzed. Successful MS confirmation was achieved using ions at m/z 101, 171, 336, and 338. No interferences were observed in either the GC/ECD or in the GC/MS analysis.

> 453188-02 R.D. Weeren and S. Pelz. (1999) Validation of DFG Methods S 19 with Modified Extraction for the Determination of the Residues of Tetraconazole in Plant Material and in Foodstuffs of Animal Origin: Study Identification Code: TET-9801V Az. 64641/98. Unpublished study sponsored by TETRACO S.r.l., 59 p.

In this study, samples of apples, grapes, tomatoes, sugar beets, wheat grain and straw, milk, meat, and egg were fortified at various levels ranging from 0.01 to 3.0 ppm, extracted using DFG Method S 19 with modified extraction, and analyzed by GC/MS. Quantitation was accomplished using ion m/z 336 and verified using ions m/z 337 and 338. Mean recoveries ranged from 75 - 106%, depending on the matrix analyzed and the level of fortification. No significant interferences from the sample matrices were detected at the retention time corresponding to tetraconazole in any of the samples analyzed.

HED's Conclusion
Adequate confirmatory methods have been provided for plant and livestock matrices. **This deficiency is now resolved.**

8. Independent Laboratory Validation of Triazole Method for Livestock Commodities

Deficiency - Conclusion 8 from Memo, D267481, W. Donovan, 12-OCT-2000:

> 8. 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 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 PMV. **This deficiency remains unresolved.**

Petitioner's Response
None.

HED's Conclusion
This deficiency remains unresolved.

9. Multiresidue Method

Deficiency - Conclusion 9 from Memo, D267481, W. Donovan, 12-OCT-2000:

9. 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. **This deficiency remains unresolved.**

Petitioner's Response

453188-03 K.H. Martin and W.B. Nixon. (2001) Assessment of Multiresidue Methodology as Presented in Pesticide Analytical Manual (PAM), Volume 1, for the Determination of Tetraconazole in Nonfatty and Fatty Plant Substrates: Project No. 468C-107. Unpublished study sponsored by Sipcam Agro USA, Inc. and conducted by Wildlife International, Ltd., 41 p.

Tetraconazole was evaluated by gas chromatography (GC) on a ZB-1 (100% methyl siloxane) column with electron capture detection (ECD). The tetraconazole retention time relative to chlorpyrifos was 1.017. 50% full scale deflection (FSD) was obtained using 0.15 and 0.21 ng of chlorpyrifos and tetraconazole, respectively. Sugar beets were fortified at 0.05 and 0.10 ppm. The procedural recoveries at 0.05 ppm were 83 and 84%, and at 0.10 ppm were 81 and 85%. However, an interfering peak was present. As required by Protocol D, a florisil clean up is necessary for compounds analyzed by ECD and matrices that produce an interfering peak at the characteristic retention time of the analyte. Low recovery of tetraconazole through florisil (<10%) precluded the possibility of a cleanup for sugar beet samples. Peanuts were fortified at 0.03 and 0.05 ppm. The fat content of the peanuts and the inability for a cleanup made it impossible for sample analysis. It is suggested that another solid support system, such as alumina, may be more amenable to recovery of tetraconazole from all matrices. However, this method is currently not encompassed by PAM Volume I.

HED's Conclusion

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.**

10. Triazole Storage Stability Data Supporting Storage Intervals of Cattle Milk and Tissue Samples.

Deficiency - Conclusion 10 from Memo, D267481, W. Donovan, 12-OCT-2000:

10. 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
None.

HED's Conclusion
This deficiency remains unresolved.

11. Crop Field Trials.

Deficiency - Conclusion 11 from Memo, D267481, W. Donovan, 12-OCT-2000:

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 0.10 lb ai/A with a 14 day PHI and a 14 day RTI. **This is a new deficiency.**

Petitioner's Response
None.

HED's Conclusion
This deficiency remains unresolved.

12. Revised Section F.

Deficiency - Conclusion 12 from Memo, D267481, W. Donovan, 12-OCT-2000:

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
None.

HED's Conclusion
This deficiency remains unresolved.

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13. Bovine Feeding Study.

Deficiency - Conclusion 13 from Memo, D267481, W. Donovan, 12-OCT-2000:

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.

Petitioner's Response
None.

HED's Conclusion
This deficiency remains unresolved.

14. Poultry Metabolism Study

Deficiency - Conclusion 14 from Memo, D267481, W. Donovan, 12-OCT-2000:

14. **The poultry metabolism study deficiency remains unresolved.** However, the sugar beet and banana petitions may proceed without the results of the poultry metabolism study.

Petitioner's Response

455044-01 G. Pizzingrilli, F. Castoldi, and D. Oriolo. (2001) The metabolism of [14C-U-triazolyl] Tetraconazole and [14C-U-phenyl]Tetraconazole in the laying hen: analytical phase. Study No. MEF.00.18. Unpublished study by ISAGRO RICERCA. Srl., 154 p.

A livestock metabolism study was carried out in laying hens treated orally with [^{14}C-U-triazolyl] Tetraconazole (specific activity 137.17 μCi/mg, radiochemical purity >97%) or [^{14}C-U-phenyl] Tetraconazole (specific activity 196.15 μCi/mg, radiochemical purity >97%) for 3 consecutive days at a dose of approximately 10 ppm in the diet. Two groups comprised of six hens each were treated separately with labeled tetraconazole. The in-life phase was performed by PTRL East, Inc. in Richmond, KY while the analytical-phase was performed by Isagro Ricerca Srl in Novara, Italy.

Liver, muscle, and eggs were extracted with acetonitrile and acetone, while fat was extracted with n-hexane, acetonitrile and acetone. All the extracts were analyzed for radioactivity content by LSC and dried residues were oxidized to determine unextractable radioactivity. Table 9 presents the averaged TRR levels found in poultry matrices when the hens were treated separately with triazole- and phenyl-labeled tetraconazole.

Table 9. TRR levels found in poultry tissues following three consecutive days of dosing at 10 ppm (800x).

Matrix	TRR	
	Triazolyl label (ppm)	Phenyl label (ppm)
Liver	3.518	3.560
Muscle	0.599	0.532
Fat	11.612	11.293
Egg yolk: 0 - 24 hrs.	0.092	0.048
Egg yolk: 24 - 48 hrs.	0.610	0.501
Egg yolk: 48 hrs - sacrifice	2.253	1.882
Egg white: 0 - 24 hrs.	0.306	0.268
Egg white: 24 - 48 hrs.	1.025	0.877
Egg white: 48 hrs. - sacrifice	1.297	1.191

The egg results presented in Tables 10 and 11 give the highest residue levels found (samples taken between 48 hours after starting tetraconazole dosing and hen sacrifice). Comparison of TLC radiochromatograms from ^{14}C-reference standards and those from the extracts allowed identification of the TRR components. Confirmation was performed by LC/MS.

Table 10. Identification/characterization of residues found in poultry metabolism study using [^{14}C-triazolyl] tetraconazole. Data are expressed in terms of tetraconazole equivalents.

Compound Identified/ Fraction Characterized	ppm (%TRR)				
	Liver	Muscle	Fat	Egg yolk	Egg white
Tetraconazole	3.214 (91.36)	0.531 (88.73)	11.031 (95.00)	1.998 (88.67)	1.187 (91.51)
M14360-DCP-3OH	0.042 (1.19)	0.006 (0.95)	ND[a]	ND	ND
Triazole	0.051 (1.44)	0.030 (5.03)	ND	0.056 (2.48)	0.042 (3.25)
M14360-DFA	0.021 (0.61)	0.004 (0.61)	ND	0.021 (0.92)	ND
Unknown (water-soluble)	0.018 (0.51)[b]	NA[c]	NA	0.044 (1.97)[d]	NA
Hydrolyzed by protease	0.028 (0.80)	NA	NA	0.027 (1.20)	NA
Bound	0.045 (1.28)	0.017 (2.84)	0.001 (0.01)	0.031 (1.38)	0.005 (0.39)

[a] Not detected.
[b] At least 5 compounds.
[c] Not applicable.
[d] At least 4 compounds.

Table 11. Identification/characterization of residues found in poultry metabolism study using [^{14}C-phenyl] tetraconazole. Data are expressed in terms of tetraconazole equivalents.

Compound	ppm (%TRR)				
	Liver	Muscle	Fat	Egg yolk	Egg white
Tetraconazole	3.438 (96.58)	0.536 (100.74)	11.855 (104.98)	1.817 (96.56)	1.242 (104.28)
M14360-DCP-3OH	0.047 (1.33)	0.003 (0.62)	ND[a]	ND	ND
M14360-DFA	0.021 (0.60)	0.004 (0.69)	ND	0.035 (1.85)	ND
Unknown (water-soluble)	0.019 (0.53)[b]	NA[c]	NA	0.043 (2.28)[d]	NA
Hydrolyzed by protease	0.040 (1.12)	NA	NA	0.036 (1.91)	NA
Bound	0.111 (3.12)	0.007 (1.32)	0.003 (0.03)	0.059 (3.13)	0.005 (0.42)

[a] Not detected.
[b] At least 5 compounds.
[c] Not applicable.
[d] At least 4 compounds.

The results shown in Tables 9-11 indicate that parent tetraconazole was the main radioactive component found in poultry tissues. Other compounds identified were also found in previous plant and livestock metabolism studies and were present in low amounts.

HED's Conclusion

The petitioner has submitted the previously requested poultry metabolism study, including results for ^{14}C labeling in the triazole and phenyl rings. The results will be presented to the HED MARC for determination of the residues of concern in poultry-derived commodities. **This deficiency is now resolved.**

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.**

Typically, tolerances are required on all livestock commodities having detectable residue levels at a 10x dosing rate or below. The MTDB to poultry based on peanut, meal (0.05 ppm (proposed tolerance level)) contributing a maximum of 25% of the diet is 0.0125 ppm. Based on this, the poultry metabolism study was conducted at an exaggerated rate of 800x. The stated LOQ for the GC/ECD method used in the bovine feeding study was 0.01 ppm for livestock commodities. Assuming that this LOQ also applies to poultry commodities, tolerance levels should be proposed for all poultry commodities having tetraconazole residues at or above 0.01 ppm from a poultry feeding study at a dosing rate of 10x or below. Thus, the petitioner should conduct the requested poultry feeding study at 1x, 3x, and 10x, indicating the LOD and LOQ of the analytical method employed in the analysis.

cc: W. Donovan, D. Vogel
RDI: G. Herndon (16-OCT-2001), RAB1 Chemists (18-OCT-2001)
W. Donovan:806R:CM#2:(703)-305-7330:MC 7509C

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