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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

DATE: 12-OCT-2000

SUBJECT: PP# 9F05066. **Tetraconazole. Additional Data to Amend HED's Residue Data and Analytical Methods Memoranda Concerning Sugar Beets, Bananas, and Peanuts (D254411, W. Donovan, 18-MAY-2000; D259205, W. Donovan, 18-MAY-2000; D259321, W. Donovan, 18-MAY-2000).** PC Code: 120603. DP Barcode: D267481. Case#: 290888. Submission#: S582336. MRID#s: 451550-01, 451550-03 thru 451550-06.

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Sipcam Agro USA, Inc. submitted additional tetraconazole-related data in response to memos identifying deficiencies in the residue chemistry database (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.

At the petitioner's request, the peanut petition (PP# 9F06023) has been separated from the sugar beet and banana petitions until completion of the poultry metabolism study. Thus, the proposed tolerance levels 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], are as follows:

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
Banana	0.2 ppm

Executive Summary of Chemistry Deficiencies

- Revised Section B.
- Sample storage information/storage stability data supporting the sugar beet and goat nature of the residue studies.
- [^{14}C-phenyl] tetraconazole sugar beet metabolism study.
- Fluorinated alkane analysis of plant and livestock metabolism samples.
- Storage stability data supporting the grape metabolism study.
- Final identification of residues of concern in plants, livestock, and rotational crops.
- Radiovalidation of proposed analytical enforcement method for plant matrices.
- Agency validation of the plant and livestock analytical methods.
- Multiresidue testing results.
- Confirmatory method for plants and livestock.
- Independent laboratory validation (ILV) of triazole method for livestock commodities*
- 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 metabolism study.
- Completed [^{14}C-phenyl] tetraconazole confined rotational crop study (with 365-DAT results and sample storage information/storage stability data).

* Contingent on conclusions about the toxicity of free triazole.

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 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, 4, 5, 6, 7, 8, 9,10, 11, 13, 14, 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 and bananas when the above deficiencies have been resolved.

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, 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.**

OPPTS GLN 860.1300: Nature of the Residue in Plants

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.**

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.**

3a. The additional identification and characterization of wheat grain and straw metabolites appears to be satisfactory. **Pending concurrence of the HED MARC, this deficiency is now resolved.**

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3b. Once the remaining metabolism-related deficiencies have been adequately addressed by the petitioner, the HED MARC will be consulted for confirmation of the previous tentative decisions regarding residues of concern (D264157, W. Donovan and D. Nixon, 19-APR-2000) and suitability of the metabolism database for tetraconazole.

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.**

OPPTS GLN 860.1300: Nature of the Residue in Livestock

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.**

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

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.**

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.**

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. 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.

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This deficiency remains unresolved.

OPPTS GLN 860.1380: Storage Stability Data

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.**

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 is a new deficiency.**

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

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

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.**

DETAILED CONSIDERATIONS

1. Proposed Uses.

Deficiency - Conclusion 1 from Memo, D254411, W. Donovan, 18-MAY-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, 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."

Petitioner's Response
None.

HED's Conclusion
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.**

2. Metabolism of Tetraconazole in Sugar Beets.

Deficiency - Conclusion 2a from Memo, D254411, W. Donovan, 18-MAY-2000:

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.

Petitioner's Response

The petitioner reduced the maximum seasonal tetraconazole application rate on the Eminent 125SL label to 0.3 lb ai/A, consisting of up to 3 individual applications at a rate of 0.1 lb ai/A, approximately corresponding to the rate used in the original sugar beet metabolism study. In addition, they submitted a protocol for a sugar beet metabolism study using ^{14}C-phenyl tetraconazole that was considered acceptable by HED (D267097, W. Donovan, 27-JUL-2000). Moreover, they submitted information (MRID # 451550-06) concerning the synthetic feasibility of [^{14}C-tetrafluoroethyl] tetraconazole, concluding that this compound cannot be synthesized in any practical manner.

HED's Conclusion

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.**

The 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.**

3. Wheat Metabolism Study

Deficiency - Conclusion 3 from Memo, D259205, W. Donovan, 18-MAY-2000:

3. The available wheat metabolism studies are not adequate to support the present petition because of the failure to characterize and/or identify the residue, and because of a lack of information pertaining to sample storage intervals. The petitioner should submit a new wheat metabolism study employing an exaggerated application rate to ensure sufficiently high radioactivity levels to allow for characterization and/or identification of the residue. The petitioner is encouraged to consult Figure 2 (Characterization/Identification of Unextractable/Bound Residues) of OPPTS 860.1300 for guidance. In future metabolism study submissions, the petitioner should include all pertinent raw data as detailed in OPPTS 860.1300.

Petitioner's Response
The petitioner submitted MRID # 451550-01, summarizing the results of previously-conducted metabolism studies concerning tetraconazole in wheat, providing details with regards to the identification/characterization of metabolites.

> 451550-01 Rizzo, F.; Pizzingrilli, G.; and Valcamonica, C. (2000) Summary: The Metabolism of Tetraconazole in Wheat: Lab Project Number: R/ABT.00.11. Unpublished study prepared by Isagro Ricerca Srl. 422 p.

Contained in the present submission were summaries of the results reported in MRID #s 442681-09 and -10, as well as report numbers R/ABT 92.05, AGR 98/974128, and R/ABT 97.05. Report R/ABT 92.05 detailed the analysis of wheat grain and straw samples collected in MRID #s 442681-09 and -10, while R/ABT 97.05 detailed the analysis of wheat straw samples collected in study AGR 98/974128. To improve the identification/characterization of metabolites, the R/ABT 92.05 and 97.05 studies made use of several radiolabeled and "cold" reference standards of metabolites in conjunction with TLC analyses and GC/MS, where appropriate. These studies confirmed that tetraconazole is the primary residue with low levels of tetraconazole-derived metabolites.

From R/ABT 92.05. Two separate applications each of [^{14}C-phenyl] tetraconazole and [^{14}C-triazole] tetraconazole at an application rate of 125 g/ha (total applied = 0.223 lb/A (0.74X maximum seasonal rate)) were made to separate wheat plots. Following harvest, samples of grain and straw were analyzed for total radioactivity by LSC after combustion, and subjected to extraction procedures in an effort to characterize/identify compounds present. The results for the grain and straw samples are summarized in Tables 1 and 2, respectively.

Table 1. Metabolism Study Results from Wheat Grain Samples.

Compound	PTG-8[a]		TTG-8[b]	
	ppm	%	ppm	%
Tetraconazole	0.048	52.17	0.042	6.29
Triazolyl alanine	—	—	0.331	50.07
Triazolyl acetic acid	—	—	0.165	24.90
Unidentified[c]	0.021	23.91	0.026	3.91
Extracted @ 37°C[d]	0.004	4.35	0.077	11.61
^{14}C-bound	0.018	19.57	0.021	3.22
TRR	0.091	100	0.662	100

a [^{14}C-phenyl] tetraconazole treated wheat grain sample.
b [^{14}C-triazole] tetraconazole treated wheat grain sample.
c At least 6 compounds.
d At least 2 compounds.

Table 2. Metabolism Study Results from Wheat Straw Samples.

Compound	PTS-7[a]		TTS-7[b]	
	ppm	%	ppm	%
Tetraconazole	2.828	49.54	3.602	49.23
M2 (M14360-alcohol)	0.033	0.58	0.044	0.60
M19 (M14360-acid)	0.093	1.63	0.134	1.83
M3+4[c]	0.150	2.62	0.176	2.4
M8+9+10+11+11+16+17+18[d]	0.676	11.83	0.640	8.76
M12+13+14+15[e]	1.040	18.24	1.494	20.40
^{14}C-Bound	0.888	15.56	1.228	16.78
TRR	5.708	100	7.318	100

a [^{14}C-phenyl] tetraconazole treated wheat straw sample.
b [^{14}C-triazole] tetraconazole treated wheat straw sample.
c Metabolites 3 and 4 were not identified.
d Metabolites 8, 9, 10, 11, 16, and 17 were not identified. Metabolite 18 had chromatographic properties corresponding to M14360-DFA.
e Metabolites 12, 13, 14, and 15 were present as conjugates. As hydrolysis with β-glucosidase was unsuccessful, apparently no conjugates with β-glucose were present. HCl hydrolysis reduced the amount of polar compounds. The free molecules correspond to M14360-alcohol, M14360(C-1)-alcohol and M14360-ketone.

From R/ABT 97.05. Three separate applications each of [^{14}C-phenyl] tetraconazole and [^{14}C-triazole] tetraconazole at an application rate of 125 g/ha (total applied = 0.335 lb/A (1.1X maximum seasonal rate)) were made to separate wheat plots. Following harvest, samples of straw were analyzed for total radioactivity by LSC after combustion, and subjected to extraction procedures in an effort to characterize/identify compounds present. The results for the straw samples are summarized in Table 3.

Table 3. Metabolism Study Results from Wheat Straw Samples.

Compound	PH straw[a]		TR straw[b]	
	ppm	%	ppm	%
Tetraconazole	7.981	69.55	7.849	63.03
S-5[c]	0.135	1.18	0.124	1.00
S-6[d]	0.110	0.96	0.102	0.82
S-8[e]	0.050	0.44	0.056	0.45
S-29[f]	0.023	0.20	0.033	0.27
S-30[g]	0.024	0.21	0.024	0.19
S-45[h]	0.273	2.38	0.322	2.59
S-53[i]	0.032	0.28	0.043	0.35
S-55[j]	0.016	0.14	0.015	2.12
^{14}C-Bound	2.189	19.08	2.619	21.03
Cellulose	0.710	6.19	0.626	5.03
Lignin	0.410	3.58	0.450	3.61
TRR	11.475	100	12.453	100

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[a] [^{14}C-phenyl] tetraconazole treated wheat straw sample.
[b] [^{14}C-triazole] tetraconazole treated wheat straw sample.
[c] S-5: M14360-dichlorophenyl-3-OH.
[d] S-6: M14360-dichlorophenyl-5-OH.
[e] S-8: M14360-alcohol.
[f] S-29: M14360-DFA.
[g] S-30: M14360-acid.
[h] S-45: M14360-CP(C-1)-alcohol.
[i] S-53: M14360-ketone.
[j] S-55: M14360(C-1)-alcohol.

The preceding analysis allowed the petitioner to construct a metabolic pathway for tetraconazole in wheat as shown in Figure 1.

HED's Conclusion
The additional identification and characterization of wheat grain and straw metabolites appears to be satisfactory. **Pending concurrence of the HED MARC, this deficiency is now resolved.**

Once the remaining metabolism-related deficiencies have been adequately addressed by the petitioner, the HED MARC will be consulted for confirmation of the previous tentative decisions regarding residues of concern (D264157, W. Donovan and D. Nixon, 19-APR-2000) and suitability of the metabolism database for tetraconazole.

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

Deficiency - Conclusion 2 from Memo, D259205, W. Donovan, 18-MAY-2000:

2. 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 for the purpose of this petition request. A comparison of the level of radioactivity and its distribution among the parent compound, metabolites, and nonextractable residues indicates that the metabolic pathway is similar for both phenyl- and triazole-labeled tetraconazole. Furthermore, it demonstrates that cleavage of the carbon chain which connects the triazole and phenyl rings does not occur during the metabolism of tetraconazole in grapes.

Petitioner's Response
None.

HED's Conclusion
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.**

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

Deficiency - Conclusion 3a from Memo, D254411, W. Donovan, 18-MAY-2000:

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.

Petitioner's Response
None.

HED's Conclusion
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.**

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

Deficiency - Conclusions 4b and 5b from Memo, D254411, W. Donovan, 18-MAY-2000:

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.

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.

Petitioner's Response

The petitioner submitted MRID#: 451550-04, describing a radiovalidation study of tetraconazole residues in milk and muscle samples from goats treated with ^{14}C-tetraconazole, as conducted by Huntingdon Life Sciences.

451550-04 Girkin, R. (2000) Tetraconazole Residue Methods Radiovalidation: Project Number 001/003179. Unpublished study prepared by Huntingdon Life Sciences Ltd, 31 p.

Re-analysis of samples from the goat metabolism studies showed that TRR values had declined by approximately 18% in milk samples and had increased approximately 16% in muscle samples compared to the original analysis. Further study of samples from [^{14}C-dichlorophenyl] tetraconazole treated goats demonstrated adequate extraction efficiency as shown in Table 4.

Table 4. Summary of Radiovalidation Results Obtained From Analysis of Goat Samples.

Matrix	Method		
	Radioactivity determined by LSC (no extraction) (ppm)	Extraction According to Metabolite Profiling Method (ppm)	Extraction According to Residue Analysis Method (ppm)
Muscle	0.081	Total 0.075	Total 0.054
		Tetraconazole 0.065	Tetraconazole 0.052
Milk	0.095	Total 0.094	Total 0.095
		Tetraconazole 0.088	Tetraconazole 0.095

The petitioner indicated they plan to submit radiovalidation data for plant samples in conjunction with a sugar beet metabolism study employing phenyl-labeled tetraconazole, now in progress.

HED's Conclusion

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.**

7. Confirmatory Method

Deficiency - Conclusion 4c from Memo, D254411, W. Donovan, 18-MAY-2000:

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)).

Petitioner's Response
None.

HED's Conclusion
This deficiency remains unresolved.

8. Independent Laboratory Validation of Triazole Method for Livestock Commodities

Deficiency - Conclusion 5c from Memo, D254411, W. Donovan, 18-MAY-2000:

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 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.

Petitioner's Response
None.

HED's Conclusion
This deficiency remains unresolved.

9. Multiresidue Method

Deficiency - Conclusion 6 from Memo, D254411, W. Donovan, 18-MAY-2000:

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.

Petitioner's Response
None.

HED's Conclusion
This deficiency remains unresolved.

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

Deficiency - Conclusion 8 from Memo, D254411, W. Donovan, 18-MAY-2000:

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.

Petitioner's Response
None.

HED's Conclusion
This deficiency remains unresolved.

11. Crop Field Trials.

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 PHI and a 14 day RTI. **This is a new deficiency.**

12. Revised Section F.

Deficiency - Conclusions 9b, 9d, 10b, 10c, 12a, 12b, 12c, and 12d from Memo, D254411, W. Donovan, 18-MAY-2000:

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).

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."

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 trial (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.

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".

Petitioner's Response
None.

HED's Conclusion
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.**

13. Bovine Feeding Study.

Deficiency - Conclusion 11 from Memo, D254411, W. Donovan, 18-MAY-2000:

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.

Petitioner's Response
None.

HED's Conclusion
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

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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. Poultry Metabolism Study

Deficiency - Conclusion 3b from Memo, D259321, W. Donovan, 18-MAY-2000:

3b. A tetraconazole poultry metabolism study was not submitted in support of this petition but is required because peanut meal is a poultry feed item.

Petitioner's Response
The petitioner has chosen to separate the peanut tolerance petition (PP# 9F6023) and its accompanying Section 3 registration action from all other tolerance and/or registration actions pertaining to tetraconazole (sugar beets, turf, and bananas), with the intention to re-prioritize the peanut petition upon submission of the poultry metabolism study.

HED's Conclusion
The poultry metabolism study deficiency remains unresolved. However, the sugar beet and banana petitions may proceed without the results of the poultry metabolism study.

15. Metabolism of [^{14}C-phenyl] Tetraconazole in Rotational Crops.

Deficiency - Conclusion 12a from Memo, D259321, W. Donovan, 18-MAY-2000:

12a. 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 for the purposes of this petition. 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.

Petitioner's Response
The petitioner has submitted the results of a study (citation listed below) investigating the metabolism of [^{14}C-phenyl] tetraconazole in rotational crops. Treated soil was aged for four periods prior to the planting of each crop: 30, 120, 223, and 365 days. This is an interim report because results are only available for the 30, 120, and 223 day plant back intervals. Once results are available for the 365 day PBI, this information will be submitted. The in-life and analytical phases of the study were conducted by Isagro Ricerca Srl (Novara, Italy).

451550-05 Rizzo, F.; Pizzingrilli, G. (2000) Uptake, Translocation and Metabolism of ((carbon-14)-Phenyl)Tetraconazole in Rotated Crops of Cereals, Carrots and Lettuce: Lab

Project Number: ABT.98.11 (Interim Report). Unpublished study prepared by Isagro Ricerca Srl. 150 p.

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). Nine 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. Carrot and lettuce were planted to the pots 30, 120, and 223 days after treatment (DAT). Sorghum was planted 223 DAT.

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 more than 80% of TRR in lettuce and carrot; it accounted for 65% TRR in sorghum. The extractable radioactivity was partitioned in n-hexane and in ethyl acetate. Table 5 summarizes the results of the interim study.

Table 5. Interim Results of [^{14}C-phenyl] Tetraconazole Confined Rotational Crop Study.

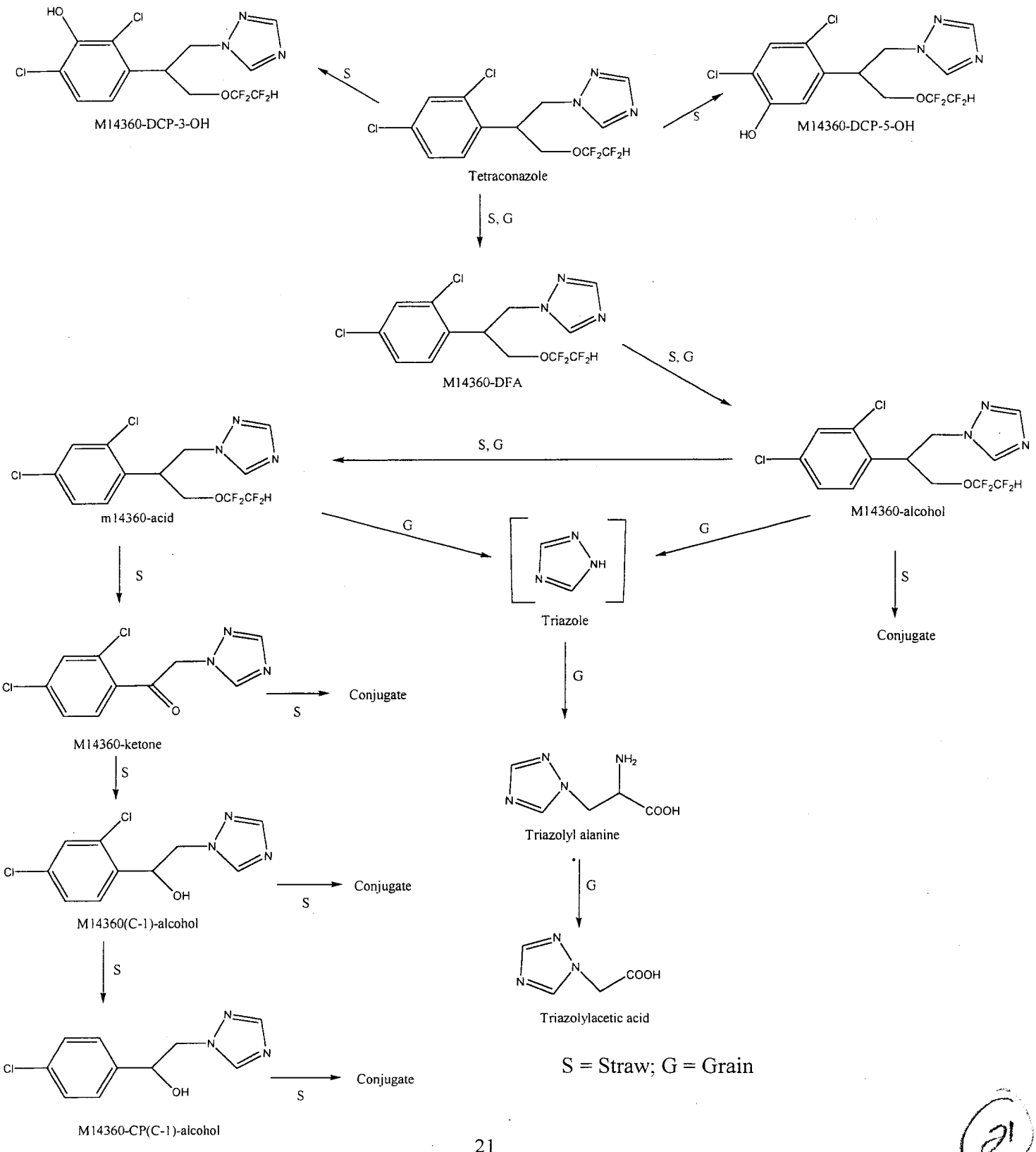
Crop (Days from planting to harvest)	Days from treatment to planting			Days from treatment to planting		
	30	120	223	30	120	223
	TRR (ppm)			Tetraconazole (ppm)		
Carrot (71)	0.031	0.022	—	0.021	0.016	---
Carrot (83)	—	—	0.014	—	—	0.012
Lettuce (53)	0.025	0.043	—	0.007	0.019	---
Lettuce (40)	—	—	0.042	—	—	0.021
Sorghum (139)	—	—	0.017	—	—	0.002

HED's Conclusion
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.**

cc: W. Donovan
RDI: G. Herndon (12-OCT-2000), G. Kramer (12-OCT-2000), RAB1 Chemists (05-OCT-2000)
W. Donovan:806R:CM#2:(703)-305-7330:MC 7509C

Figure 1. Metabolic Pathway of Tetraconazole in Wheat.



S = Straw; G = Grain