Residue Chemistry Review

Comments:

Subject: Fipronil in or on Corn and Animal RACs. Review of Residue Data and Analytical Methodology. MRID#s 434011-04 to -12, 433857-01 & -02, 429186-11 and 435086-01. Case 286075. CBTS# 15436

Document Class:

Product Chem:
- 830.1550 Product Identity and composition
- 830.1600 Description of materials used to produce the product
- 830.1620 Description of production process
- 830.1650 Description of formulation process
- 830.1670 Discussion of formation of impurities
- 830.1700 Preliminary analysis
- 830.1750 Certified limits
- 830.1800 Enforcement analytical method
- 830.6302 Color
- 830.6303 Physical state
- 830.6304 Odor
- 830.6313 Stability to sunlight, normal and elevated temperatures, metals, and metal ions
- 830.7000 pH of water solutions or suspensions
- 830.7200 Melting point/melting range
- 830.7220 Boiling point/boiling range
- 830.7300 Density/relative density/bulk density
- 830.7370 Dissociation constant in water
- 830.7550 Partition coefficient (n-octanol/water), shake flask method
- 830.7560 Partition coefficient (n-octanol/water), generator column method
- 830.7570 Partition coefficient (n-octanol/water), estimation by liquid chromatography
- 830.7840 Water solubility: Column elution method, shake flask method
- 830.7860 Water solubility, generator column method
- 830.7950 Vapor pressure

Residue Chem:
- 860.1200 Directions for use
- 860.1300 Nature of the residue - plants, livestock
- 860.1340 Residue analytical method
- 860.1360 Multiresidue method
- 860.1380 Storage stability data
- 860.1480 Meat/milk/poultry/eggs
- 860.1500 Crop field trials
- 860.1520 Processed food/feed
- 860.1550 Proposed tolerances

Biochemicals:

DP Barcode: D214376
MRIDs: 42918611, 43385701, 43385702, 43401104, 43401105, 43401106, 43401107, 43401108, 43401109, 43401110, 43401111, 43401112, 43508601

PC Codes: 129121 1H-Pyrazole-3-carbonitrile, 5-amino-1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-4-((trifluoromethyl)sulfi

Activates/Inerts CAS #: 120068-37-3

Commodities: Corn; Cattle, Fat; Cattle, Kidney; Cattle, Liver; Cattle, Meat; Poultry, fat; Poultry, Liver; Poultry, Meat; Milk; Egg; Goat, fat; Goat, Liver; Goat, MBYP; Goat, Meat; Hog, Fat; Hog, Liver; Hog, MBYP; Hog, Meat; Horse, Meat; Horse, MBYP; Horse, Liver; Horse, Fat; Sheep, Fat; Sheep, Liver; Sheep, MBYP; Sheep, Meat; Poultry, MBYP

Administrative #: 5F04426

Reviewers: G. F. Kramer
MEMORANDUM


FROM: G.F. Kramer, Ph.D., Chemist
Tolerance Petition Section III
Chemistry Branch I, Tolerance Support
Health Effects Division (7509C)

THRU: M.S. Metzger, Branch Chief
Chemistry Branch I, Tolerance Support
Health Effects Division (7509C)

TO: Rick Keigwin, Product Manager
Ann Sibold, Team 10 Reviewer
Registration Division (7505C)

And

Jane Smith, Acting Section Head
Registration Section, RCAB
Health Effects Division (7509C)

Rhône-Poulenc has submitted an application for permanent tolerances for the insecticide fipronil (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(1R,S)-(trifluoromethyl)sulfinyl]-1H-pyrazole-3-carbonitrile) or its metabolites MB46136 (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfonyl]-1H-pyrazole-3-carbonitrile) or MB45950 (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)thio]-1H-pyrazole-3-carbonitrile) on/in corn. The petitioner has proposed the following tolerances for corn and animal RACs (expressed as parent or metabolites MB45950 or MB46136):

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<tr>
<th></th>
<th>ppm</th>
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</thead>
<tbody>
<tr>
<td>Corn Grain</td>
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<td>Corn Fodder</td>
<td>0.15</td>
</tr>
<tr>
<td>Corn Forage</td>
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<td>Liver'</td>
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<tr>
<td>Milk'</td>
<td>0.02</td>
<td>Eggs</td>
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</tr>
<tr>
<td>Fat'</td>
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<td>Poultry Skin/Fat</td>
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<tr>
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<td>Poultry Muscle</td>
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</tr>
<tr>
<td>Kidney'</td>
<td>0.02</td>
<td>Poultry Liver</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Dairy Cows

Rhône-Poulenc has also submitted an application for registration of the end-use product, Regent 1.5G Insecticide.

In review of a request for an EUP and temporary tolerances for fipronil on corn (PP#3G4263), CBTS identified the deficiencies which must be addressed by the registrant in order for us to be
able to recommend in favor of permanent tolerances (Memo, G. Kramer 6/7/94). In the Detailed Considerations section of this Memo, the outstanding deficiencies, listed as presented in the Memo of G. Kramer (6/7/94), are followed by the petitioner's response and our conclusions.

There are no permanent tolerances established for residues of fipronil to date.

Executive Summary of Chemistry Deficiencies

• Revise label deleting forage/fodder feeding restrictions and crop rotation restrictions.
• Need confined crop rotation study.
• Need plant metabolism study with label in pyrazole ring.
• Additional information for ruminant metabolism study.
• Revision and additional validation of analytical method for plants and submission of analytical reference standards.
• Agency validation of analytical method for animals.
• Additional information for ruminant feeding study.
• Revised Section F.

CONCLUSIONS

1a. The following product chemistry data requirements remain outstanding: i) for GLN § 61-3, provide a theoretical discussion on the formation of impurities that might hypothetically occur but were not found in the TGAI (i.e., RPA200060, RPA098028, RPA109263, MB45897, MB45513 and MB46058- impurities reported as not being observed in the TGAI during the preliminary analysis); ii) for GLN § 62-1, the manufacturing process is being revised to eliminate the formation of a specific impurity. The preliminary analysis should thus be repeated on five batches of fipronil produced by the revised process; iii) for GLN § 63-13, submit data on the sensitivity of the TGAI to metal ions and legible thermograms for each metal tested.
1b. CBTS concludes that the impurities listed on the CSF do not present a residue problem on the subject crop when fipronil is formulated into Regent Insecticide and used as directed. Based on the chemistry employed during the manufacturing process, the formation of nitrosamines or dioxin is unlikely.

2. The following deficiencies in the directions for use were noted: a) Restrictions against the feeding of forage and fodder to livestock are considered impractical and should be removed from the label. b) Limiting crop rotation to corn only is not practical as the majority of corn in the U.S. is grown in rotation with other crops. Crop rotation restrictions, based on the results of the required crop rotation studies, may be required. A revised Section B is required.

3. No rotational crop studies were submitted with this petition. As limiting crop rotation to corn only is not practical, the registrant must submit a confined crop rotation study (GRN 165-1). The results of this study will be used to determine the appropriate crop rotation restrictions and/or the need for limited rotational crop field trials.

4a. The registrant has reanalyzed the samples generated from the corn metabolism study reviewed in conjunction with the EUP (MRID# 429186-65).

4b. By reanalysis of the stored phenyl-labelled samples, the registrant has successfully addressed the deficiencies in metabolite identification. However, based on the data submitted, storage stability cannot be demonstrated for the samples of this study. It appears that MB46136 and RPA200766 are the major metabolites of fipronil in corn, but conclusions on the relative amounts of the parent and metabolites and on the presence of RPA105048 can not be reached. However, CBTS will not require the registrant to repeat the phenyl-labelled study if the results of the required corn metabolism study using pyrazole-labelled fipronil (see below) are qualitatively the same as the results with the phenyl label.

4c. Fipronil contains two rings but the registrant has performed metabolism studies using only [¹⁴C]phenyl-labelled fipronil. In order to fully characterize the nature of the residue in corn, the registrant should perform a metabolism study in corn using [¹³C]pyrazole-labelled fipronil.

5a. In the ruminant metabolism study, [phenyl(U)-¹⁴C]-fipronil was administered orally to lactating goats. The goats were dosed at a rate of 0.02 ppm, 2 ppm or 10 ppm. Doses were administered twice daily for 7 consecutive days. Of the administered radioactivity, 18-64% was recovered in feces, 1-5% in the milk and 8-25% in the tissues. The greatest tissue residues were observed in fat (1.9
ppm at 10 ppm dose).

5b. Fipronil per se was the predominant component of the residue in milk, muscle and fat accounting for 60-73% of the TRR. The metabolites RPA200766, MB45950 and MB46136 were also identified, accounting for 0.6-7%, 5-12% and 17-23% of the TRR, respectively. MB46136 was the predominant component of the residue in liver and kidney accounting for 53-75% of the TRR. Fipronil and the metabolite RPA200766 were also identified, accounting for 2-3% and 0-11% of the TRR, respectively. A total of 66-97% of the TRR was identified.

5c. The actual dates of sample extraction and analysis were not provided in this report so that the sample storage interval could not be calculated. As fipronil and its metabolites comprised >90% of the TRR in the milk, fat and muscle samples, storage stability is not an issue for these matrices. However, the liver sample contains numerous unidentified compounds which account for a large portion of the TRR (33%, 0.28 ppm). The actual dates of sample analysis should thus be provided. If the liver samples were stored for longer than 6 months, then evidence of storage stability should be provided.

5d. Providing that storage stability can be demonstrated for the liver samples, CBTS will not require a ruminant metabolism study to be performed using [14C]pyrazole-labelled fipronil as there was no evidence for ring cleavage in this study.

6a. In the poultry metabolism study, [phenyl(U)-14C]-fipronil was administered orally to laying hens. The hens were dosed at a rate of 0.02 ppm, 2 ppm or 10 ppm. There were five birds in each dosing group. Doses were administered daily for 28 consecutive days. Of the administered radioactivity, 28-42% was recovered in excreta, 15-18% in the eggs and 1-5% was recovered in the tissues. The total recovery was 52-58%. The greatest tissue residues were observed in fat (56 ppm at 10 ppm dose). Residues in eggs were also extremely high (30 ppm in yolks at 10 ppm dose) and had not plateaued by the end of the study (28 days).

6b. MB46136 was the predominant component of the residue in tissues and eggs, accounting for 95-99% of the TRR. Fipronil was also identified in egg yolk, skin, fat and liver, accounting for 1-3% of the TRR. A total of 95-100% of the TRR was identified.

6c. The actual dates of sample extraction and analysis were not provided in this report so that the sample storage interval could not be calculated. As fipronil and its metabolite MB46136 comprised >90% of the TRR in all egg and tissue samples, storage stability is not an issue.

6d. CBTS will not require a poultry metabolism study to be
performed using [\(^{14}\text{C}\)]pyrazole-labelled fipronil as there was no
evidence for ring cleavage in this study.

6e. Provided that storage stability of the goat liver samples can
be demonstrated, the nature of the residue in animals is considered
to be understood. Fipronil and MB46136 are the primary components
of the residue, accounting for 54-99% of the TRR. CBTS will refer
to the Metabolism Committee on the toxicological significance of
metabolites once the deficiencies associated with ruminant
metabolism have been addressed. A decision by CBTS concerning
which residues to regulate will then follow. A tolerance based on
the parent and metabolites MB46136 and MB45950 may not be
appropriate; in such an instance a revised Section F and additional
feeding studies, analytical methodology, and storage stability data
may be needed.

7a. Proposed enforcement method EC-93-236 for corn RACs was
submitted with PP# 3G04263. An ILV of this method was performed by
Colorado Analytical Research and Development Co. The method and
ILV were sent to Beltsville for PMV (Memo, G. Kramer 9/12/94).

7b. The method has been validated by ACL (Memo, G. Kramer
3/28/95). Acceptable recoveries were obtained for fipronil,
MB45950, MB46136, RPA105048 and RPA200766. The LOQ for each
compound is 0.01 ppm in grain and 0.02 ppm in forage and fodder.
The registrant should submit a revised version of the proposed
analytical enforcement method specified in conclusions 1-3 of Memo,
G. Kramer (3/28/95). Analytical standards of fipronil and its
metabolites should be sent to the EPA Repository, RTP. Until the
receipt of the standards and the revised method, the requirements
for analytical enforcement methodology will remain unfulfilled.

7c. A report on Multiresidue testing of fipronil and its
metabolites MB45950 and MB46136 (MRID# 434011-07) has been received
and forwarded to FDA (Memo, G. Kramer 5/9/95). Acceptable
recoveries of fipronil and its metabolites were obtained in corn
grain using Protocol E. Recoveries in forage were 38-65% using
Protocol E.

7d. The specificity of the proposed analytical enforcement method
was demonstrated by performing an interference study with 45 of the
pesticides for which tolerances are established on corn. The
registrant reports that 10 of these compounds were found to have
"minor interferences" with fipronil or its metabolites. The
registrant should submit chromatograms in which fipronil and its
metabolites are injected both with and without these pesticides so
that CBTS can determine whether the reported "minor interferences"
are of concern.

7e. No radiovalidation data have been submitted. Samples of
radiolabelled forage and fodder from the metabolism study must be
analyzed with the proposed enforcement method. Based on the
results of the plant metabolism study, only 25% of the TRR in fodder samples would be extractable by the proposed enforcement method.

7f. The registrant has included conditions for separation on a different GC column (HP-5 instead of HP-1) and use of GC/MS as confirmatory techniques. The Multiresidue Method is also available as a confirmatory technique.

8a. Proposed enforcement method EC-94-258 for animal RACs was submitted with this petition. Acceptable recoveries were obtained for all analytes in eggs and beef fat and kidney. The LOQs were reported to be 0.002 ppm for fipronil, 0.003 ppm for MB45950 and 0.004 ppm for MB46136 in eggs; 0.004 ppm for fipronil, 0.003 ppm for MB45950 and 0.002 ppm for MB46136 in kidney; and 0.020 ppm for fipronil and MB45950 and 0.008 ppm for MB46136 in fat.

8b. An ILV of this method was performed by CYAL, Inc. Acceptable recoveries were obtained by the laboratory for all analytes. The method and ILV were sent to Beltsville for PMV (Memo, G. Kramer 5/11/95). CBTS will withhold a final conclusion on the adequacy of this method as analytical enforcement method pending receipt of the PMV report.

8c. The specificity of the proposed analytical enforcement method was demonstrated by performing an interference study with 115 of the pesticides for which tolerances are established on animal commodities. None was found to interfere with fipronil or its metabolites.

8d. Samples of radiolabelled goat fat from the metabolism study were analyzed by the proposed enforcement method. As the results obtained from the metabolism study and the enforcement method do not differ significantly, CBTS concludes that this method has been successfully radiovalidated.

8e. The registrant has included conditions for separation on a different GC column (DB-5ms instead of DB-1701) as a confirmatory technique.

9. Samples of corn grain, forage, fodder, silage, crude oil, refined oil, grain dust, meal and starch were spiked with 0.1 ppm of fipronil MB45950, MB46136, RPA105048 and RPA200766 and stored frozen at <-10 °C. The results demonstrate that residues of fipronil and its metabolites are stable during storage in corn substrates for up to 12 months. As the samples from the field residue trials and processing study were stored for a maximum of 10 months, storage stability is not an issue for this petition.

10a. Ten corn field trials were conducted in nine different states in 1993. Fipronil was applied as a 1.5% granular formulation prior to planting at a rate of 0.13 lbs. ai/A (1X). Forage samples were
harvested 44-47 days after planting. Silage samples were harvested at the dent stage, 96-129 days after planting. Grain and fodder samples were harvested 124-171 days after planting. The levels of fipronil and its metabolites were below the LOQ in all grain samples. In forage, the maximum levels of fipronil observed were 0.056 ppm; of MB45950, were <0.02 ppm; of MB46136, were 0.044 ppm; of RPA105058, were <0.02 ppm; and of RPA200766, were 0.031 ppm. In silage, the maximum levels of fipronil observed were 0.022 ppm; of MB45950, were <0.02 ppm; of MB46136, were 0.036 ppm; and of RPA200766, were <0.02 ppm. In fodder, the maximum levels of fipronil observed were 0.020 ppm; of MB45950, were <0.02 ppm; of MB46136, were 0.073 ppm; and of RPA200766, were 0.030 ppm.

10b. Between these trials and those submitted previously, the registrant has submitted a total of 20 field corn residue trials. These trials were located in Regions 1 (1 trial), 2 (2 trials), 5 (13 trials), 6 (1 trial), 8 (1 trial), 10 (1 trial) and 11 (1 trial). This distribution does not correspond with that suggested for field corn in EPA Guidance on Number and Location of Domestic Crop Field Trials for Establishment of Pesticide Residue Tolerances, 6/2/94: Regions 1 (1 trial), 2 (1 trial), 5 (17 trials) and 6 (1 trial). However, as the states in which these trials were performed represented >80% of U.S. corn acreage in 1991 (Agricultural Statistics, 1992), CBTS concludes that the number of trials and the geographic representation are adequate to establish tolerances for fipronil and its metabolites on corn. The residue data support the proposed tolerances of 0.02 ppm for grain, 0.15 ppm for forage and fodder.

10c. Further residue data may be required if other metabolites are determined to be of regulatory significance. Existing samples could be analyzed in order to determine metabolite levels, provided storage stability can be demonstrated.

10d. The registrant has proposed tolerances based on the sum of the actual residue levels or LOQ of each metabolite. However, tolerances are proposed for fipronil or its metabolites. The registrant should thus propose tolerances for "fipronil and its metabolites MB45950 and MB46136." A revised Section F which includes this tolerance expression is required. This revised Section F should also contain the chemical names of fipronil, MB45950 and MB46136. Section F may need further revision in accordance with the decision of the Metabolism Committee on the metabolites of toxicological concern.

11a. Corn was treated with fipronil at a rate of 20X and the grain processed after harvest. No detectable residues of fipronil, MB46136 or MB45950 were observed in any processed fraction. No feed or food additive tolerances are thus required for this petition. Low, but detectable residues of two metabolites not
proposed to be included in the tolerance expression were found in some processed fractions. However, due to the exaggerated application rate, it is unlikely measurable residues would be present at a 1X use rate.

11b. This processing study is acceptable provided that there are no metabolites determined to be of toxicological concern by the Metabolism Committee which were not accounted for in this study.

12a. Holstein dairy cows were dosed daily for 35 consecutive days with fipronil at levels of 0, 0.04, 0.13 and 0.43 ppm in the diet. At the 0.43 ppm dietary burden, quantifiable residues of fipronil were observed only in fat (0.051 ppm); while quantifiable residues of MB46136 were observed in milk (0.052 ppm), liver (0.172 ppm), muscle (0.059 ppm), kidney (0.035 ppm) and fat (0.554 ppm). No quantifiable residues were of MB45950 were observed in any tissue.

12b. As there are no established permanent tolerances for fipronil residues to date, the maximum dietary burden for dairy cattle, 0.36 ppm, results from a diet comprised solely of corn forage and fodder. The maximum dietary burden for beef cows, 0.21 ppm, results from a diet comprised of corn forage, grain and fodder. Based on extrapolation of the results from 0.13 and 0.43 ppm to the 1X level, the appropriate tolerances for fipronil and its metabolites MB45950 and MB46136 are:

| Milkfat (reflecting 0.06 ppm in whole milk) | -- | 2.0 ppm |
| Fat | -- | 0.40 ppm |
| Meat | -- | 0.04 ppm |
| Meat By-Products (except liver) | -- | 0.02 ppm |
| Liver | -- | 0.10 ppm |

'of cattle, goats, horses, hogs and sheep

The registrant has proposed tolerances only for dairy cow RACs and not in terms of meat and meat by-products. However, tolerances are required for beef cattle, goats, horses, hogs and sheep. A Revised Section F is required. The above terminology should be included. Section F may need further revision in accordance with the decision of the Metabolism Committee on the metabolites of toxicological concern.

12c. Note that the milk tolerance should be expressed in terms of "milkfat" as fipronil is fat soluble. The recommended tolerance for milkfat is derived from the estimated maximum residue in whole milk (0.06 ppm) and a theoretical concentration factor of 31X. However, the registrant should report the actual fat content of the milk in this study. The milkfat tolerance should then be revised, if necessary, based on the actual fat content of the milk from the cow in which residues were the greatest.
12d. Ruminant feeding studies employing higher dosing levels may be required in the future if tolerances are proposed on other crops which increase the potential dietary exposure.

13a. Leghorn hens were dosed daily for 42 consecutive days with fipronil at levels of 0, 0.010, 0.031 and 0.103 ppm in the diet. At the 0.103 ppm dietary burden, quantifiable residues of MB46136 were observed in eggs (0.116 ppm), liver (0.072 ppm), muscle (0.014 ppm) and fat (0.214 ppm). No quantifiable residues of fipronil or MB45950 were observed in any tissue.

13b. As there are no established permanent tolerances for fipronil residues to date, the maximum dietary burden, 0.02 ppm, results from a poultry diet comprised solely of corn grain and milled by-products. Based on extrapolation of the results from 0.010, 0.031 and 0.103 ppm to the 1X level, the appropriate tolerances for fipronil and its metabolites MB45950 and MB46136 are:

<table>
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<tr>
<th></th>
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<td>0.02</td>
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<tr>
<td>Poultry Meat By-Products</td>
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</table>

**A Revised Section F is required.** A terminology revision is also required as tolerances were not proposed in terms of meat, fat and meat by-products of poultry. Section F may need further revision in accordance with the decision of the Metabolism Committee on the metabolites of toxicological concern.

14. There is no Codex proposal, nor Canadian or Mexican limits for residues of fipronil and its metabolites in corn. Therefore, a compatibility issue is not relevant to the proposed tolerance. A copy of the IRLS is attached to this memorandum.

15. A DRES run will need to be conducted, but will not be requested until all deficiencies affecting the proposed tolerance levels have been resolved.

**RECOMMENDATIONS**

CBTS recommends against the proposed tolerances for fipronil on corn and animal RACs for reasons detailed in conclusions 1a, 2a, 2b, 3, 4b, 4c, 5b, 5c, 6e, 7b, 7d, 7e, 8b, 10c, 10d, 11b, 12b, 12c and 13b.
DETAILED CONSIDERATIONS

Product Chemistry

Deficiency - Conclusion 1a (from Memo, G. Kramer 6/7/94)

1a) for GLN § 61-1, submit a revised CSF for the TGA1 in which impurities MB45897 and MB46513 are deleted. Also, the registrant should provide information on the relative pesticidal activity of the two enantiomers of fipronil. If one stereoisomer is found not to be pesticidally active, then it should be listed as an impurity in the revised CSF.

Petitioner's Response: Both enantiomers have pesticidal activity. They are quantified as a single a.i. in the enforcement method. A revised CSF is included in MRID# 433857-01. See the confidential appendix for details.

CBTS' Conclusion: The requested information has been provided. This deficiency is now resolved.

Deficiency - Conclusion 1b (from Memo, G. Kramer 6/7/94)

1b) for GLN § 61-2, provide copies of the Material Safety Data Sheets for all of the starting materials.

Petitioner's Response: The Material Safety Data Sheets are included in MRID# 433857-01.

CBTS' Conclusion: The requested information has been provided. This deficiency is now resolved.

Deficiency - Conclusion 1c (from Memo, G. Kramer 6/7/94)

1c) for GLN § 61-3, provide a theoretical discussion on the formation of RPA200766, the formation of the three impurities found at levels <0.1% (MB45897, MB46513 and MB46058), the formation of impurities that might hypothetically occur but were not found in the TGA1, possible degradation products of the TGA1, and the potential for starting materials to carry over to the TGA1.

Petitioner's Response: Further discussion of impurities is included in MRID# 433857-01. See the confidential appendix for details.

CBTS' Conclusion: All of the requested information has been provided except for the formation of impurities that might hypothetically occur but were not found in the TGA1 (i.e., RPA200060, RPA098028, RPA109263, MB45897, MB46513 and MB46058-
impurities reported as not being observed in the TGAI during the preliminary analysis). **This deficiency remains outstanding.**

**Deficiency - Conclusion 1d (from Memo, G. Kramer 6/7/94)**

1d) for GLN § 62-1, report the results of batch analyses of fipronil TGAI once full production starts. The CSF for the TGAI may need to be revised if the results of the new batch analyses differ from those done previously.

**Petitioner's Response:** The results of a five-batch analysis was included in MRID# 433857-01. See the confidential appendix for details.

**CBTS' Conclusion:** The requested information has been provided. However, the manufacturing process is being revised to eliminate the formation of impurity RPA097965. The preliminary analysis should thus be repeated on five batches of fipronil produced by the revised process. **This deficiency remains outstanding.**

**Deficiency - Conclusion 1e (from Memo, G. Kramer 6/7/94)**

1e) for GLN § 62-2, provide an explanation as to how all the certified limits were determined.

**Petitioner's Response:** The certified limits are based on results of the five-batch analysis, statistical variations and control limits for future production.

**CBTS' Conclusion:** The requested information has been provided. This deficiency is now resolved.

**Deficiency - Conclusion 1f (from Memo, G. Kramer 6/7/94)**

1f) for GLN § 62-3, demonstrate the accuracy of Method M-647-08-91(E) for measurement of fipronil.

**Petitioner's Response:** This method is also used to verify the certified limits of the a.i. in the end-use product. When validating the method, known amounts of fipronil were added to the end-use product and the recovery determined (MRID# 429186-11). The recovery was 98.35 ± 1.46% (n=3).

**CBTS' Conclusion:** The requested information has been provided. This deficiency is now resolved.

**Deficiency - Conclusion 1g (from Memo, G. Kramer 6/7/94)**
for GLN § 63-13, submit data on the sensitivity of the TGAI to metal ions and legible thermograms for each metal tested.

**Petitioner's Response:** This information will be submitted at a later date.

**CBTS' Conclusion:** The requested information has not been provided. This deficiency remains outstanding.

### Table 1- PRODUCT CHEMISTRY DATA SUMMARY

**Chemical No.** 129121  
**Product:** Fipronil TGAI

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<th>Guideline Number</th>
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<td>433857-01</td>
</tr>
<tr>
<td>62-1</td>
<td>Preliminary Analysis</td>
<td>N⁶</td>
<td>429186-02</td>
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<tr>
<td>62-2</td>
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<td>Y</td>
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</tr>
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<td></td>
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<td>62-3</td>
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<td>Y</td>
<td>429186-02</td>
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<td>433857-02</td>
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<tr>
<td>63-2</td>
<td>Color</td>
<td>Y</td>
<td>429779-01</td>
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<tr>
<td>63-3</td>
<td>Physical State</td>
<td>Y</td>
<td>429779-01</td>
</tr>
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<td>63-4</td>
<td>Odor</td>
<td>Y</td>
<td>429779-01</td>
</tr>
<tr>
<td>63-5</td>
<td>Melting Point</td>
<td>Y</td>
<td>429779-01</td>
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<tr>
<td>63-6</td>
<td>Boiling Point</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>63-7</td>
<td>Density, Bulk Density or Specific Gravity</td>
<td>Y</td>
<td>429779-01</td>
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<tr>
<td>63-8</td>
<td>Solubility</td>
<td>Y</td>
<td>429186-03, 429186-04</td>
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<tr>
<td>63-9</td>
<td>Vapor Pressure</td>
<td>Y</td>
<td>429186-05</td>
</tr>
<tr>
<td>63-10</td>
<td>Dissociation Constant</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>63-11</td>
<td>Octanol/Water Partition Coefficient</td>
<td>Y</td>
<td>429186-06</td>
</tr>
<tr>
<td>63-12</td>
<td>pH</td>
<td>Y</td>
<td>429186-07</td>
</tr>
<tr>
<td>63-13</td>
<td>Stability</td>
<td>N⁶</td>
<td>429186-08</td>
</tr>
</tbody>
</table>

⁶ Y = Yes; N = No; N/A = Not Applicable.

⁶ Discussion incomplete.

⁶ The preliminary analysis should be repeated on five batches of fipronil produced by the revised process.

⁶ Data required on the sensitivity of the TGAI to metals and metal ions.
**Formulation:** Fipronil is formulated as Regent 1.5G Insecticide, a granular formulation containing 1.5% a.i.

**Product Chemistry Conclusions:** CBTS concludes that the impurities listed on the CSF do not present a residue problem on the subject crop when fipronil is formulated into Regent Insecticide and used as directed. Based on the chemistry employed during the manufacturing process, the formation of nitrosamines or dioxin is unlikely.

**Proposed Use**

Fipronil 1.5G granules are incorporated into the soil at planting by in-furrow or T-band application. The maximum use rate is 0.13 lbs. ai/A (8.7 lbs. Regent 1.5G/A). Only one application may be made per season.

The label contains the following restrictions: "Do not feed treated corn forage or fodder to livestock," "Do not allow livestock to graze treated fields," "Do not harvest within 90 days of application," and "Do not plant any crop other than field corn the year following application of Regent 1.5G."

The following deficiencies in the directions for use were noted: a) Restrictions against the feeding of forage and fodder to livestock are considered impractical and should be removed from the label. b) Limiting crop rotation to corn only is not practical as the majority of corn in the U.S. is grown in rotation with other crops. Crop rotation restrictions, based on the results of the required crop rotation studies, must be added to the label. A revised Section B is required.

**Rotational Crop Studies**

No studies were submitted with this petition.

As limiting crop rotation to corn only is not practical, the registrant must submit a confined crop rotation study (GRN 165-1). After treatment of the soil with labelled fipronil at a 1X rate, rotational crops (root and leafy vegetables and small grain) should be planted at 30, 120 and 365 day intervals. The nature of the residue should be determined in the edible portions of all crops. This study should include the use of fipronil labelled in both
rings, either as a single compound or as separate studies using material labelled in the phenyl or pyrazole ring. The results of this study will be used to determine the appropriate crop rotation restrictions and/or the need for limited rotational crop field trials.

**Nature of Residue- Plants**

**Deficiency - Conclusions 4b(i-iii) (from Memo, G. Kramer 6/7/94)**

4b(i) The storage stability of the samples in this study has not been demonstrated. The data that were presented by the registrant indicated that fipronil per se may not be stable during storage in fodder and forage. The registrant must show that the nature of the residue in the samples has not changed during storage (over 2 years) by presenting representative chromatographic separations performed early in the study and at the conclusion of the study. If such data do not exist or if significant changes in the metabolite profile occurred during storage, the registrant may be required to repeat this corn metabolism study. (ii) Unknown metabolites 1, 2 and 3 accounted for significant portions of the TRR in corn RACs. The registrant should identify these compounds. (iii) Significant portions of the TRR in forage and grain were found to be extractable but were not characterized by HPLC. The registrant should characterize any of these fractions which contain >0.05 ppm (methanol extract of forage and methanol reflux fraction of grain).

**Petitioner's Response:** Submitted with this petition:

Metabolic Fate and Distribution of $^{14}$C-Fipronil in Corn. (171-4 Nature of the Residue- Plants). **Amended Report** MRID# 434011-04

The registrant has reanalyzed the samples generated from the metabolism study reviewed in conjunction with the EUP (MRID# 429186-65). The structures of reference standards are shown in figure 1 (copied from pp. 51-52 of MRID# 434011-04).

**In-Life Phase:** [Phenyl(U)-$^{14}$C]-fipronil (19.62 mCi/mmole) with radiolabel uniformly distributed in the phenyl ring (diluted to 7.79 mCi/mmole with cold fipronil) had a radiopurity of greater than 99.5%. The test solutions were prepared by mixing the labelled compound with inert ingredients to simulate a 1.69% granulation formulation.

Sweet corn was planted in sandy loam soil and grown in the greenhouse. [$^{14}$C]-Fipronil was applied to the soil prior to planting at a rate of 170 g a.i./A (2.9X) or 1.7 Kg a.i./A (29X). Forage samples were taken after 42 days when the plants were 38-42 inches tall and grain and fodder samples were harvested at maturity.
TRR: As sufficient radioactivity was found in the plants treated with 170 g a.i./A, the plants treated at the higher rate were not analyzed. The tissues were ground to a powder and the TRR was determined by combustion (Tables 2 & 3).
Table 2- Extraction and fractionation of TRR in corn forage and fodder.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Forage ppm</th>
<th>% TRR</th>
<th>Fodder ppm</th>
<th>% TRR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial ppm</td>
<td>0.21</td>
<td>100</td>
<td>3.70</td>
<td>100</td>
</tr>
<tr>
<td>ACN</td>
<td>0.13</td>
<td>63.9</td>
<td>1.05</td>
<td>28.5</td>
</tr>
<tr>
<td>ACN:H$_2$O (8:2)</td>
<td>0.02</td>
<td>9.6</td>
<td>1.94</td>
<td>52.4</td>
</tr>
<tr>
<td>ACN:H$_2$O (2:8)</td>
<td>&lt;0.01</td>
<td>1.8</td>
<td>0.45</td>
<td>12.3</td>
</tr>
<tr>
<td>3 N HCl Reflux</td>
<td>&lt;0.01</td>
<td>1.6</td>
<td>0.47</td>
<td>12.6</td>
</tr>
<tr>
<td>Total Extracted</td>
<td>0.16</td>
<td>76.9</td>
<td>3.92</td>
<td>105.8</td>
</tr>
<tr>
<td>Bound Residues</td>
<td>0.04</td>
<td>20.6</td>
<td>0.19</td>
<td>5.2</td>
</tr>
</tbody>
</table>

**Extraction and Fractionation:** The procedures are outlined in figures 2-4 (copied from p. 64-66 of MRID# 434011-04). Tissues were initially extracted with acetonitrile (ACN). Extractions of forage and fodder samples were repeated using ACN:water (8:2) and ACN:water (2:8). The residuum was then refluxed in acidic methanol (3N HCl). Extractions of grain were repeated using water and methanol. The residuum was then refluxed in methanol. The results of these procedures are shown in Tables 2 & 3. The majority of the TRR was readily extractable and bound residues comprised 5-21% of the TRR.

Table 3- Extraction and fractionation of TRR in corn grain.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Grain ppm</th>
<th>% TRR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial ppm</td>
<td>0.16</td>
<td>100</td>
</tr>
<tr>
<td>ACN</td>
<td>0.01</td>
<td>5.7</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>0.10</td>
<td>62.0</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.05</td>
<td>28.8</td>
</tr>
<tr>
<td>Methanol Reflux</td>
<td>&lt;0.01</td>
<td>2.2</td>
</tr>
<tr>
<td>Total Extracted</td>
<td>0.16</td>
<td>98.7</td>
</tr>
<tr>
<td>Bound Residues</td>
<td>0.02</td>
<td>12.8</td>
</tr>
</tbody>
</table>

**Metabolite Identification:** The soluble fractions were cleaned-up for analysis independently as outlined in figures 2-4. The extracts were partitioned against hexane and the resulting aqueous fraction was partitioned with methylene chloride. The methylene chloride and aqueous extracts were then analyzed using HPLC. Fipronil and eight metabolites were resolved on a C-18 column with
gradient elution. The identity of all metabolites found in the corn samples was confirmed by TLC and MS.

**Nature of the Residue in Forage:** The methylene chloride extracts were analyzed on HPLC. Fipronil per se was found to comprise 39.9% of the TRR; MB46136, 8.7%; and RPA200766, 12.7% (Table 4) for a total of 61.3% of the TRR identified. The aqueous fractions were not chromatographed so that 4.8% of the TRR was found to be extractable but not further analyzed.

**Nature of the Residue in Fodder:** The methylene chloride extracts were analyzed on HPLC. The methylene chloride and aqueous extracts of the 8:2 ACN:water partitioning and the aqueous extract of the 2:8 ACN:water partitioning were subjected to enzymatic (β-glucosidase) and acid hydrolysis prior to analysis on HPLC. Fipronil per se was found to comprise 12.1% of the TRR; MB46136, 27.6%; RPA200761, 7.7%; MB45950, 1.7%; and RPA200766, 25.3% (Table 4) for a total of 74.4% of the TRR identified. A total of seven unknown peaks were also observed, accounting for a total of 11.1% of the TRR. The aqueous fractions which were not chromatographed comprised 17% of the TRR.
Table 4- Metabolite identification of extractable residues in corn RACs.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Forage</th>
<th></th>
<th>Fodder</th>
<th></th>
<th>Grain</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppm</td>
<td>% TRR</td>
<td>ppm</td>
<td>% TRR</td>
<td>ppm</td>
<td>% TRR</td>
</tr>
<tr>
<td>Fipronil</td>
<td>0.08</td>
<td>39.9</td>
<td>0.45</td>
<td>12.1</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>MB46136</td>
<td>0.02</td>
<td>8.7</td>
<td>1.02</td>
<td>27.6</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>RPA200766</td>
<td>0.03</td>
<td>12.7</td>
<td>0.94</td>
<td>25.3</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>RPA200761</td>
<td>ND</td>
<td></td>
<td>0.29</td>
<td>7.7</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>MB45950</td>
<td>ND</td>
<td></td>
<td>0.06</td>
<td>1.7</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>RPA200766</td>
<td>ND</td>
<td></td>
<td>ND</td>
<td></td>
<td>0.14</td>
<td>87.5</td>
</tr>
<tr>
<td>Conjugate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown 1</td>
<td>&lt;0.01</td>
<td></td>
<td>0.20</td>
<td>5.42</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Unknown 2</td>
<td>ND</td>
<td></td>
<td>0.02</td>
<td>0.7</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Unknown 3</td>
<td>ND</td>
<td></td>
<td>0.02</td>
<td>0.7</td>
<td>ND</td>
<td></td>
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<tr>
<td>Unknown 4</td>
<td>&lt;0.01</td>
<td></td>
<td>0.01</td>
<td>0.3</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Unknown 5</td>
<td>ND</td>
<td></td>
<td>0.10</td>
<td>2.8</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Unknown 6</td>
<td>ND</td>
<td></td>
<td>0.02</td>
<td>0.6</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Unknown 7</td>
<td>ND</td>
<td></td>
<td>0.02</td>
<td>0.6</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>TRR not</td>
<td>0.01</td>
<td>4.8</td>
<td>0.63</td>
<td>17.0</td>
<td>0.01</td>
<td>5.7</td>
</tr>
<tr>
<td>Analyzed</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Identified</td>
<td>0.13</td>
<td>61.3</td>
<td>2.68</td>
<td>74.4</td>
<td>0.14</td>
<td>87.5</td>
</tr>
</tbody>
</table>

ND = Not Detected

**Nature of the Residue in Grain:** The methylene chloride extracts were acid-hydrolyzed and analyzed on HPLC. A conjugate of RPA200766 (87.5% of the TRR, Table 4) was the only metabolite identified. The aqueous fractions were not chromatographed so that 5.7% of the TRR was found to be extractable but not further analyzed.

**Bound Residues:** The levels of bound residues did not exceed 10% of the TRR and 0.05 ppm in any sample so that further characterization was not required.

**Storage Stability:** The registrant has reanalyzed the samples using the same extraction procedures utilized in the original report, thus allowing comparison of samples analyzed after 11-13 months of storage with samples stored for 34-38 months. The report includes a comparison of quantitative results from the initial analysis with qualitative results of the second analysis and a comparison of chromatograms from the two analyses. Inspection of these chromatograms reveals substantial differences. Also the question
of storage stability can be addressed by comparison of the quantitative analysis of the samples in this report (Table 4) with the results of the initial report (Table 5, copied from Memo of G. Kramer 6/7/94). Again, the results are substantially different. For grain, >38% of the TRR was identified as fipronil per se initially, while 88% of the TRR was identified as a conjugate of RPA200766 in the second analysis. Metabolite RPA105048 was a major component of the forage and fodder residue in the initial analysis but was not found in the second analysis. The registrant attributes this finding to misidentification of RPA200761 as RPA105048 in the initial analysis as a result of similar HPLC retention times. However, CBTS has reexamined the original report and notes that in the HPLC method used, these compounds were very well resolved and that the identity of the peak in question as RPA105048 was confirmed by MS. Furthermore, RPA105048 was initially identified as a major metabolite of forage (24% of the TRR), while neither RPA200761 or RPA105048 were identified in forage in the second analysis.

Table 5- Results of initial metabolite identification of extractable residues in corn RACs as reported in Memo, G. Kramer 6/7/94.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Forage ppm</th>
<th>% TRR</th>
<th>Fodder ppm</th>
<th>% TRR</th>
<th>Grain ppm</th>
<th>% TRR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fipronil</td>
<td>0.06</td>
<td>23.93</td>
<td>0.24</td>
<td>6.31</td>
<td>0.08</td>
<td>38.28</td>
</tr>
<tr>
<td>RPA105048</td>
<td>0.05</td>
<td>23.57</td>
<td>0.52</td>
<td>13.92</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>RPA200766</td>
<td>ND</td>
<td>-</td>
<td>0.44</td>
<td>11.74</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>MB46136</td>
<td>ND</td>
<td>-</td>
<td>0.42</td>
<td>11.25</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>MB45950</td>
<td>ND</td>
<td>-</td>
<td>0.08</td>
<td>2.24</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>Unknown 1</td>
<td>ND</td>
<td>-</td>
<td>1.20</td>
<td>32.12</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>Unknown 2</td>
<td>ND</td>
<td>-</td>
<td>0.18</td>
<td>4.83</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>Unknown 3</td>
<td>0.02</td>
<td>7.86</td>
<td>ND</td>
<td>-</td>
<td>0.06</td>
<td>28.21</td>
</tr>
<tr>
<td>Unknown 4</td>
<td>ND</td>
<td>-</td>
<td>0.03</td>
<td>0.67</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>TRR not Analyzed</td>
<td>0.06</td>
<td>26.33</td>
<td>0.07</td>
<td>1.80</td>
<td>0.06</td>
<td>28.21</td>
</tr>
<tr>
<td>Total Identified</td>
<td>0.11</td>
<td>47.50</td>
<td>1.70</td>
<td>45.46</td>
<td>0.08</td>
<td>38.28</td>
</tr>
</tbody>
</table>

ND = Not Detected

**CBTS' Conclusion:** By reanalysis of the stored phenyl-labelled corn samples, the registrant has successfully addressed the deficiencies in metabolite identification, (ii) and (iii). However, based on the data submitted, storage stability can not be demonstrated for
the samples of this study. It appears that MB46136 and RPA200766 are the major metabolites of fipronil in corn, but conclusions on the relative amounts of the parent and metabolites and on the presence of RPA105048 can not be reached. However, CBTS will not require the registrant to repeat the phenyl-labelled study if the results of the required corn metabolism using pyrazole-labelled fipronil (see below) are qualitatively the same as the results with the phenyl label. The proposed metabolic pathway is shown in figure 5 (copied from p. 69 of MRID# 434011-04).

Deficiency - Conclusion 4b(iv) (from Memo, G. Kramer 6/7/94)

(iv) Fipronil contains two rings but the registrant has performed metabolism studies using only [14C]phenyl-labelled fipronil. In order to fully characterize the nature of the residue in corn, the registrant should perform a metabolism study in corn using [14C]pyrazole-labelled fipronil.

Petitioner's Response: None

CBTS' Conclusion: The requested information has not been provided. This deficiency remains outstanding.

CBTS will refer to the Metabolism Committee on the toxicological significance of metabolites once the deficiencies associated with plant metabolism have been addressed. A decision by CBTS concerning which residues to regulate will then follow. A tolerance based on the parent and metabolites MB46136 and MB45950 may not be appropriate; in such an instance a revised Section F and additional field studies, analytical methodology, and storage stability data may be needed.

Nature of Residue- Animals

Deficiency - Conclusion 5 (from Memo, G. Kramer 6/7/94)

5. The nature of the residue in animals has not been reported. These data will not be required for this EUF due to the label restrictions against the feeding of treated RACs to livestock and the limited number of acres involved. However, acceptable nature of the residue studies in ruminants and poultry will be required for the permanent tolerance petition. These studies should utilize fipronil labelled in both rings or separate studies should be performed using [14C]phenyl- and [14C]pyrazole-labelled fipronil. If there are significant fipronil metabolites formed in corn which are not also formed in animals, then CBTS may also require metabolism studies using any such metabolites.
Petitioner's Response - Ruminants: Submitted with this petition:

\(^{14}\)C-M&B46.030: Absorption, Distribution, Metabolism and Excretion Following Repeat Oral Administration to the Dairy Goat. Performing Laboratory: Hazleton Europe. MRID# 434011-05

In-Life Phase: [Phenyl(\(U\)-\(^{14}\)C)-fipronil (19.2 mCi/mmol) was isotopically diluted, placed in a gelatin capsule and administered orally to lactating goats (weight of 45-60 kg, age 3-7 years) with the aid of a balling gun. The goats were dosed at a total rate of 0.05 ppm, 2 ppm or 10 ppm per day. Doses were administered twice daily for 7 consecutive days. The animals were sacrificed approximately 24 hours after administration of the final dose.

Quantitation of Total Radioactivity: Milk was collected twice daily. Tissues were obtained after sacrifice. The distribution of the radioactivity is shown in Table 6. Of the administered radioactivity, 18-64% was recovered in feces, 1-5% in the milk and 8-25% was recovered in the tissues. The total recovery was 50-83%. The TRR in tissues and milk is shown in Table 7. The greatest tissue residues were observed in fat (1.9 ppm at 10 ppm dose).

Table 6- Total recovery of radioactivity from lactating goats treated with phenyl-labelled fipronil at a dietary burden of 0.05, 2 or 10 ppm for 7 consecutive days.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>% of Total Radioactivity Administered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.05 ppm</td>
</tr>
<tr>
<td>Urine</td>
<td>ND</td>
</tr>
<tr>
<td>Feces</td>
<td>64.16</td>
</tr>
<tr>
<td>Milk</td>
<td>0.86</td>
</tr>
<tr>
<td>Cage Wash/Debris</td>
<td>ND</td>
</tr>
<tr>
<td>Tissues</td>
<td>18.31</td>
</tr>
<tr>
<td>Total</td>
<td>83.32</td>
</tr>
</tbody>
</table>

ND = Not Detected
Table 7- TRR in goat milk and tissues following treatment with phenyl-labelled fipronil at a dietary burden of 0.05, 2 or 10 ppm for 7 consecutive days.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>TRR (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.05 ppm</td>
</tr>
<tr>
<td>Liver</td>
<td>0.004</td>
</tr>
<tr>
<td>Kidney</td>
<td>ND</td>
</tr>
<tr>
<td>Renal Fat</td>
<td>0.009</td>
</tr>
<tr>
<td>Omental Fat</td>
<td>0.008</td>
</tr>
<tr>
<td>Skeletal Muscle</td>
<td>0.003</td>
</tr>
<tr>
<td>Milk'</td>
<td>0.001</td>
</tr>
</tbody>
</table>

'Day 7 sample
ND = Not Detected

Extraction: All milk, tissue and feces samples (from the 2 and 10 ppm dose) were extracted in acetonitrile. The tissue extracts were washed with hexane. Approximately 90% of the TRR in all samples was organic-soluble. As the minimal loss of radioactivity appeared to be procedural, the TRR value was corrected for the % recovery prior to analysis.

Metabolite Identification: Organic-soluble residues were analyzed by HPLC and the retention times compared with those of possible metabolites (figure 1). The identity of metabolites was confirmed by GC-MS. The results presented below are for the 10 ppm dose level. The results for the 2 ppm dose level were similar with the exception that a greater percentage of fipronil was metabolized to MB46136 in the milk, muscle and fat samples at 2 ppm.

Nature of the Residue in Feces: MB461363 was the major component of the residue, accounting for 44% of the TRR. Fipronil, RPA200766 and MB45950 were also identified, accounting for 25%, 0.7% and 15% of the TRR, respectively.

Nature of the Residue in Milk: Fipronil per se was the predominant component of the residue, accounting for 59.8% of the TRR (Table 8). The metabolites MB46136 and MB45950 were also identified, accounting for 11.7% and 22.5% of the TRR, respectively. A total of 94% of the TRR was identified.

Nature of the Residue in Muscle: Fipronil per se was the
predominant component of the residue, accounting for 60.8% of the TRR (Table 8). The metabolites RPA200766, MB45950 and MB46136 were also identified, accounting for 7.2%, 8.3% and 20.5% of the TRR, respectively. A total of 97% of the TRR was identified.

**Nature of the Residue in Kidney:** MB46136 was the predominant component of the residue, accounting for 75.1% of the TRR (Table 8). Fipronil was also identified, accounting for 3.2% of the TRR. A total of 78% of the TRR was identified.

**Nature of the Residue in Omental Fat:** Fipronil *per se* was the predominant component of the residue, accounting for 73.2% of the TRR (Table 8). The metabolites RPA200766, MB45950 and MB46136 were also identified, accounting for 0.6%, 5.5% and 16.8% of the TRR, respectively. A total of 96% of the TRR was identified.

**Nature of the Residue in Renal Fat:** Fipronil *per se* was the predominant component of the residue, accounting for 72.7% of the TRR (Table 8). The metabolites RPA200766, MB45950 and MB46136 were also identified, accounting for 0.7%, 6.0% and 18.0% of the TRR, respectively. A total of 97% of the TRR was identified.

**Nature of the Residue in Liver:** MB46136 was the predominant component of the residue, accounting for 52.9% of the TRR (Table 8). Fipronil and RPA200766 were also identified, accounting for 1.5% and 11.3% of the TRR, respectively. A total of 7 unknown peaks were also observed. The most abundant peak, U24, accounted for 10.7% of the TRR. However, as this peak was comprised of more than one compound, there was no single unknown which comprised >10% of the TRR. Further efforts to identify these unknown metabolites will thus not be required. A total of 66% of the TRR was identified.
Table 8- Metabolite identification in goat milk and tissues following treatment with phenyl-labelled fipronil at a dietary burden of 10 ppm for 7 consecutive days

<table>
<thead>
<tr>
<th>Metabolite/Unknown (Retention Time)</th>
<th>10 ppm Diet ppm</th>
<th>% TRR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Milk</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fipronil</td>
<td>0.099</td>
<td>59.77</td>
</tr>
<tr>
<td>MB45950</td>
<td>0.019</td>
<td>11.68</td>
</tr>
<tr>
<td>MB46136</td>
<td>0.037</td>
<td>22.52</td>
</tr>
<tr>
<td>U64</td>
<td>0.003</td>
<td>1.53</td>
</tr>
<tr>
<td>Total Identified</td>
<td>0.155</td>
<td>93.97</td>
</tr>
<tr>
<td><strong>Muscle</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPA200766</td>
<td>0.006</td>
<td>7.22</td>
</tr>
<tr>
<td>Fipronil</td>
<td>0.048</td>
<td>60.76</td>
</tr>
<tr>
<td>MB45950</td>
<td>0.007</td>
<td>8.26</td>
</tr>
<tr>
<td>MB46136</td>
<td>0.016</td>
<td>20.51</td>
</tr>
<tr>
<td>U61</td>
<td>0.001</td>
<td>1.79</td>
</tr>
<tr>
<td>Total Identified</td>
<td>0.077</td>
<td>96.75</td>
</tr>
<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U22</td>
<td>0.002</td>
<td>1.51</td>
</tr>
<tr>
<td>U24</td>
<td>0.005</td>
<td>3.22</td>
</tr>
<tr>
<td>U25</td>
<td>0.005</td>
<td>3.40</td>
</tr>
<tr>
<td>Fipronil</td>
<td>0.005</td>
<td>3.21</td>
</tr>
<tr>
<td>MB46136</td>
<td>0.113</td>
<td>75.06</td>
</tr>
<tr>
<td>U32</td>
<td>0.001</td>
<td>0.39</td>
</tr>
<tr>
<td>Total Identified</td>
<td>0.118</td>
<td>78.27</td>
</tr>
<tr>
<td><strong>Omental Fat</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPA 200766</td>
<td>0.012</td>
<td>0.64</td>
</tr>
<tr>
<td>Fipronil</td>
<td>1.405</td>
<td>73.19</td>
</tr>
<tr>
<td>MB45950</td>
<td>0.105</td>
<td>5.47</td>
</tr>
<tr>
<td>MB46136</td>
<td>0.323</td>
<td>16.85</td>
</tr>
<tr>
<td>U62</td>
<td>0.018</td>
<td>0.94</td>
</tr>
<tr>
<td>Total Identified</td>
<td>1.740</td>
<td>96.15</td>
</tr>
<tr>
<td>Metabolite/Unknown (Retention Time)</td>
<td>10 ppm Diet</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ppm</td>
<td>% TRR</td>
</tr>
<tr>
<td>Renal Fat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPA200766</td>
<td>0.014</td>
<td>0.74</td>
</tr>
<tr>
<td>Fipronil</td>
<td>1.414</td>
<td>72.72</td>
</tr>
<tr>
<td>MB45950</td>
<td>0.117</td>
<td>6.04</td>
</tr>
<tr>
<td>MB46136</td>
<td>0.349</td>
<td>17.95</td>
</tr>
<tr>
<td>U62</td>
<td>0.018</td>
<td>0.94</td>
</tr>
<tr>
<td>Total Identified</td>
<td>1.894</td>
<td>97.45</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U19</td>
<td>0.039</td>
<td>4.49</td>
</tr>
<tr>
<td>RPA200766</td>
<td>0.098</td>
<td>11.32</td>
</tr>
<tr>
<td>U21</td>
<td>0.023</td>
<td>2.67</td>
</tr>
<tr>
<td>U22</td>
<td>0.052</td>
<td>6.00</td>
</tr>
<tr>
<td>U23</td>
<td>0.018</td>
<td>2.13</td>
</tr>
<tr>
<td>U24</td>
<td>0.092</td>
<td>10.70</td>
</tr>
<tr>
<td>U25</td>
<td>0.021</td>
<td>2.42</td>
</tr>
<tr>
<td>U26</td>
<td>0.005</td>
<td>0.53</td>
</tr>
<tr>
<td>Fipronil</td>
<td>0.013</td>
<td>1.54</td>
</tr>
<tr>
<td>MB46136</td>
<td>0.456</td>
<td>52.93</td>
</tr>
<tr>
<td>U41</td>
<td>0.019</td>
<td>2.22</td>
</tr>
<tr>
<td>Total Identified</td>
<td>0.567</td>
<td>65.79</td>
</tr>
</tbody>
</table>

**Storage Stability:** The actual dates of sample extraction and analysis were not provided in this report so that the sample storage interval could not be calculated. As fipronil and its metabolites comprised >90% of the TRR in the milk, fat and muscle samples, storage stability is not an issue for these matrices. However, the liver sample contains numerous unidentified compounds which account for a large portion of the TRR (33%, 0.28 ppm). The actual dates of sample analysis should thus be provided. If the liver samples were stored for longer than 6 months, then evidence of storage stability should be provided.

**CBTS' Conclusion:** Provided that storage stability of the goat
liver samples can be demonstrated, the nature of the residue in ruminants is considered to be understood. Fipronil, RPA200766, MB45950 and MB46136 are the primary components of the residue, accounting for 66-97% of the TRR. Metabolism of fipronil proceeds via: 1) oxidation of the parent sulfoxide to the sulfone, MB46136, which is conjugated and excreted in the urine; 2) reduction of the parent sulfoxide to the sulfide, MB45950; and 3) hydrolysis of the parent nitrile to the amide, RPA200761. These three pathways were also observed in corn.

For compounds with multiple rings, CBTS generally requires that metabolism studies be performed with each ring labelled. However, as long as there is no evidence of ring cleavage, further animal metabolism studies for fipronil will not be required.

**Poultry:** Submitted with this petition:

\(^{14}\)C-M&B46.030: Distribution, Metabolism and Excretion Following Repeat Oral Administration to the Laying Hen. Performing Laboratory: Hazleton Europe. MRID# 434011-06

**In-Life Phase:** [Phenyl(U)-\(^{14}\)C]-fipronil (19.2 mCi/mmol) was isotopically diluted, placed in a gelatin capsule and administered orally to laying hens (weight of 1.72 ± 0.14 kg, age 19 weeks). The hens were dosed at a rate of 0.05 ppm, 2 ppm or 10 ppm. There were five birds in each dosing group. Doses were administered daily for 28 consecutive days. The animals were sacrificed approximately 24 hours after administration of the final dose.

**Quantitation of Total Radioactivity:** Eggs were collected twice daily. Tissues were obtained after sacrifice. The results presented are the average of the values for the individual birds of each dosing group. The distribution of the radioactivity is shown in Table 9. Of the administered radioactivity, 28-42% was recovered in excreta, 15-18% in the eggs and 1-5% was recovered in the tissues. The total recovery was 52-58%. The TRR in tissues and eggs is shown in Table 10. The greatest tissue residues were observed in fat (56 ppm at 10 ppm dose). Residues in eggs were also extremely high (30 ppm in yolks at 10 ppm dose) and had not plateaued by the end of the study (28 days).
Table 9- Average total recovery of radioactivity from laying hens treated with phenyl-labelled fipronil at a dietary burden of 0.05, 2 or 10 ppm for 28 consecutive days.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>% of Total Radioactivity Administered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.05 ppm</td>
</tr>
<tr>
<td>Excreta</td>
<td>28.35</td>
</tr>
<tr>
<td>Egg White</td>
<td>1.99</td>
</tr>
<tr>
<td>Egg Yolk</td>
<td>16.11</td>
</tr>
<tr>
<td>Cage Wash/Debris</td>
<td>0.04</td>
</tr>
<tr>
<td>Tissues</td>
<td>5.40</td>
</tr>
<tr>
<td>Total</td>
<td>51.90</td>
</tr>
</tbody>
</table>

Table 10- Average TRR in hen eggs and tissues following treatment with phenyl-labelled fipronil at a dietary burden of 0.05, 2 or 10 ppm for 28 consecutive days.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>TRR (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.05 ppm</td>
</tr>
<tr>
<td>Skin</td>
<td>0.101</td>
</tr>
<tr>
<td>Fat</td>
<td>0.286</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.005</td>
</tr>
<tr>
<td>Liver</td>
<td>0.030</td>
</tr>
<tr>
<td>Egg White'</td>
<td>0.008</td>
</tr>
<tr>
<td>Egg Yolk'</td>
<td>0.177</td>
</tr>
</tbody>
</table>

*Day 28 sample
ND = Not Detected

**Extraction:** All egg, tissue and excreta samples were extracted in acetonitrile. The tissue extracts were washed with hexane. Approximately 90% of the TRR in all tissue and egg samples was organic-soluble. As the minimal loss of radioactivity appeared to be procedural, the TRR value was corrected for the % recovery prior to analysis.
Metabolite Identification: Organic-soluble residues were analyzed by HPLC and the retention times compared with those of possible metabolites (figure 1). The identity of metabolites was confirmed by GC-MS. The results presented below are for the 10 ppm dose level. The results for the 0.05 and 2 ppm dose level were very similar.

Nature of the Residue in Excreta: Fipronil was the major component of the residue, accounting for 51% of the TRR. MB461363 was also identified, accounting for 34% of the TRR.

Nature of the Residue in Egg Yolk: MB46136 was the predominant component of the residue, accounting for 96.4% of the TRR (Table 11). Fipronil was also identified, accounting for 2.6% of the TRR. A total of 99% of the TRR was identified.

Nature of the Residue in Egg White: MB46136 was the only component of the residue, accounting for 94.6% of the TRR (Table 11).

Nature of the Residue in Skin: MB46136 was the predominant component of the residue, accounting for 98.3% of the TRR (Table 11). Fipronil was also identified, accounting for 1.6% of the TRR. A total of 99.9% of the TRR was identified.

Nature of the Residue in Muscle: MB46136 was the only component of the residue, accounting for 99.9% of the TRR (Table 11).

Nature of the Residue in Peritoneal Fat: MB46136 was the predominant component of the residue, accounting for 97.1% of the TRR (Table 11). Fipronil was also identified, accounting for 1.9% of the TRR. A total of 99% of the TRR was identified.

Nature of the Residue in Liver: MB46136 was the predominant component of the residue, accounting for 98.5% of the TRR (Table 11). Fipronil was also identified, accounting for 1.4% of the TRR. A total of 99.9% of the TRR was identified.
Table 11: Metabolite identification in hen eggs and tissues following treatment with phenyl-labelled fipronil at a dietary burden of 10 ppm for 28 consecutive days

<table>
<thead>
<tr>
<th>Metabolite/Unknown (Retention Time)</th>
<th>10 ppm Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppm</td>
</tr>
<tr>
<td><strong>Egg Yolk</strong></td>
<td></td>
</tr>
<tr>
<td>Fipronil</td>
<td>0.789</td>
</tr>
<tr>
<td>MB46136</td>
<td>29.020</td>
</tr>
<tr>
<td>Total Identified</td>
<td>29.809</td>
</tr>
<tr>
<td><strong>Egg White</strong></td>
<td></td>
</tr>
<tr>
<td>Fipronil</td>
<td>ND</td>
</tr>
<tr>
<td>MB46136</td>
<td>0.939</td>
</tr>
<tr>
<td>Total Identified</td>
<td>0.939</td>
</tr>
<tr>
<td><strong>Skin</strong></td>
<td></td>
</tr>
<tr>
<td>Fipronil</td>
<td>0.279</td>
</tr>
<tr>
<td>MB46136</td>
<td>16.742</td>
</tr>
<tr>
<td>Total Identified</td>
<td>17.022</td>
</tr>
<tr>
<td><strong>Peritoneal Fat</strong></td>
<td></td>
</tr>
<tr>
<td>Fipronil</td>
<td>0.820</td>
</tr>
<tr>
<td>MB46136</td>
<td>54.916</td>
</tr>
<tr>
<td>Total Identified</td>
<td>55.996</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
</tr>
<tr>
<td>Fipronil</td>
<td>0.066</td>
</tr>
<tr>
<td>MB46136</td>
<td>4.815</td>
</tr>
<tr>
<td>Total Identified</td>
<td>4.882</td>
</tr>
<tr>
<td><strong>Muscle</strong></td>
<td></td>
</tr>
<tr>
<td>Fipronil</td>
<td>ND</td>
</tr>
<tr>
<td>MB46136</td>
<td>0.730</td>
</tr>
<tr>
<td>Total Identified</td>
<td>0.730</td>
</tr>
</tbody>
</table>

ND = Not Detected
Storage Stability: The actual dates of sample extraction and analysis were not provided in this report so that the sample storage interval could not be calculated. As fipronil and its metabolite MB46136 comprised >90% of the TRR in all egg and tissue samples, storage stability is not an issue.

CBTS' Conclusion: The nature of the residue in poultry is considered to be understood. Fipronil and MB46136 are the primary components of the residue, accounting for 95-100% of the TRR. Metabolism of fipronil proceeds via oxidation of the parent sulfoxide to the sulfone, MB46136. For compounds with multiple rings, CBTS generally requires that metabolism studies be performed with each ring labelled. However, as long as there is no evidence of ring cleavage, further animal metabolism studies for fipronil will not be required.

CBTS will refer to the Metabolism Committee on the toxicological significance of metabolites once the deficiencies associated with ruminant metabolism have been addressed. A decision by CBTS concerning which residues to regulate will then follow. A tolerance based on the parent and metabolites MB46136 and MB45950 may not be appropriate; in such an instance a revised Section F and additional feeding studies, analytical methodology, and storage stability data may be needed.

Analytical Methodology- Plants

Submitted with PP# 3G04263:

Fipronil- Validation of Method of Analysis for Fipronil and Its Metabolites in Field Corn. EC-93-236. MRID# 433234-01

Procedure: Samples (25 g) are extracted by homogenization in 100 ml acetonitrile. Solids are removed by filtration and NaCl is added to the extract. The extraction step is repeated once. After clean-up by liquid/liquid partitioning with hexane, the acetonitrile is removed by rotary evaporation. The aqueous solution is then extracted with dichloromethane. The dichloromethane solution is concentrated and cleaned-up using a silica gel and charcoal column with elution in dichloromethane and 50% dichloromethane in acetonitrile. Fipronil and its metabolites are then analyzed in a single chromatographic separation using GC with ECD.

Results: Acceptable recoveries were obtained for all analytes except MB45950 (average recovery of 65%). Reproducible recoveries
of MB45950 in the range of 50-70% may be acceptable as this metabolite does not appear to comprise a significant portion of the total fipronil residues in corn. Note that validation using metabolites MB46513, RPA105048 and RPA200766 was also successful.

ILV: An ILV of this method was performed by Colorado Analytical Research and Development Co. Acceptable recoveries were obtained by the laboratory for all analytes except MB45950 (average recovery of 56.7% at 0.04 ppm). The method and ILV were sent to Beltsville for PMV (Memo, G. Kramer 9/12/94).

PMV: The method has been validated by ACL (Memo, G. Kramer 3/28/95). Acceptable recoveries were obtained for fipronil, MB45950, MB46136, RPA105048 and RPA200766. The LOQ for each compound is 0.01 ppm in grain and 0.02 ppm in forage and fodder. The registrant should submit a revised version of the proposed analytical enforcement method specified in conclusions 1-3 of Memo, G. Kramer (3/28/95) and send analytical standards of fipronil and its metabolites to the EPA Repository, RTP. Until the receipt of the standards and the revised method, the requirements for analytical enforcement methodology will remain unfulfilled.

Multiresidue Method Testing: A report on Multiresidue testing of fipronil and its metabolites MB45950 and MB46136 (MRID# 434011-07) has been received and forwarded to FDA (Memo, G. Kramer 5/9/95). Acceptable recoveries of fipronil and its metabolites were obtained in corn grain using Protocol E. Recoveries in forage were 38-65% using Protocol E.

Specificity: The specificity of the proposed analytical enforcement method was demonstrated by performing an interference study with 45 of the pesticides for which tolerances are established on corn. The registrant reports that 10 of these compounds were found to have "minor interferences" with fipronil or its metabolites. The registrant should submit chromatograms in which fipronil and its metabolites are injected both with and without these pesticides so that CBTS can determine whether the reported "minor interferences" are of concern.

Radiovalidation: No data have been submitted. Samples of radiolabelled forage and fodder from the metabolism study must be analyzed with the proposed enforcement method. Based on the results of the plant metabolism study, only 25% of the TRR in fodder samples would be extractable by the proposed enforcement method.

Confirmatory Method: The registrant has included conditions for separation on a different GC column (HP-5 instead of HP-1) and use of GC/MS as confirmatory techniques. The Multiresidue Method is also available as a confirmatory technique.
Conclusions: CBTS concludes that Method EC-93-236 is adequate for data gathering purposes. A conclusion on the adequacy of the method for enforcement of the proposed tolerances will be withheld pending satisfactory method validation (radiovalidation and interference study).

Analytical Methodology- Animals

Submitted with this petition:

Fipronil- Validation of Method of Analysis for Fipronil and Its Metabolites in Animal Tissues. EC-94-258. MRID# 434011-10

Procedure: Samples (25 g) are extracted by homogenization in 100 ml 30% acetone in acetonitrile. The extract is concentrated and cleaned-up using a silica gel and charcoal column with acetonitrile elution. Fipronil and its metabolites are then analyzed in a single chromatographic separation using GC with ECD.

Results: Acceptable recoveries were obtained for all analytes in eggs and beef fat and kidney. The LOQs were reported to be 0.002 ppm for fipronil, 0.003 ppm for MB45950 and 0.004 ppm for MB46136 in eggs; 0.004 ppm for fipronil, 0.003 ppm for MB45950 and 0.002 ppm for MB46136 in kidney; and 0.020 ppm for fipronil and MB45950 and 0.008 ppm for MB46136 in fat.

ILV: An ILV of this method was performed by CYAL, Inc (MRID# 434011-10). Acceptable recoveries were obtained by the laboratory for all analytes.

PMV: The method and ILV were sent to Beltsville for PMV (Memo, G. Kramer 5/11/95). CBTS will withhold a final conclusion on the adequacy of this method as analytical enforcement method pending receipt of the PMV report.

Specificity: The specificity of the proposed analytical enforcement method was demonstrated by performing an interference study with 115 of the pesticides for which tolerances are established on animal commodities. None was found to interfere with fipronil or its metabolites.

Radiovalidation: Samples of radiolabelled goat fat from the metabolism study were analyzed by the proposed enforcement method:

<table>
<thead>
<tr>
<th>Study</th>
<th>ppm Fipronil</th>
<th>ppm MB45950</th>
<th>ppm MB46136</th>
<th>Total ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolism</td>
<td>1.405</td>
<td>0.105</td>
<td>0.323</td>
<td>1.833</td>
</tr>
<tr>
<td>-----------------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Enforcement Method</td>
<td>1.285</td>
<td>0.103</td>
<td>0.335</td>
<td>1.723</td>
</tr>
</tbody>
</table>

As the results obtained from the metabolism study and the enforcement method do not differ significantly, CBTS concludes that this method has been successfully radiovalidated.

**Confirmatory Method:** The registrant has included conditions for separation on a different GC column (DB-5ms instead of DB-1701) as a confirmatory technique.

**Conclusions:** CBTS concludes that Method EC-94-258 is adequate for data gathering purposes. A conclusion on the adequacy of the method for enforcement of the proposed tolerances will be withheld pending satisfactory method validation (PMV).

**Storage Stability**

**Deficiency - Conclusion 8 (from Memo, G. Kramer 6/7/94)**

8. No storage stability data were submitted with this petition. For the permanent tolerance petition, the registrant must demonstrate storage stability for fipronil and every metabolite of toxicological concern in corn RACs and processed fractions and animal RACs for a period of time which corresponds to the maximum storage interval in the respective magnitude of the residue study.

**Petitioner's Response:** Submitted with this petition:

Storage Stability of Fipronil and Its Metabolites in Field Corn Substrates and Corn Processed Fractions. MRID# 435086-01

Samples of corn grain, forage, fodder, silage, crude oil, refined oil, grain dust, meal and starch were spiked with 0.1 ppm of fipronil, MB45950, MB46136, RPA105048 and RPA200766 and stored frozen at <-10 °C. Samples were maintained frozen and two subsamples were removed and analyzed at various intervals for residues using the proposed enforcement method over the course of 1 year. Each analysis included one freshly fortified control. The results demonstrate that residues of fipronil and its metabolites are stable during storage in corn substrates for up to 12 months. As the samples from the field residue trials and processing study were stored for a maximum of 10 months, storage stability is not an issue for this petition. Fipronil and its metabolites MB45950, MB46136, RPA105048 and RPA200766 in/on corn RACs and processed commodities are stable in frozen storage for at least 1 year.
Magnitude of Residue - Plants

Submitted with this petition:

Fipronil: Magnitude of the Residues on Corn Resulting from Applications of Granular Formulated Product (1993) MRID# 434011-12

Ten field corn trials were conducted in nine different states in 1993. Fipronil was applied as a 1.5% granular formulation prior to planting at a rate of 0.13 lbs. ai/A (0.12 oz ai/100 ft., 1X). T-band application was used at all sites and in-furrow application was also used at five sites. Three treated samples and one untreated control sample per matrix were harvested per trial. The forage, fodder and silage samples consisted of at least 12 randomly harvested plants per sample. The grain samples consisted of at least 12 randomly harvested ears per sample. Forage samples were harvested 44-47 days after planting. Silage samples were harvested at the dent stage, 96-129 days after planting. Grain and fodder samples were harvested 124-171 days after planting. The "silage" samples were not actually ensiled and are thus forage samples collected at the early dent stage. Samples were kept frozen prior to analysis. Fipronil and its metabolites were determined by the proposed analytical enforcement method. The method was validated in each RAC at the LOQ and 5X the LOQ. Untreated samples fortified at the LOQ were also analyzed with each set of samples. The average concurrent recoveries for fipronil were 81% in grain, 100% in forage, 95% in fodder and 105% in silage; for MB45950, 81% in grain, 93% in forage, 93% in fodder and 88% in silage; for MB46136, 108% in grain, 117% in forage, 103% in fodder and 108% in silage; for RPA105048, 97% in grain, 114% in forage, 112% in fodder and 118% in silage; and for RPA200766, 102% in grain, 116% in forage, 102% in fodder and 118% in silage. The results of the analysis of the field samples are summarized in Table 12. The levels of fipronil and its metabolites were below the LOQ in all grain samples. In forage, the maximum levels of fipronil observed were 0.056 ppm; of MB45950, were <0.02 ppm; of MB46136, were 0.044 ppm; of RPA105058, were <0.02 ppm; and of RPA200766, were 0.031 ppm. In silage, the maximum levels of fipronil observed were 0.022 ppm; of MB45950, were <0.02 ppm; of MB46136, were 0.036 ppm; and of RPA200766, were <0.02 ppm. In fodder, the maximum levels of fipronil observed were 0.020 ppm; of MB45950, were <0.02 ppm; of MB46136, were 0.073 ppm; and of RPA200766, were 0.030 ppm.
Table 12- Summary of field residue data for fipronil and its metabolites in field corn RACs.

<table>
<thead>
<tr>
<th>Trial</th>
<th>RAC</th>
<th>PHI</th>
<th>Maximum Residue (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fipronil</td>
</tr>
<tr>
<td>TX</td>
<td>Grain</td>
<td>148</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Forage</td>
<td>45</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>Silage</td>
<td>113</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>Fodder</td>
<td>148</td>
<td>0.020</td>
</tr>
<tr>
<td>NC</td>
<td>Grain</td>
<td>140</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Forage</td>
<td>45</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Silage</td>
<td>103</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>Fodder</td>
<td>140</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>NC</td>
<td>Grain</td>
<td>125</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Forage</td>
<td>44</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>Silage</td>
<td>96</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>Fodder</td>
<td>125</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>WA</td>
<td>Grain</td>
<td>168</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Forage</td>
<td>NC</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Silage</td>
<td>128</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Fodder</td>
<td>168</td>
<td>ND</td>
</tr>
<tr>
<td>NY</td>
<td>Grain</td>
<td>128</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Forage</td>
<td>45</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>Silage</td>
<td>106</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Fodder</td>
<td>128</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>CA</td>
<td>Grain</td>
<td>124</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Forage</td>
<td>45</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>Silage</td>
<td>109</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>Fodder</td>
<td>124</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>CO</td>
<td>Grain</td>
<td>171</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Forage</td>
<td>45</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>Silage</td>
<td>115</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Fodder</td>
<td>171</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>NE</td>
<td>Grain</td>
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<td>ND</td>
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<tr>
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<td>Forage</td>
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<td>0.028</td>
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<td>Silage</td>
<td>129</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Fodder</td>
<td>171</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>IA</td>
<td>Grain</td>
<td>131</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Forage</td>
<td>47</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>Silage</td>
<td>103</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>Fodder</td>
<td>131</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>IL</td>
<td>Grain</td>
<td>140</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Forage</td>
<td>45</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>Silage</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>----------</td>
<td>--------</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Fodder</td>
<td>140</td>
<td>&lt;0.02</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = Not Detected, Below the Limit of Detection (0.001-0.008 ppm, depending on the metabolite and RAC)
NC = Not Collected

**Conclusions:** Between these trials and those submitted previously, the registrant has submitted a total of 20 field corn residue trials. These trials were located in Regions 1 (1 trial), 2 (2 trials), 5 (13 trials), 6 (1 trial), 8 (1 trial), 10 (1 trial) and 11 (1 trial). This distribution does not correspond with that suggested for field corn in *EPA Guidance on Number and Location of Domestic Crop Field Trials for Establishment of Pesticide Residue Tolerances, 6/2/94: Regions 1 (1 trial), 2 (1 trial), 5 (17 trials) and 6 (1 trial)*. However, as the states in which these trials were performed represented >80% of U.S. corn acreage in 1991 (*Agricultural Statistics, 1992*), CBTS concludes that the number of trials and the geographic representation are adequate to establish tolerances for fipronil and its metabolites on corn. The residue data support the proposed tolerances of 0.02 ppm for grain, 0.15 ppm for forage and fodder. However, further residue data may be required if other metabolites are determined to be of regulatory significance. Existing samples could be analyzed in order to determine metabolite levels, provided storage stability can be demonstrated.

The registrant has proposed tolerances based on the sum of the actual residue levels or LOQ of each metabolite. However, tolerances are proposed for fipronil or its metabolites. The registrant should thus propose tolerances for "fipronil and its metabolites MB45950 and MB46136." A revised Section F which includes this tolerance expression is required. This revised Section F should also contain the chemical names of fipronil, MB45950 and MB46136. Section F may need further revision in accordance with the decision of the Metabolism Committee on the metabolites of toxicological concern.

Note that for the EUP, CBTS requested that the tolerance for grain be expressed as "residues of fipronil or..." However, for this petition in which quantifiable residues have been observed, this approach would result in overestimation of the dietary burden. The appropriate tolerance for fipronil or its metabolites MB45950 or MB46136 in forage and fodder would be 0.10 ppm. The dietary burden would be calculated using 0.30 ppm (the sum of 0.1 ppm each of fipronil, MB45950 and MB46136), twice that of the tolerance for fipronil and MB45950 and MB46136.
Magnitude of the Residue- Processed Fractions

Deficiency - Conclusion 10 (from Memo, G. Kramer 6/7/94)

10a. Corn was treated with fipronil at a rate of 20X and the grain processed after harvest. No detectable residues of fipronil, MB46136 or MB45950 were observed in any processed fraction. No feed or food additive tolerances are thus required for this EUP petition. Low, but detectable residues of two metabolites not proposed to be included in the tolerance expression were found in some processed fractions. However, due to the exaggerated application rate, it is unlikely measurable residues would be present at a 1X use rate. Thus, food additive tolerances would not be required for this temporary tolerance request.

10b. This processing study can also be used to support the permanent tolerance petition provided that: i) there are no metabolites determined to be of toxicological concern which are not accounted for in this study, and ii) the registrant submits adequate storage stability data for fipronil and all metabolites of toxicological concern in the processed fractions.

Petitioner's Response: Submitted with this petition:

Storage Stability of Fipronil and Its Metabolites in Field Corn Substrates and Corn Processed Fractions. MRID# 435086-01

The results demonstrate that residues of fipronil and its metabolites are stable during storage in corn substrates for up to 12 months.

Conclusions: As the samples from the processing study were stored for a maximum of 7 months, storage stability is not an issue for this petition. This processing study is acceptable provided that there are no metabolites determined to be of toxicological concern which were not accounted for.

Magnitude of the Residue- Ruminants

Submitted with this petition:

Fipronil: Magnitude of the Residues in Meat and Milk of Lactating Dairy Cows. Performing Laboratory: Southwest Bio-Labs, Inc. MRID# 434011-08

Holstein dairy cows were dosed daily for 35 consecutive days with fipronil at levels of 0, 0.04, 0.13 and 0.43 ppm in the diet. Each treatment group had three cows. Milk samples were taken twice daily. The cows were sacrificed 7 hours after administration of the final dose. Samples were stored for a maximum of 3 months prior to analysis. A concurrent storage stability study
demonstrated that fipronil and its metabolites were stable in animal matrices for this time period. Samples of milk and tissues were analyzed with the proposed enforcement method. The method was validated in milk and tissues over a range of 0.01-0.50 ppm. The overall recovery ranged from 79.8% to 113.5%. The results are expressed in terms of MB46136 equivalents. The maximum residues observed in milk and tissues are shown in Table 13. At the 0.43 ppm dietary burden, quantifiable residues of fipronil were observed only in fat (0.051 ppm); while quantifiable residues of MB46136 were observed in milk (0.052 ppm), liver (0.172 ppm), muscle (0.059 ppm), kidney (0.035 ppm) and fat (0.554 ppm). Residues in milk appeared to plateau by day 29. No quantifiable residues were of MB45950 were observed in any tissue.

Table 13- Maximum residues in cow tissues following 35 days of administration of fipronil at dietary burdens of 0.04, 0.13 and 0.43 ppm.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Maximum Residues (ppm) at Dietary Burden of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.04 ppm</td>
</tr>
<tr>
<td></td>
<td>Fipronil</td>
</tr>
<tr>
<td>Milk</td>
<td>ND</td>
</tr>
<tr>
<td>Liver</td>
<td>ND</td>
</tr>
<tr>
<td>Kidney</td>
<td>ND</td>
</tr>
<tr>
<td>Muscle</td>
<td>ND</td>
</tr>
<tr>
<td>Fat</td>
<td>&lt;0.010</td>
</tr>
</tbody>
</table>

**Dietary Burden:** As there are no established permanent tolerances for fipronil residues to date, the maximum dietary burden for dairy cows results from a diet comprised solely of corn forage and fodder:

<table>
<thead>
<tr>
<th>Feed Item</th>
<th>% Diet</th>
<th>Proposed Tolerance</th>
<th>% DM</th>
<th>ppm in Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forage</td>
<td>90</td>
<td>0.15 ppm</td>
<td>40</td>
<td>0.34</td>
</tr>
<tr>
<td>Fodder</td>
<td>10</td>
<td>0.15 ppm</td>
<td>83</td>
<td>0.02</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
<td></td>
<td>0.36</td>
</tr>
</tbody>
</table>

Whereas, the maximum dietary burden for beef cattle results from a diet comprised of corn forage, grain and fodder:

<table>
<thead>
<tr>
<th>Feed Item</th>
<th>% Diet</th>
<th>Proposed Tolerance</th>
<th>% DM</th>
<th>ppm in Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forage</td>
<td>40</td>
<td>0.15 ppm</td>
<td>40</td>
<td>0.15</td>
</tr>
<tr>
<td>Fodder</td>
<td>25</td>
<td>0.15 ppm</td>
<td>83</td>
<td>0.05</td>
</tr>
<tr>
<td>Grain</td>
<td>35</td>
<td>0.02 ppm</td>
<td>88</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Conclusions: Based on the estimated maximum dietary burden for beef cattle of 0.21 ppm, the dietary feeding levels in this study were 0.2X, 0.6X and 2X. Based on the estimated maximum dietary burden of 0.36 ppm for dairy cows, the dietary feeding levels in this study were 0.1X, 0.4X and 1.2X. Based on extrapolation of the results in Table 13 to the 1X level, the appropriate tolerances for fipronil and its metabolites MB45950 and MB46136 are:

- Milkfat (reflecting 0.06 ppm in whole milk) -- 2.0 ppm
- Fat' -- 0.40 ppm
- Meat' -- 0.04 ppm
- Meat By-Products (except liver)' -- 0.02 ppm
- Liver' -- 0.10 ppm

'of cattle, goats, horses, hogs and sheep

The registrant has proposed tolerances only for dairy cow RACs and not in terms of meat and meat by-products. However, tolerances are required for beef cattle, goats, horses, hogs and sheep. A Revised Section F is required. The above terminology should be included. Section F may need further revision in accordance with the decision of the Metabolism Committee on the metabolites of toxicological concern.

Note that the milk tolerance should be expressed in terms of "milkfat" as fipronil is fat soluble. The recommended tolerance for milkfat is derived from the estimated maximum residue in whole milk (0.06 ppm) and a theoretical concentration factor of 31X. However, the registrant should report the actual fat content of the milk in this study. The milkfat tolerance should then be revised, if necessary, based on the actual fat content of the milk from the cow in which residues were the greatest.

Ruminant feeding studies employing higher dosing levels may be required in the future if tolerances are proposed on other crops which increase the potential dietary exposure.

Magnitude of the Residue- Poultry

Submitted with this petition:

Fipronil: Magnitude of the Residues in Meat and Eggs of Laying Hens. Performing Laboratory: Southwest Bio-Labs, Inc. MRID#
Leghorn hens were dosed daily for 42 consecutive days with fipronil at levels of 0, 0.010, 0.031 and 0.103 ppm in the diet. Each treatment group had 10 hens. Egg samples were taken daily. The hens were sacrificed 3 hours after administration of the final dose. Samples were stored for a maximum of 3 months prior to analysis. A concurrent storage stability study demonstrated that fipronil and its metabolites were stable in animal matrices for this time period. Samples of eggs and tissues were analyzed with the proposed enforcement method. The method was validated in eggs and tissues over a range of 0.01-0.50 ppm. The overall recovery ranged from 67.5% to 117.1%. The results are expressed in terms of MB46136 equivalents. The maximum residues observed in eggs and tissues are shown in Table 14. At the 0.103 ppm dietary burden, quantifiable residues of MB46136 were observed in eggs (0.116 ppm), liver (0.072 ppm), muscle (0.014 ppm) and fat (0.214 ppm). Residues in eggs appeared to plateau by day 28. No quantifiable residues of fipronil or MB45950 were observed in any tissue.

Table 14- Maximum residues in hen tissues following 42 days of administration of fipronil at dietary burdens of 0.010, 0.031 and 0.103 ppm.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Maximum Residues (ppm) at Dietary Burden of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.010 ppm</td>
</tr>
<tr>
<td></td>
<td>Fipronil</td>
</tr>
<tr>
<td>Eggs</td>
<td>ND</td>
</tr>
<tr>
<td>Liver</td>
<td>ND</td>
</tr>
<tr>
<td>Muscle</td>
<td>ND</td>
</tr>
<tr>
<td>Skin/Fat</td>
<td>ND</td>
</tr>
</tbody>
</table>

**Dietary Burden:** As there are no established permanent tolerances for fipronil to date, the maximum dietary burden results from a poultry diet comprised solely of corn grain and milled by-products:

<table>
<thead>
<tr>
<th>Feed Item</th>
<th>% Diet</th>
<th>Recommended Tolerance</th>
<th>ppm in Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain</td>
<td>80</td>
<td>0.02 ppm</td>
<td>0.016</td>
</tr>
<tr>
<td>Milled Bypdts</td>
<td>20</td>
<td>0.02 ppm</td>
<td>0.004</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
<td>0.02</td>
</tr>
</tbody>
</table>

**Conclusions:** Based on the estimated maximum dietary burden of 0.02 ppm, the dietary feeding levels in this study were 0.5X, 1.6x and 5X. Based on extrapolation of the results in Table 14 to the 1X
Other Considerations

There is no Codex proposal, nor Canadian or Mexican limits for residues of fipronil and its metabolites in corn. Therefore, a compatibility issue is not relevant to the proposed tolerance. A copy of the IRLS is attached to this memorandum.

A DRES run will need to be conducted, but will not be requested until all deficiencies affecting the proposed tolerance levels have been resolved.

Attachments:

Figures 1-5
Attachment 1- IRLS sheet
Attachment 2- Confidential Appendix

cc (with all attachments): PP#5F04426, Kramer, R.F.
cc (without attachment 2): Circ.
RDI: F.D. Griffith (7/5/95), M.J. Nelson for R.A. Loranger (7/19/95), M.S. Metzger (7/24/95)
G.F. Kramer: 804T:CM#2: (703) 305-5079:7509C