Residue Chemistry Review 6/7/1994

Comments:

Subject: New Chemical- Fipronil in/on Corn RACs. Evaluation of residue data and analytical methods. MRID#s 428186 -01 to -12 and -65 to -68, and 429779-01. CBTS# 12927.

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
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- 830.7200 Melting point/melting range
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- 830.7300 Density/relative density/bulk density
- 830.7370 Dissociation constant in water
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- 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

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- 860.1200 Directions for use
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Biochemicals:

DP Barcode: D197451

MRIDs: 42818601, 42818602, 42818603, 42818604, 42818605, 42818606, 42818607, 42818608, 42818609, 42818610, 42818611, 42818612, 42818665, 42818666, 42818667, 42818668, 42977901

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

Actives/Inerts

CAS #: 120068-37-3

Commodities: Corn; Corn, Field

Administrative #: 3G04263

Reviewers: G. F. Kramer

Review Approver: Esther Saito **Approved on:** June 7, 1994

WP Document:  - Fipronil_005.wpd

MEMORANDUM

SUBJECT: PP# 3G04263. New Chemical- Fipronil in/on Corn RACs. Evaluation of residue data and analytical methods. MRID#s 428186 -01 to -12 and -65 to -68, and 429779-01. Barcode D197451. CBTS# 12927.

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

THRU: E.C. Saito, Branch Chief
Chemistry Branch I, Tolerance Support
Health Effects Division (7509C)

TO: Richard Mountfort, Product Manager
Daphne Waldo, Team 10 Reviewer
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And

Albin Kocialski, Head
Registration Section, CCB
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Rhône-Poulenc has submitted an application for an EUP and temporary tolerances for the insecticide fipronil (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(1R,S)-(trifluoromethyl)sulfinyl]-1H-pyrazole-3-carbonitrile) and its metabolites MB46136 (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfonyl]-1H-pyrazole-3-carbonitrile) and 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 RACs (expressed as parent plus metabolites MB45950 and MB46136):

Corn, Field, Grain	-	0.02 ppm
Corn, Forage	-	0.05 ppm
Corn, Fodder	-	0.07 ppm

BACKGROUND

Fipronil belongs to a new class of insecticides known as phenylpyrazoles. These compounds effect the central nervous system (CNS) by interfering with the passage of chloride through gamma aminobutyric acid (GABA)-regulated chloride channels, thereby disrupting CNS activity. Fipronil displays tighter binding to the insect GABA-regulated chloride channels than those of vertebrates, providing selective toxicity. Fipronil has been developed as a soil corn insecticide and has activity against corn rootworms and wireworms. According to the petitioner, commercial development of

fipronil could result in a significant reduction in the use rate of soil corn insecticides since the fipronil use rate is one tenth that of the two most common products of this type currently in use (terbufos and chlorpyrifos).

CONCLUSIONS

1. The product chemistry is adequate for this EUP only. For the permanent tolerance petition, the registrant must: **a)** for GLN § 61-1, submit a revised CSF for the TGAI 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; **b)** for GLN § 61-2, provide copies of the Material Safety Data Sheets for all of the starting materials; **c)** 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 TGAI, possible degradation products of the TGAI, and the potential for starting materials to carry over to the TGAI; **d)** 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; **e)** for GLN § 62-2, provide an explanation as to how all the certified limits were determined; **f)** for GLN § 62-3, demonstrate the accuracy of Method M-647-08-91(E) for measurement of fipronil; **g)** for GLN § 63-13, submit data on the sensitivity of the TGAI to metal ions and legible thermograms for each metal tested

2a. Fipronil 1.5G granules are incorporated into the soil at planting. The maximum use rate is 0.13 lbs. ai/A (8.7 lbs. Fipronil 1.5G/A).

2b. **The directions for use are not adequate for this EUP.** The registrant should add the following restrictions to the label: 1) "Do not plant any rotational crops except corn in treated fields within one year of fipronil application," 2) "Do not apply to sweet corn or pop corn," 3) "Do not feed treated corn grain, silage or stover to livestock," 4) "After grain harvest, plough under all remaining plant material" and 5) "Grain is to be used only for processing into corn by-products." **A revised Section B is required.**

2c. The proposed EUP program covers 50 trials in eight different states which together accounted for 73% of the U.S. corn acreage in 1991 (*Agricultural Statistics*, 1992). A total of 130 lbs. of a.i.

will be applied to 1000 acres.

3. No rotational crop studies were submitted with this petition. For the purposes of this EUP, crop rotation may be restricted by a label amendment. However, for the permanent tolerance the registrant must submit a confined crop rotation study. The results of this study will be used to determine the appropriate crop rotation restrictions and/or the need for limited field trials.

4a. Corn was grown in soil treated with [^{14}C]phenyl-labelled fipronil at a rate of 2.8X. The TRR in forage was 0.23 ppm; in grain, 0.21 ppm; and in fodder, 3.74 ppm.

4b. The nature of the residue in corn is understood for the purposes of this EUP only. Approximately one half of the TRR was identified as fipronil and its metabolites MB46136, MB45950, RPA200766 and RPA105048. For the permanent tolerance petition, the petitioner must address the following deficiencies in the corn metabolism study: **i)** The storage stability of the samples in this study has not been demonstrated. The data that was 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). **iv)** Fipronil contains two rings but the registrant has performed metabolism studies using only [^{14}C]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 [^{14}C]pyrazole-labelled fipronil.

5. The nature of the residue in animals has not been reported. This data will not be required for this EUP 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 [^{14}C]phenyl- and [^{14}C]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.

6a. The registrant has proposed an analytical enforcement method

for corn RACs in which fipronil and its metabolites are resolved on a single GC chromatograph. Acceptable recoveries were obtained for all analytes except MB45950 (average recovery of 60% at 0.02 ppm). 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.

6b. 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 have been sent to Beltsville for PMV (Memo, G. Kramer 3/16/94). CBTS will withhold a final conclusion on the adequacy of this method as analytical enforcement method pending receipt of the PMV report.

6c. For the permanent tolerance petition, the registrant must submit the results of Multiresidue testing for fipronil and all of its metabolites that are determined to be of regulatory concern.

7. Since no temporary tolerances have been proposed for animal RACs, an analytical enforcement method for animals is not required for this EUP. If, however, animal metabolism/feeding studies demonstrate a potential for transfer of residues to meat, milk or eggs, then the registrant will be required to propose permanent tolerances for these RACs and develop the appropriate analytical enforcement methodology. Any required enforcement methods for meat, milk and eggs will need successful ILVs and PMVs before being judged to be acceptable by CBTS.

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.

9a. The magnitude of the residue in corn RACs was determined in 10 field trials conducted in 10 different states which together accounted for 78% of the U.S. corn acreage in 1991 (*Agricultural Statistics*, 1992). The levels of fipronil and its metabolites were below the LOQ in all grain, forage and silage samples. Detectable residues of MB46136 (up to 0.042 ppm) were observed in fodder samples.

9b. The field residue data is adequate to support this EUP proposal. The registrant has proposed tolerances in grain and forage based on the sum of the LOQ of each metabolite as the observed residues were below the LOQ. In the case of fodder, the actual residue levels were used for MB46136. However, if tolerances are proposed for fipronil or its metabolites, then the LOQ can be used for the tolerances. Also, due to the feeding restrictions on the label, tolerances for stover and forage are not necessary for this EUP. The registrant should thus propose a tolerance for

"fipronil or its metabolites MB45950 or MB46136" of 0.02 ppm in/on Corn, field, grain. **A revised Section F which includes these tolerances is required for this EUP.** This revised Section F should also contain the chemical names of fipronil, MB45950 and MB46136.

9c. For the permanent tolerance petition, Section F may need further revision in accordance with the decision of the Metabolism Committee on the metabolites of toxicological concern and with the observed levels of such metabolites in the supporting field trials. Tolerances for forage and stover will also be required for the permanent tolerance petition.

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.

11. The magnitude of the residue in animals has not been reported. This data will not be required for this EUP due to the label restrictions against the feeding of treated RACs to livestock and the limited number of acres involved. However, acceptable magnitude of the residue studies in ruminants and poultry will be required for the permanent tolerance petition. If there are significant fipronil metabolites formed in corn which are not also formed in animals, then CBTS may also require feeding studies using any such metabolites.

12. 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 the memorandum.

RECOMMENDATIONS

CBTS will recommend in favor of the proposed temporary tolerances for fipronil and its metabolites MB46136 and MB45950 on corn RACs provided the registrant submits revised Sections B and F as detailed in conclusions 2b and 9b. A DRES run can be initiated

using the tolerance expression in conclusion 9b.

For the permanent tolerance petition, the registrant must: **1)** satisfactorily resolve all deficiencies in the product chemistry (conclusion 1), corn metabolism study (conclusion 4b), analytical methodology (conclusions 6b and 6c) and the processing study (conclusion 10b); **2)** submit acceptable nature and magnitude of the residue in animals, rotational crop and storage stability studies; and, if necessary, **3)** propose tolerances for animal RACs and develop appropriate analytical enforcement methodology.

DETAILED CONSIDERATIONS

Product Chemistry

§ 61-1 Product Identity and Disclosure of Ingredients

The active ingredient (a.i.) is fipronil (MB46030) or 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(1R,S)-(trifluoromethyl)sulfinyl]-1H-pyrazole-3-carbonitrile. The chemical structure is shown in figure 1 (copied from p. 50 of MRID# 429186-65). Other identifying characteristics are:

Empirical Formula:	$C_{12}H_4Cl_2F_6N_4OS$
Molecular Weight:	437.15
CAS Registration No.:	120068-37-3
Common Name:	Fipronil

The registrant has submitted a CSF for both the TGAI and end use product. Nominal concentrations were provided for all inert and three out of five impurities. However, the CSF for the TGAI lists two impurities for which no certified limits or nominal concentrations were provided. Since these impurities were found at a level well below 0.1%, they should be deleted from the CSF. A revised CSF for the TGAI is thus required. **The requirements for GLN § 61-1 are fulfilled for the purposes of this EUP only.** For the permanent tolerance petition, the registrant should submit a revised CSF for the TGAI. Also, the registrant should determine 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.

§ 61-2 Beginning Materials and Manufacturing Process

The registrant has submitted the names and addresses of the suppliers of the starting materials. Copies of the Material Safety

Data Sheets of the starting materials were, however, not included. A list of the beginning materials is included in the confidential appendix.

Included in the report are the chemical equations for each reaction of each step in the process, the amounts of each starting material, the equipment used, the parameters controlled and the steps at which analytical methods were used to make quality control measurements. Details of each step are given in the confidential appendix. **The requirements for GLN § 61-2 are fulfilled for the purposes of this EUP only.** For the permanent tolerance petition, the registrant must provide copies of the Material Safety Data Sheets for all of the starting materials.

§ 61-3 Discussion of the Formation of Impurities

The registrant has analyzed the TGAI for a total of six possible impurities. Three of these were not found at a level of >0.1%. The registrant has provided a discussion of the formation of two of three impurities that were found at >0.1% in the TGAI (RPA200766 was omitted from this discussion). No discussion of the formation of the other three impurities (MB45897, MB46513 and MB46058) was provided. The registrant has also failed to include discussions of impurities that might hypothetically occur but were not found in the TGAI, possible degradation products, and the potential for starting materials to carry over to the TGAI. Details are provided in the confidential appendix. **The requirements for GLN § 61-3 are fulfilled for the purposes of this EUP only.** For the permanent tolerance petition, the registrant should discuss 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 TGAI, possible degradation products of the TGAI, and the potential for starting materials to carry over to the TGAI.

§ 62-1 Preliminary Analysis of Product Samples

The registrant has submitted data from the analysis of 3 batches of the TGAI. **The requirements for GLN § 62-1 are fulfilled for the purposes of this EUP only.** For the permanent tolerance petition, the registrant should report the results of batch analyses of fipronil TGAI once full production for this proposed use has occurred. The CSF may need to be revised if the results of the new batch analyses differ from those done previously.

§ 62-2 Certified Limits

The registrant has submitted data and a CSF dated 7/21/93 which establishes certified limits for the a.i., residual solvents and all impurities present at a level >0.1% (see the confidential appendix). However, the CSF for the TGAI lists two impurities for which no certified limits or nominal concentrations were provided.

Since these impurities were found at a level well below 0.1%, they should be deleted from the CSF. A revised CSF for the TGAI is thus required. **The requirements for GLN § 62-2 are fulfilled for the purposes of this EUP only.** To satisfy GLN § 62-2 for the permanent tolerance petition, the registrant must also provide an explanation as to how all the certified limits were determined.

§ 62-3 Analytical Methods to Verify Certified Limits

The registrant has submitted a non-confidential method (Method M-647-08-91(E), MRID# 429186-02) for determination of the a.i. in the TGAI. The method involves HPLC using isocratic elution from an C-18 column with UV detection at 220 nm. The registrant also included a validation of this method. Linearity of the calibration curve was demonstrated (correlation coefficient = 1.000) for 5 concentrations over a range of 0.001 to 0.100%. Repeatability was determined by performing 6 determinations on a single sample of the TGAI. The precision was found to be 0.79%. The registrant has not reported on the accuracy of this method. CBTS concludes that this method is adequate to enforce the certified limits of the a.i. in the TGAI for this EUP only. For the permanent tolerance petition, the registrant should determine the accuracy of Method M-647-08-91(E) by performing at least five determinations of fipronil in the analytical grade standard.

The registrant has also submitted a non-confidential method (Method F-658-10-91(E), MRID# 429186-02) for determination of MB45950, MB46136, RPA200766 and toluene in the TGAI. The method involves HPLC using isocratic elution from an C-18 column with UV detection at 220 nm. The registrant also included a validation of this method. Linearity of the calibration curve for each compound was demonstrated (correlation coefficient = 1.000) for 5 concentrations. Repeatability was determined by performing 6 determinations on a single sample of the TGAI. The precision was found to be 2.8% for MB45950, 3.1% for MB46136, 28% RPA200766 and 5.3% for toluene. The registrant has determined the accuracy of this method by spiking a sample of the TGAI with a known amount of each impurity. The accuracy for MB45950 at 1% added was 115%, for MB46136 at 3% added was 98%, for RPA200766 at 0.04% added was 79% and for toluene at 0.08% added was 84%. CBTS concludes that this method is adequate to enforce the certified limits of impurities in the a.i. in the TGAI.

Quantitation of water was performed using Karl Fischer Titration. The average precision when applied to the TGAI was 21.2% and the average recovery of water spiked into the TGAI at levels of 0.25-0.5% was 98.0 ± 5.0%. CBTS concludes that this method is adequate for determination of the residual water present in the TGAI.

The requirements for GLN § 62-3 are fulfilled for the purposes of this EUP only. To satisfy GLN § 62-3 for the permanent tolerance petition, the registrant must demonstrate the accuracy of Method M-647-08-91(E) for measurement of fipronil.

§ 63 Physical and Chemical Characteristics of the TGAI

§ 63-2 Color: Visual inspection was used to determine that the color of the TGAI was white. These data fulfill the requirements for GLN § 63-2.

§ 63-3 Physical State: The TGAI was observed to be a powder at 23 °C. These data fulfill the requirements for GLN § 63-3.

§ 63-4 Odor: The TGAI was observed to have a mouldy odor at 23 °C. These data fulfill the requirements for GLN § 63-4.

§ 63-5 Melting Point: The melting point was determined using a ACD DuPont 990 Thermal Analyzer. The melting point range of the TGAI was 195.5 to 203 °C. These data fulfill the requirements for GLN § 63-5.

§ 63-7 Density: The specific gravity and density relative to water at 20 °C was determined by pyknometry. The density of the TGAI at 20 °C was 1.6262 g/ml and the specific gravity was 1.6292. These data fulfill the requirements for GLN § 63-7.

§ 63-8 Solubility: The solubility in distilled water was determined by the OECD column elution method. The solubility of the TGAI was 1.9 mg/l at 20 °C. The same method was also used to determine the solubility in buffered aqueous solutions and the shake flask method used to determine solubility in organic solvents (HPLC grade):

Solvent	Solubility, g/l
Water, pH 5	0.0024
Water, pH 9	0.0022
Acetone	545.9
2-Propanol	36.2
Dichloromethane	22.3
Ethyl Acetate	264.9
Hexane	0.028
Methanol	137.5
Toluene	3.0
Octanol	12.2

These data fulfill the requirements for GLN § 63-8.

§ 63-9 Vapor Pressure: The registrant has submitted a report on the vapor pressure of the TGAI. Using the gas saturation method (OECD 1-104), the vapor pressure was found to be 2.8×10^{-9} mm Hg at 25 °C. These data fulfill the requirements for GLN § 63-9.

§ 63-10 Dissociation Constant: N/A

§ 63-11 Octanol/Water Partition Coefficient ($P_{o/w}$)

The $P_{o/w}$ of analytical grade fipronil (99.3% pure) was determined by the shake flask method in accordance with OECD GLN 107 in distilled water. The observed $P_{o/w}$ was 10,154 ± 1326 ($\log P_{o/w} = 4.01$). These data fulfill the requirements for GLN § 63-11.

§ 63-12 pH

The pH of a 1% w/v aqueous suspension of the TGAI containing 2% v/v acetonitrile was found to be 5.90-6.10 at 23 °C. These data fulfill the requirements for GLN § 63-12.

§ 63-13 Stability

Stability to metals was investigated by mixing the TGAI with powders of Fe, Sn and Al. These samples were then subjected to differential thermal analysis over a range of 30-150 °C. However, the thermograms submitted by the registrant are illegible so that CBTS is unable to reach a conclusion on the stability of the TGAI to metals. No data on the stability of the TGAI to metal ions was submitted. Stability in sunlight was determined by exposing the TGAI to light from a xenon lamp for 12 days at 23 °C. Slight decomposition from 96.53% a.i. to 93.79% a.i. was observed

concomitant with a color change from white to orange in the upper layers of the sample. Stability at normal and elevated temperatures was determined by incubating the TGAI for 1 year at room temperature, 92 days at 35 °C, 30 days at 50 °C and 14 days at 54 °C. No significant decomposition was observed at any temperature.

The requirements for GLN § 63-13 are fulfilled for the purposes of this EUP only. To satisfy GLN § 63-13 for the permanent tolerance petition, the registrant must submit data on the sensitivity of the TGAI to metal ions and legible thermograms for each metal tested.

Note: The registrant has also submitted data on the end-use product for GLNs § 61,62 and 63 (MRID#s 429186-10, -11 & -12). These data requirements are under purview of RD and are thus not reviewed herein. The product chemistry status is summarized in Table 1.

Table 1- PRODUCT CHEMISTRY DATA SUMMARY
Chemical No. 129121
Product: Fipronil TGAI

Guideline Number	Requirement	Are Data Requirements Fulfilled?[a]	MRID Number
61-1	Product Identity and Disclosure of Ingredients	N[b]	429186-01
61-2	Beginning Materials and Manufacturing Process	N[c]	429186-01
61-3	Discussion of Formation of Impurities	N[d]	429186-01
62-1	Preliminary Analysis	N[e]	429186-02
62-2	Certification of Ingredient Limits	N[f]	429186-02
62-3	Analytical Methods to Verify the Certified Limits	N[g]	429186-02
63-2	Color	Y	429779-01
63-3	Physical State	Y	429779-01
63-4	Odor	Y	429779-01
63-5	Melting Point	Y	429779-01
63-6	Boiling Point	N/A	
63-7	Density, Bulk Density or Specific Gravity	Y	429779-01
63-8	Solubility	Y	429186-03, 429186-04
63-9	Vapor Pressure	Y	429186-05
63-10	Dissociation Constant	N/A	
63-11	Octanol/Water Partition Coefficient	Y	429186-06
63-12	pH	Y	429186-07
63-13	Stability	N[h]	429186-08

[a] Y = Yes; N = No; N/A = Not Applicable.
[b] Revised CSF required.
[c] Copies of the Material Safety Data Sheets required for all of the starting materials.
[d] Discussion incomplete.
[e] Only three batches of the TGAI were analyzed.
[f] Discussion of origin of certified limits required.
[g] Additional validation data required for a.i.method.
[h] Data required on the sensitivity of the TGAI to metals and metal ions.

Formulation: Fipronil is formulated as Fipronil 1.5% Granular Soil Insecticide, containing 1.5% a.i.

Proposed Use

Fipronil 1.5G granules are incorporated into the soil at planting. The maximum use rate is 0.13 lbs. ai/A (8.7 lbs. Fipronil 1.5G/A).

The label contains the following restrictions: "Do not feed treated corn or fodder to livestock," "Do not allow livestock to graze treated fields," and "Do not harvest within 90 days of

application." There are no rotational crop restrictions.

The directions for use are not adequate for this EUP. The registrant should add the following restrictions to the label: 1) "Do not plant any rotational crops except corn in treated fields within one year of fipronil application," 2) "Do not apply to sweet corn or pop corn," 3) "Do not feed treated corn grain, silage or stover to livestock," 4) "After grain harvest, plough under all remaining plant material" and 5) "Grain is to be used only for processing into corn by-products." **A revised Section B is required.**

The proposed EUP program covers 50 trials in eight different states (IA, IL, IN, MN, NE, OH, SD and WI) which together accounted for 73% of the U.S. corn acreage in 1991 (*Agricultural Statistics*, 1992). A total of 130 lbs. of a.i. will be applied to 1000 acres (0.001% of the U.S. corn acreage).

Rotational Crop Studies

No studies were submitted with this petition.

For the purposes of this EUP, crop rotation may be restricted by a label amendment (see above). However, for the permanent tolerance the registrant must submit a confined crop rotation study. The results of this study will be used to determine the appropriate crop rotation restrictions and/or the need for limited field trials.

Nature of Residue- Plants

Submitted with this petition:

> Metabolic Fate and Distribution of ^{14}C-Fipronil in Corn. (171-4 Nature of the Residue- Plants). MRID# 429186-65

In-Life Phase: [Phenyl(U)-^{14}C]-fipronil (19.62mCi/mmole) with radiolabel uniformly distributed in the phenyl ring (diluted to 7.79 mCi/mmole with cold fipronil) had a radiopurity of than 99.5%. The test solutions were prepared by mixing the labelled compound with inert ingredients to simulate a 1.69% granular 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 (Table 2).

Table 2- TRR in corn RACs resulting from application of ^{14}C-phenyl-labelled fipronil to soil at a rate of 170 g ai/A (2.9X).

RAC	PHI (Days)	TRR (ppm)
Forage	42	0.23
Grain	98	0.21
Fodder	106	3.74

Extraction and Fractionation: Tissues were ground in methylene chloride and the debris removed by centrifugation. The debris was reextracted at least twice using the same solvent. Extractions were repeated using methanol and water. Debris which still contained >0.01 ppm was refluxed in methanol. If bound residues remained, then this procedure was repeated using acidic methanol (3N HCl). The results of these procedures are shown in Table 3. The majority of the TRR was readily extractable and bound residues comprised 6-19% of the TRR.

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

Fraction	Forage		Fodder		Grain	
	ppm	% TRR	ppm	% TRR	ppm	% TRR
$MeCl_2$	0.13	55.37	0.78	20.76	0	1.03
MeOH	0.05	21.49	0.91	24.24	0.14	66.49
H_2O	0	1.67	1.06	28.23	0.01	4.35
MeOH Reflux	-	-	0.35	9.38	0.05	22.2
MeOH Acid Reflux	0.01	2.69	0.22	5.8	-	-
Total Extracted	0.19	81.22	3.31	88.41	0.20	94.06
Bound Residues	0.04	18.78	0.43	11.59	0.01	5.94

Metabolite Identification: The soluble fractions were cleaned-up for analysis independently. Methylene chloride and methanol extracts were concentrated and filtered. Methylene chloride extracts were cleaned-up using a Sep-Pak. Water extracts were concentrated by lyophilization. The water and refluxed fractions

were cleaned and concentrated using solvent exchange. The methanol refluxates were further cleaned by liquid/liquid partitioning. The cleaned-up samples were then analyzed using HPLC. Fipronil and eight metabolites were resolved on a C-18 column with isocratic elution. The structures of these metabolites are shown in figure 1. The identity of all metabolites found in the corn samples was confirmed by MS.

Nature of the Residue in Forage: The methylene chloride extracts were cleaned-up and analyzed on HPLC. Fipronil *per se* was found to comprise 23.9% of the TRR and RPA105048, 23.6% (Table 4) for a total of 47.5% of the TRR identified. Another 7.9% of the TRR was comprised of Unknown 3, a compound which eluted between RPA200766 and fipronil on HPLC. The methanol, water and refluxate fractions were not chromatographed so that 26.33% of the TRR was found to be extractable but not further analyzed.

Table 4- Metabolite identification of extractable residues in corn RACs.

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

ND = Not Detected

Nature of the Residue in Fodder: Analysis of the soluble residues on HPLC showed that Fipronil *per se* was 6.3% of the TRR, RPA105048 was 13.9%, RPA200766 was 11.7%, MB46136 was 11.2% and MB45950 was 2.2% (Table 4) for a total of 45.6% of the TRR identified. Another 32.1% of the TRR was comprised of Unknown 1; 4.8%, Unknown 2; and 0.7%, Unknown 4. Unknowns 1 and 2 were polar compounds which eluted between RPA104615 and RPA105048 and Unknown 4, a nonpolar compound which eluted after the standards.

Nature of the Residue in Grain: The methanol extracts were cleaned-up and analyzed on HPLC. Fipronil *per se* was found to comprise 38.3% of the TRR (Table 4). Another 28.2% of the TRR was comprised of Unknown 3. The methylene chloride, water and refluxate fractions were not chromatographed so that 28.2% of the TRR was found to be extractable but not further analyzed.

Bound Residues: The bound residues were further characterized by enzymatic digestion. Fodder samples were chosen as a test substrate since this RAC contained the highest level of bound radioactivity (0.43 ppm). The residue was treated sequentially with the following procedures: 1) Extraction of water-soluble polysaccharides and proteins- The sample was sonicated in aqueous buffer and then extracted with methanol/chloroform. 2) Extraction of starch- The residues were treated with α-amylase. 3) Extraction of proteins- The residue was treated with pronase. 4) Extraction of pectins- The residue was treated with pectinase. 5) Extraction of lignins- The residue was treated with DMSO. 6) Extraction of hemicellulose- The residue was extracted with 24% KOH for 24 hrs., brought to pH 3.4 and treated with hemicellulase. These procedures released 0.02 ppm (0.6% of the TRR) as water-soluble protein, 0.02 ppm (0.6% of the TRR) as lignin and 0.10 ppm (2.7% of the TRR) as hemicellulose. The remaining bound residues comprised 7.7% of the TRR. CBTS concludes that the registrant has provided adequate characterization of the bound residues.

Storage Stability: Samples were stored for 25 months between harvest and the final extractions. The registrant attempted to demonstrate storage stability by spiking samples with ^{14}C-fipronil. One year later, the samples were extracted and analyzed. In grain, 99.8% of the TRR was identified as fipronil; in fodder, 63.8% of the TRR was identified as fipronil; and in forage, 67.6% of the TRR was identified as fipronil.

Conclusions: The nature of the residue in corn is understood for the purposes of this EUP only. Approximately one half of the TRR was identified as fipronil and its metabolites MB46136, MB45950, RPA200766 and RPA105048. For the permanent tolerance petition, the petitioner must address the following deficiencies in the corn metabolism study: **a)** The storage stability of the samples in this study has not been demonstrated. The data that was 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. **b)** Unknown metabolites 1 (32.1% of the TRR in fodder), 2 (4.8% of the TRR in fodder) and 3 (28.2% of the TRR in grain) accounted for significant portions of the TRR in corn RACs. The registrant should identify

these compounds. **c)** 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). **d)** Fipronil contains two rings but the registrant has performed metabolism studies using only [^{14}C]phenyl-labelled fipronil. The possibility of uptake of pryazole-containing metabolites derived from ring cleavage has thus not been investigated. The failure of the registrant to find phenyl-labelled cleavage products does not exclude the possibility of ring cleavage since pyrazole-containing cleavage products could be formed in the soil and preferentially taken-up by the plants. In order to fully characterize the nature of the residue in corn, the registrant should perform a metabolism study using [^{14}C]pyrazole-labelled fipronil.

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

No studies were submitted with this petition.

The nature of the residue in animals has not been reported. This data will not be required for this EUP 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 [^{14}C]phenyl- and [^{14}C]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.

Analytical Methodology- Plants

Submitted with this petition:

Fipronil Independent Laboratory Method Validation MRID# 429186-66

Procedure: Samples are extracted by homogenization in acetonitrile. Solids are removed by filtration and NaCl is added to the extract. 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. 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 60% at 0.02 ppm) (Table 5). 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 RPA105048 and RPA200766 was also successful.

Table 5- Validation data for proposed analytical enforcement method for residues of fipronil and its metabolites MB46136 and MB45950 in corn RACs. For all % recoveries, n=5.

RAC	Spike (ppm)	% Recovery ± s.d.		
		Fipronil	MB45950	MB46136
Grain	0.010	90 ± 5	ND	ND
	0.050	78 ± 7	ND	ND
Forage	0.020	74 ± 7	ND	ND
	0.100	70 ± 7	ND	ND
Fodder	0.020	78 ± 6	60 ± 7	93 ± 21
	0.100	82 ± 5	59 ± 17	92 ± 17

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 have been sent to Beltsville for PMV (Memo, G. Kramer 3/16/94). The registrant claims that the LOD for fipronil and its metabolites to be 0.01 ppm in grain and 0.02 ppm in forage and fodder. However, validation data was not provided for the metabolites at 0.01 ppm. ACL is thus unable to validate the method for the metabolites at 0.01 ppm in grain since this data was not submitted by the registrant (H.

Hundley, Personal Communication 3/23/94). All validations using the metabolites will be at conducted at 0.02 ppm. CBTS will withhold a final conclusion on the adequacy of this method as analytical enforcement method pending receipt of the PMV report.

Multiresidue Method Testing: No reports on Multiresidue testing of fipronil or its metabolites have been received. For the permanent tolerance petition, the registrant must submit the results of Multiresidue testing for fipronil and all of its metabolites that are determined to be of regulatory concern.

Analytical Methodology- Animals

No analytical method has been submitted by the registrant

Since no temporary tolerances have been proposed for animal RACs, an analytical enforcement method for animals is not required for this EUP. If, however, animal metabolism/feeding studies demonstrate a potential for transfer of residues to meat, milk or eggs, then the registrant will be required to propose tolerances for these RACs and develop the appropriate analytical enforcement methodology. Any required enforcement methods for meat, milk and eggs will need successful ILVs and PMVs before being judged to be acceptable by CBTS.

Storage Stability Studies

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.

Magnitude of Residue- Plants

Submitted with this petition:

> Fipronil: Magnitude of the Residues on Corn Resulting from Ground Applications of Granular Formulated Product (1992) MRID# 429186-67

Ten field trials were conducted in 10 different states which together accounted for 78% of the U.S. corn acreage in 1991 (*Agricultural Statistics*, 1992). Fipronil was applied as a 1.5% granular formulation prior to planting at a rate of 0.13 lbs. ai/A

(1X). Three treated samples and one untreated control sample were harvested per trial. Forage samples were harvested 45-71 days after planting. Silage samples were harvested at the early dent stage, 102-137 days after planting. Grain and fodder samples were harvested 146-191 days after planting. 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. 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. The average recoveries for fipronil were 81% in grain, 80% in forage, 82% in fodder and 84% in silage; for MB45950, 62% in grain, 54% in forage, 75% in fodder and 66% in silage; for MB46136, 91% in grain, 96% in forage, 114% in fodder and 96% in silage; for RPA105048, 103% in grain, 113% in forage, 120% in fodder and 113% in silage; and for RPA200766, 99% in grain, 107% in forage, 104% in fodder and 99% in silage. The results of the analysis of the field samples are summarized in Table 6. The levels of fipronil and its metabolites were below the LOQ in all grain, forage and silage samples. Detectable residues of MB46136 (up to 0.042 ppm) were observed in fodder samples.

Conclusions: The field residue data are adequate to support this EUP proposal. The registrant has proposed tolerances in grain and forage based on the sum of the LOQ of each metabolite as the observed residues were below the LOQ. In the case of fodder, the actual residue levels were used for MB46136. However, if tolerances are proposed for fipronil or its metabolites, then the LOQ can be used for the tolerances. Also, due to the feeding restrictions on the label, tolerances for stover and forage are not necessary for this EUP. The registrant should thus propose a tolerance for "fipronil or its metabolites MB45950 or MB46136" of 0.02 ppm in/on Corn, field, grain. **A revised Section F which includes these tolerances is required for this EUP.** This revised Section F should also contain the chemical names of fipronil, MB45950 and MB46136.

For the permanent tolerance petition, Section F may need further revision in accordance with the decision of the Metabolism Committee on the metabolites of toxicological concern and with the observed levels of such metabolites in the supporting field trials. Tolerances for forage and stover will also be required for the permanent tolerance petition.

Table 6- Summary of field residue data for fipronil and its metabolites in corn RACs.

Trial	RAC	PHI	Maximum Residue (ppm)				
			Fipronil	MB45950	MB46136	RPA105048	RPA200766
IA	Grain	146	ND	ND	ND	ND	ND
	Forage	45	<0.02	ND	ND	ND	<0.02
	Silage	110	ND	ND	<0.02	ND	<0.02
	Fodder	146	<0.02	ND	0.022	ND	<0.02
MO	Grain	157	ND	ND	ND	ND	ND
	Forage	45	ND	ND	ND	ND	<0.02
	Silage	109	ND	ND	<0.02	ND	<0.02
	Fodder	157	<0.02	ND	0.033	ND	<0.02
OH	Grain	191	ND	ND	ND	ND	ND
	Forage	45	<0.02	ND	ND	ND	<0.02
	Silage	137	<0.02	<0.02	<0.02	ND	ND
	Fodder	191	<0.02	ND	<0.02	<0.02	ND
MN	Grain	153	ND	ND	ND	ND	ND
	Forage	45	<0.02	ND	ND	ND	<0.02
	Silage	117	ND	ND	ND	ND	ND
	Fodder	153	<0.02	ND	<0.02	ND	ND
SD	Grain	156	ND	ND	ND	ND	ND
	Forage	45	ND	ND	ND	ND	ND
	Silage	102	ND	ND	ND	ND	ND
	Fodder	156	ND	<0.02	ND	**0.020**	ND
NE	Grain	159	ND	ND	ND	ND	ND
	Forage	45	<0.02	ND	ND	ND	ND
	Silage	129	ND	ND	ND	ND	<0.02
	Fodder	159	<0.02	ND	<0.02	ND	<0.02
KS	Grain	151	<0.01	ND	ND	ND	ND
	Forage	45	<0.02	ND	<0.02	<0.02	<0.02
	Silage	115	<0.02	ND	<0.02	ND	<0.02
	Fodder	151	<0.02	ND	0.024	ND	<0.02
IN	Grain	173	ND	ND	ND	ND	ND
	Forage	45	<0.02	ND	<0.02	ND	<0.02
	Silage	121	ND	<0.02	<0.02	ND	ND
	Fodder	173	<0.02	<0.02	**0.042**	ND	<0.02
WI	Grain	162	ND	ND	ND	ND	ND
	Forage	46	<0.02	ND	ND	ND	<0.02
	Silage	130	ND	ND	<0.02	ND	ND
	Fodder	162	<0.02	ND	<0.02	ND	<0.02
IL	Grain	169	ND	ND	ND	ND	ND
	Forage	71	<0.02	ND	ND	ND	<0.02
	Silage	126	<0.02	ND	<0.02	ND	<0.02
	Fodder	169	<0.02	ND	0.038	<0.02	**0.020**

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

Magnitude of the Residue- Processed Fractions

Submitted with this petition:

> Fipronil: Magnitude of the Residue in Corn Processing Fractions Resulting From Ground Applications of Granular Formulated Product (1992) MRID# 429186-68

Corn was grown at a test site in NE. Fipronil was applied at a rate of 2.6 lbs. ai/A (20X) at the time of planting. Samples were collected 179 days after planting. Three replicate treated grain samples were harvested for dry milling, three were harvested for wet milling and one untreated control sample was harvested for each process. Samples were stored frozen for approximately 4 months prior to shipment to TX A & M Univ. for processing. Processed fractions were stored ≈2.5 months prior to analysis. Fipronil and its metabolites were determined by the proposed analytical enforcement method. The method was validated in each fraction at the LOQ and 5X the LOQ. The average recoveries for fipronil and its metabolites were comparable to those observed in the RACs. The LOQ was 0.01 ppm for oil and grain and 0.02 ppm for starch. The results of the analysis of the processed fractions is summarized in Table 7. Residues were nondetectable in grain (RAC). The only metabolites detected in a processed fraction were RPA200766 which was found in refined oil (0.018-0.030 ppm) and MB46513 which was found in meal (0.010 ppm) and flour (0.018 ppm). One untreated control sample of refined oil (dry processing) contained 0.027 ppm RPA200766.

Table 7- Summary of residue data for fipronil and its metabolites in processed corn fractions.

Milling Process	Fraction	Maximum Residue (ppm)					
		Fipronil	MB45950	MB46136	RPA105048	RPA200766	MB46513
Dry	Grain	ND	ND	ND	ND	ND	ND
	Grits	ND	ND	ND	ND	ND	ND
	Meal	ND	ND	ND	ND	ND	0.010
	Flour	ND	ND	ND	ND	ND	0.018
	Crude Oil	<0.01	ND	ND	ND	ND	ND
	Refined Oil	<0.01	ND	ND	ND	0.030	ND
	Grain Dust	ND	ND	ND	ND	ND	ND
Wet	Grain	ND	ND	ND	ND	ND	ND
	Starch	ND	ND	ND	ND	ND	ND
	Crude Oil	ND	ND	ND	ND	<0.01	ND
	Refined Oil	<0.01	ND	ND	ND	0.018	ND
	Grain Dust	<0.01	ND	<0.01	ND	ND	ND

ND = Not Detected, Below the Limit of Detection (0.002 ppm, 0.004 ppm for starch)

Conclusions: 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 of these metabolites would be present at a 1X use rate. It is thus not likely that food additive tolerances would be required for these metabolites even if they are determined to be of toxicological concern. This processing study can also be used to support the permanent tolerance petition provided that: a) there are no metabolites determined to be of toxicological concern which are not accounted for in this study, and b) the registrant submits adequate storage stability data for fipronil and all metabolites of toxicological concern in the processed fractions.

Magnitude of the Residue- Animals

No studies were submitted with this petition.

The magnitude of the residue in animals has not been reported. This data will not be required for this EUP due to the label restrictions against the feeding of treated RACs to livestock and the limited number of acres involved. However, acceptable magnitude of the residue studies in ruminants and poultry will be required for the permanent tolerance petition. Proposed enforcement methodology for meat, milk and eggs and an ILV will also be required. If there are significant fipronil metabolites formed in corn which are not also formed in animals, then CBTS may also require feeding studies using any such metabolites.

Attachment: Confidential Appendix

cc (without attachment): circ.
cc (with attachment): PP#3G04263, Kramer, R.F.
RDI: P.V. Errico (5/25/94), R.A. Loranger (6/6/94)
G.F. Kramer:804T:CM#2:(703)305-5079:7509C