7-14-97

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

**MEMORANDUM**

DATE: 7/14/97

SUBJECT: PP# 6F04664. Isoxaflutole in/on Field Corn and Animal RACs. Amendment of 12/2/96. Revised Sections B & F, New Analytical Method for Animal Tissues and Storage Stability Data. MRID#s 441690-01 thru -07. Barcode D232139. Chemical 123000. Case 287353.

FROM: George F. Kramer, Ph.D., Chemist
RABI/HED (7509C)

THROUGH: Melba Morrow, Branch Senior Scientist
RAB1/HED (7509C)

TO: Barbara Madden
RCAB/HED (7509C)

Rhône-Poulenc Ag Company has proposed permanent tolerances for the combined residues of the herbicide isoxaflutole and its metabolites 1-(2-methylsulfonyl-4-trifluoromethylphenyl-2-cyano-3-cyclopropyl propane-1,3-dione (RPA 202248) and 2-methylsulfonyl-4-trifluoromethyl benzoic acid (RPA 203328), calculated as the parent compound, in/on:

| | |
|---|---|
| Field Corn, Grain -- 0.20 ppm | Field Corn, Fodder -- 0.50 ppm |
| Field Corn, Forage -- 1.0 ppm | |

Tolerances are also proposed for the combined residues of the herbicide isoxaflutole and its metabolite RPA 202248, calculated as the parent compound, in/on:

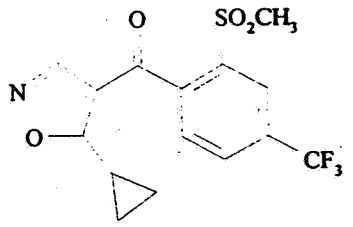
| | |
|---|---|
| Milk -- 0.02 ppm | Liver* -- 2.0 ppm |
| Poultry, Liver - 2.0 ppm | Kidney* -- 0.40 ppm |
| Meat Byproducts (except liver and kidney)* -- 0.20 ppm | |

*of cattle, goat, hogs, poultry and sheep

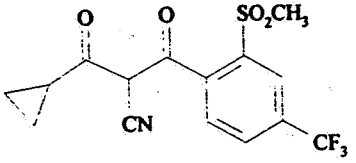
The current amendment addresses deficiencies identified in CBTS's previous review (Memo, G. Kramer 8/14/96; D224213). The structure

of isoxaflutole and its metabolites are shown below:

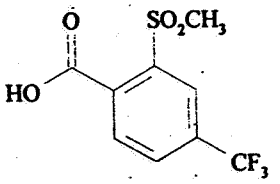
**ISOXAFLUTOLE**



**RPA 202248**



**RPA 203328**



## Executive Summary of Chemistry Deficiencies

- Field accumulation studies in rotational crops.
- HED Metabolism Committee decision.
- Submission analytical standards to the EPA repository.
- Revised version of the analytical enforcement method for plants.
- Agency validation of analytical method for animals.
- Revised Section F.

## RECOMMENDATIONS

**RAB1 continues to recommend against the proposed tolerances for isoxaflutole and its metabolites in/on field corn and animal RACs for reasons detailed in conclusions 1b, 2b, 3b, 4b, 5, 6b, and 9.**

A preliminary DRES run can be initiated at this time at the following residue levels:

| | | | |
|---|---|---|---|
| Corn, Grain -- | 0.20 ppm | Poultry Liver -- | 0.30 ppm |
| Milk -- | 0.02 ppm | Liver* -- | 0.50 ppm |
| Meat Byproducts (except liver)* -- | 0.10 ppm | | |

*of cattle, goat, hogs, horses and sheep

Note: residues are not expected in sweet corn.

**CONCLUSIONS**

1a. Supplemental storage stability data for the confined rotational crop study indicate that isoxaflutole was extensively metabolized to RPA 202248 and RPA 203328 during storage. As RPA 202248 and RPA 203328 were the only metabolites identified and these metabolites are determined in the proposed enforcement method, the petitioner will not be required to repeat the confined rotational crop study. Due to uncertainties in the composition of the samples at harvest, RAB1 will base its conclusions from this study on the TRR. The results of this study show that residues are ≥ 0.01 ppm in all crops at the 12-month plantback interval.

1b. Field accumulation studies in rotational crops are required to determine the appropriate plantback intervals and/or the need for rotational crop tolerances. These studies should be performed in accordance with OPPTS Test Guidelines 860.1900.

2a. The petitioner has submitted a revised label in which the planting of rotational crops is limited to the following season.

2b. RAB1 is unable to assess the adequacy of the proposed rotational crop restrictions until the requisite limited field trials for rotational crops are performed and submitted for our review.

3a. The nature of the residue in corn is now considered to be understood. RPA 202248 and RPA 203328 are the primary components of the residue, accounting for 64-91% of the TRR. Metabolism of isoxaflutole in corn proceeds via: 1) hydrolysis of the isoxazole ring to form RPA 202248; 2) further hydrolysis to produce RPA 203328.

3b. RAB1 need not defer to the HED Metabolism Committee on the toxicological significance of isoxaflutole metabolites identified in corn and rotational crops as the only metabolites identified, RPA 202248 and RPA 203328, are included in the tolerance expression. However, the HED Metabolism Committee will consider the possible formation of metabolites of toxicological concern which were not identified in these studies.

4a. The nature of the residue in poultry is now considered to be understood. RPA 202248, RPA 207048, RPA 203328, and RPA 205834 are the primary components of the residue, accounting for up to 93% of the TRR. Metabolism of isoxaflutole proceeds in poultry via: 1) hydrolysis of the isoxazole ring to form RPA 202248 and RPA 205834;

2) further hydrolysis to produce RPA 207048 and RPA 203328.

4b. RAB1 will defer to the HED Metabolism Committee on the toxicological significance of metabolites in animal commodities. A decision concerning which residues to regulate will then follow. A tolerance based on the parent and RPA 202248 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.

5. The proposed analytical enforcement method for corn RACs has been validated by ACL, Beltsville (Memo, G. Kramer 8/20/96; D228481). However, the petitioner should submit standards of isoxaflutole (including metabolites and the GC standard) to the EPA repository in RTP along with the MSDS, and a revised version of the proposed analytical enforcement method as specified in conclusions 1-5 of the aforementioned Memo. Until the receipt of the standard and the revised method, the requirements for analytical enforcement methodology will remain unfulfilled.

6a. A new HPLC/UV enforcement method for meat, milk and eggs (EC. 96-340) has been submitted by the petitioner. Adequate validation data (recovery, ILV and radiovalidation) were also submitted. The method and ILV have been sent to Beltsville for PMV (Memo, G. Kramer 1/16/97).

6b. RAB1 will withhold a final conclusion on the adequacy of this method as an analytical enforcement method pending receipt of the PMV report.

6c. The method used for data gathering in the animal feeding studies was shown to extract only 53% of the TRR. Therefore, in order to obtain an accurate interpretation of the residue data from the ruminant and poultry magnitude of residue studies, adjustments in the results from the LC-MS-MS data gathering method were made to correct for the lower extraction efficiency. The above adjusted data necessitate revision of the proposed tolerances for meat and meat byproducts (see below).

7. The submitted storage stability data for corn processed commodities indicate that there were no significant losses of isoxaflutole, RPA 202248, or RPA 203328 during storage under freezer conditions. Tolerances on these commodities are not required.

8. The submitted storage stability data for milk indicate that RPA 202248, RPA 205834 and RPA 203328 show no indication of degradation during the conditions of the study. Isoxaflutole appears to degrade with an estimated half life of approximately 111 days. The results for the tissues indicate that RPA 207048 does degrade in tissue matrices. The other analytes appear to be stable in the kidney, muscle and fat tissues. For liver, isoxaflutole and RPA 202248 appear to be generally stable, whereas RPA 205834 and RPA

207048 appear to degrade with an estimated half life of about 3 months. The results for egg indicate that RPA 202248 is stable in the egg matrix.

9. The samples from the feeding studies were stored for a maximum of 3 months. The results of the feeding study have been recalculated, correcting for the ≈50% extraction efficiency of the LC-MS-MS data gathering method and the decline of residues observed in some tissue/metabolite combinations The appropriate tolerances are:

| | | | |
|---|---|---|---|
| Milk | -- 0.02 ppm | Liver* | -- 0.50 ppm |
| Meat Byproducts (except liver)* | -- 0.10 ppm | | |
| Poultry, Liver - 0.30 ppm | | | |

*of cattle, goat, hogs, horses and sheep

**A revised Section F is required for this petition.** Further revisions to Section F will be required if additional metabolites are determined to be of toxicological significance by the HED Metabolism Committee.

## DETAILED CONSIDERATIONS

### Deficiency - Conclusion 2 (from Memo, G. Kramer 8/14/96)

2. The following deficiency in the Balance label was noted: Crop rotation restrictions are required. Limited field trials will be necessary in order to determine the appropriate plantback intervals (see below). A revised Section B is required.

**Petitioner's Response:** Submission of a revised label in which the planting of rotational crops is limited to the following season.

**RAB1's Conclusion:** RAB1 is unable to assess the adequacy of the proposed rotational crop restrictions until the requisite limited field trials for rotational crops are performed and submitted for our review.

### Deficiency - Conclusion 3c & 3d (from Memo, G. Kramer 8/14/96)

3c. One major deficiency in this study was noted: storage stability was not demonstrated. Such information is needed in order for the confined study to be acceptable.

3d. As the petitioner has proposed to have no plantback restrictions, CBTS can conclude that limited field trials will be required since the total of isoxaflutole and its metabolites included in the tolerance expression exceeded 0.01 ppm in all crops in the confined study at the shortest plantback interval (34 days). These trials should be conducted in accordance with the draft 860 Guidelines (8/95).

Conclusions on the nature of the residue in rotational crops will be withheld pending resolution of deficiencies regarding storage stability.

**Petitioner's Response:** Submission of:

Supplemental Report: $^{14}$C-RPA201772: Accumulation Study on Confined Rotational Crops. MRID# 441690-02.

Samples of each crop matrices were spiked with a mixture of $^{14}$C-isoxaflutole, $^{14}$C-RPA 202248, and $^{14}$C-RPA 203328. The total concentration was approximately 2 ppm (49% isoxaflutole, 33% RPA 202248, and 18% RPA 203328). The samples were analyzed on day 0 and day 700 using methodology described in the initial submission. The results indicate that isoxaflutole is not stable in storage as shown by the decrease from ca. 49% (at 0-DAT) to 10% (at 700-DAT) of the total peak area (Table 1). These results confirmed those reported in the corn metabolism report (MRID# 43573249) where a decrease in isoxaflutole of up to 27% during a ca. 7-month storage period was reported. In contrast to the corn metabolism study, however, RPA 202248 was found to be somewhat susceptible to degradation over the longer storage period in this study. Although an average of ≈9% increase was realized (from 33.3 to 42.0%), a 30% increase in RPA 203328 was also demonstrated suggesting that degradation from isoxaflutole to RPA 202248 and subsequently from RPA 202248 to RPA 203328 had occurred.

Table 1. HPLC Profiles of extracts of plant matrices fortified with Isoxaflutole and its metabolites, RPA 202248 and RPA 203328, stored in the freezer for periods of 0- and 700-days after treatment (DAT).

| MATRIX | PERCENT OF TOTAL PEAK AREA | | | | | |
|---|---|---|---|---|---|---|
| | RPA201772 | | RPA202248 | | RPA203328 | |
| | 0 DAT | 700 DAT | 0 DAT | 700 DAT | 0 DAT | 700 DAT |
| Sorghum forage | 49.7 | 29.3 | 34.2 | 26.4 | 16.1 | 43.5 |
| Lettuce | 51.8 | 4.9 | 25.2 | 57.6 | 23.0 | 40.0 |
| Radish leaf | 54.7 | 0.0 | 26.4 | 48.7 | 19.0 | 51.0 |
| Radish root | 43.8 | 5.1 | 39.2 | 41.9 | 17.1 | 53.0 |
| Sorghum grain | 42.8 | 8.2 | 40.5 | 31.4 | 16.8 | 59.9 |
| Sorghum stover | 50.0 | 12.9 | 34.7 | 46.1 | 15.4 | 40.9 |
| AVERAGE | 48.8 | 10.0 | 33.3 | 42.0 | 17.9 | 48.0 |

**RAB1's Conclusion:** The petitioner has provided stability data only for the parent and 2 metabolites instead of investigating the stability of the metabolite profile present in the samples at harvest. Further, the data submitted indicate that isoxaflutole was extensively metabolized to RPA 202248 and RPA 203328 during storage. As RPA 202248 and RPA 203328 were the only metabolites identified and these metabolites are determined in the proposed enforcement method, the petitioner will not be required to repeat the confined rotational crop study. Due to uncertainties in the composition of the samples at harvest, RAB1 will base its

conclusions from this study on the TRR. The results of this study show that residues are ≥ 0.01 ppm in all crops at the 12-month plantback interval. Field accumulation studies in rotational crops are required to determine the appropriate plantback intervals and/or the need for rotational crop tolerances. These studies should be performed in accordance with OPPTS Test Guidelines 860.1900.

**Deficiency - Conclusion 4a &4b (from Memo, G. Kramer 8/14/96)**

4a. The samples from the corn metabolism study were stored for up to 7 months prior to extraction and the extracts were stored for up to 3 months prior to analysis. The petitioner must submit data which demonstrates that the metabolite profile of these samples remained unchanged during the storage conditions employed in this study.

4b. CBTS will defer to the HED Metabolism Committee on the toxicological significance of metabolites once the deficiencies associated with plant metabolism and confined rotational crops have been addressed. A decision concerning which residues to regulate will then follow. A tolerance based on the parent and metabolites RPA 202248 and RPA 203328 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.

**Petitioner's Response:** Storage stability data were previously submitted for samples fortified with a mixture of $^{14}$C-isoxaflutole and $^{14}$C-RPA 202248.

**RAB1's Conclusion:** These storage stability data were reviewed previously (Memo, P. Errico 12/7/95; CBTS# 15430). A decrease in isoxaflutole of up to 27% with a concomitant increase in $^{14}$C-RPA 202248 was reported. These data provide no information on the stability of the total metabolite profile as is generally required for metabolism studies. However, as 64-91% of the TRR in corn RACs was comprised of isoxaflutole metabolites which are measured in the proposed enforcement method and no unidentified fraction exceeded our trigger for identification (10% of the TRR and 0.05 ppm), RAB1 will not require further evidence of storage stability. The nature of the residue in corn is now considered to be understood. RPA 202248 and RPA 203328 are the primary components of the residue, accounting for 64-91% of the TRR. Metabolism of isoxaflutole in corn proceeds via: 1) hydrolysis of the isoxazole ring to form RPA 202248; 2) further hydrolysis to produce RPA 203328.

RAB1 need not defer to the HED Metabolism Committee on the toxicological significance of isoxaflutole metabolites identified in corn and rotational crops as the only metabolites identified, RPA 202248 and RPA 203328, are included in the tolerance expression. However, the HED Metabolism Committee will consider the possible formation of metabolites of toxicological concern which were not identified in these studies.

**Deficiency - Conclusion 6a-c (from Memo, G. Kramer 8/14/96)**

6a. For the poultry metabolism study, the petitioner should submit the dates of sample collection, extraction and analysis. For any matrix stored longer than 6 months, evidence of storage stability should be provided. CBTS can not translate the excreta storage stability results to other matrices as RPA 202248 was the only compound present in excreta and some degradation of this compound was observed.

6b. Provided that storage stability of the hen samples can be demonstrated, the nature of the residue in poultry is considered to be understood. RPA 202248, RPA 207048, RPA 203328, and RPA 205834 are the primary components of the residue, accounting for up to 93% of the TRR. Metabolism of isoxaflutole proceeds in poultry via: 1) hydrolysis of the isoxazole ring to form RPA 202248 and RPA 205834; 2) further hydrolysis to produce RPA 207048 and RPA 203328.

6c. CBTS will defer to the HED Metabolism Committee on the toxicological significance of metabolites once the deficiencies associated with poultry metabolism have been addressed. A decision concerning which residues to regulate will then follow. A tolerance based on the parent and metabolites RPA 202248 and RPA 203328 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.

**Petitioner's Response:** Sample analysis was initiated within 4 months of collection and continued for another 15 months. A comparison of chromatographic separations performed after 4 and 11 months of storage showed no differences in the metabolite profiles.

**RAB1's Conclusion:** These data do not cover stability for the entire interval of storage as is generally required for metabolism studies. However, as the metabolites identified in poultry correspond well with those identified in ruminants, RAB1 will not require further evidence of storage stability. The nature of the residue in poultry is now considered to be understood. RPA 202248, RPA 207048, RPA 203328, and RPA 205834 are the primary components of the residue, accounting for up to 93% of the TRR. Metabolism of isoxaflutole proceeds in poultry via: 1) hydrolysis of the isoxazole ring to form RPA 202248 and RPA 205834; 2) further hydrolysis to produce RPA 207048 and RPA 203328.

RAB1 will defer to the HED Metabolism Committee on the toxicological significance of metabolites in animal commodities. A decision concerning which residues to regulate will then follow. A tolerance based on the parent and RPA 202248 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.

**Deficiency - Conclusion 7d & 7f (from Memo, G. Kramer 8/14/96)**

7d. The specificity of the proposed analytical enforcement method was investigated by performing an interference study with 115 different pesticides. None were found to interfere with isoxaflutole. These compounds included all those for which tolerances are established on corn with the exception of rimsulfuron, flumiclorac-pentyl, halosulfuron, thifensulfuron-methyl, tridiphane, 4-aminopyridine, cyprazine, prosulfuron and 2-(thiocyanomethylthio)benzothiazole. The petitioner should provide interference data for these nine pesticides or provide a rationale for why these data are not needed.

7f. Provided that deficiencies pertaining to the interference study are resolved,

a confirmatory method will not be required.

**Petitioner's Response:** A supplemental study containing data on the nine requested compounds has been completed and is included with this submission:

> HERBICIDES: RPA 201772 Interference Study with Nine Additional Pesticides Used on Corn According to the "Analytical Method for the Determination of Residues of RPA 201772, RPA 202248 and RPA 203328 in Maize Forage, Silage, Grain and Fodder" MRID#441690-03.

None of the nine additional pesticides screened interfered with the analysis of Isoxaflutole, RPA 202248 and RPA 203328 above the limit of detection of 0.002 mg $kg^{-1}$ Isoxaflutole.

**RAB1's Conclusion:** The requested information has been provided. This deficiency is now resolved.

**Deficiency - Conclusion 7g (from Memo, G. Kramer 8/14/96)**

7g. CBTS concludes that Method P/93011 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 and completed interference study).

**Petitioner's Response:** none

**RAB1's Conclusion:** The proposed analytical enforcement method for corn RACs has been validated by ACL, Beltsville (Memo, G. Kramer 8/20/96; D228481). However, the petitioner should submit standards of isoxaflutole (including metabolites and the GC standard) to the EPA repository in RTP along with the MSDS, and a revised version of the proposed analytical enforcement method as specified in conclusions 1-5 of the aforementioned Memo. Until the receipt of the standard and the revised method, the requirements for analytical enforcement methodology will remain unfulfilled.

**Deficiency - Conclusion 8d & 8e (from Memo, G. Kramer 8/14/96)**

8d. A sample from the ruminant metabolism study was analyzed with the proposed enforcement method. In liver, 36% of the TRR was extractable. RPA 202248 comprised 13% of the TRR; isoxaflutole, 11%. These values do not correspond with the results of the metabolism study in which RPA 202248 comprised 86% of the TRR; isoxaflutole, 0%; and RPA 207048, 12%. CBTS concludes that the radiovalidation of this method was not successful. The petitioner should explain this discrepancy or develop a new enforcement method for meat, milk and eggs.

8e. A conclusion on the adequacy of this method for enforcement of the proposed animal RAC tolerances will be withheld pending satisfactory method validation (PMV and radiovalidation).

**Petitioner's Response:** A new enforcement method has been developed and independent laboratory evaluation conducted. The study reports

are included with this submission:

Isoxaflutole- Validation of Method of Analysis for Isoxaflutole and Its Metabolite in Animal Tissues. MRID# 441690-04.

Independent Method Validation of RPA 201772 and RPA 202248 in/on Bovine Kidney/Liver Tissue. Mckenzie Labs. MRID# 441690-05.

**Procedure:** Milk samples are extracted by homogenization in acidified acetonitrile. The extract is purified with a C-8 cartridge column. RPA 203328 is eluted in the first fraction; isoxaflutole, RPA 205834 and RPA 202248 are eluted in the second. These two fractions are then analyzed on two different HPLC systems, both of which employ a C-18 column with UV-Vis detection (270 or 300 nm). Egg samples are extracted by homogenization in acetonitrile. The extract is purified with a C-8 cartridge column. RPA 202248 is eluted in the second fraction and analyzed with HPLC as described above. Tissue samples are analyzed by a common moiety technique. The samples are extracted by homogenization in 0.1% aqueous trifluoroacetic acid. The extracts of fat samples are partitioned against hexane. In all samples, isoxaflutole is converted to RPA 202248 by base hydrolysis. The extract is then purified with a C-18 cartridge column. RPA 202248 is eluted and analyzed with HPLC as described above. The LOQ is 0.01 ppm for milk and eggs; 0.40 ppm for beef and poultry liver, 0.20 ppm for beef and poultry muscle and fat; and 0.20 ppm for beef kidney.

**Results:** Acceptable recoveries were obtained in all matrices (Table 2).

**ILV:** An ILV of this method was performed by Mckenzie Labs, Phoenix, AZ. Acceptable recoveries were obtained by the laboratory.

**Specificity:** The specificity of the proposed analytical enforcement method was investigated by performing an interference study with 205 different pesticides. None were found to interfere with isoxaflutole.

**Radiovalidation:** A goat milk sample from the ruminant metabolism study was used to determine extraction efficiency of the proposed tolerance enforcement method of analysis for milk and eggs. Three samples of goat milk containing grown-in residues of radiolabeled isoxaflutole, and one untreated control (UTC) cow milk were extracted using the proposed tolerance enforcement method of analysis for milk and eggs. The method was shown to extract 88% of the TRR and is adequate to extract residues of toxicological significance in milk and eggs. This result is comparable with that of the 87% extraction efficiency in the 1995 validation study submitted previously. No further analysis of this sample was performed. A goat liver sample from the ruminant metabolism study

was used to determine extraction efficiency of the proposed tolerance enforcement method of analysis. Three samples of goat liver containing grown-in residues of radiolabeled isoxaflutole, and one untreated control (UTC) cow liver were extracted using the proposed tolerance enforcement method of analysis. The method was shown to extract 93.8 % of the total radioactive residue (TRR). The percent of extracted radioactivity in the metabolism study is 99.5%. The extraction efficiency of the tolerance enforcement method of analysis is 94% and is adequate to extract residues of toxicological significance.

Table 2- Results of validation of proposed enforcement method for meat, milk and eggs.

| Animal | RAC | Fortification Level (ppm) | Average Recovery ± s.d. (n) |
|---|---|---|---|
| Cow | Fat | 0.20 | 84 ± 10% (3) |
| | | 1.0 | 75 ± 1% (2) |
| | Kidney | 0.20 | 73 ± 3% (2) |
| | | 1.0 | 80 ± 4% (2) |
| | Liver | 0.40 | 83 ± 4% (5) |
| | | 2.0 | 80 ± 3% (5) |
| | Muscle | 0.20 | 92 ± 13% (2) |
| | | 1.0 | 72 ± 0% (2) |
| | Milk | 0.01 | 98 ± 26% (5) |
| | | 0.05 | 91 ± 20% (6) |
| Poultry | Eggs | 0.01 | 74 ± 10% (5) |
| | | 0.05 | 93 ± 16% (5) |
| | Liver | 0.40 | 80 ± 1% (2) |
| | | 2.0 | 83 ± 2% (2) |
| | Fat + Skin | 0.20 | 86 ± 7% (5) |
| | | 1.0 | 83 ± 16% (5) |
| | Muscle | 0.20 | 67 ± 10% (2) |
| | | 1.0 | 78 ± 3% (2) |

**Confirmatory Method:** The petitioner has included conditions for separation on a different HPLC column (phenyl-SB) as a confirmatory technique. The method used for data gathering (LC/MS) is also available as a confirmatory technique.

**RAB1's Conclusion:** A new HPLC/UV enforcement method for meat, milk and eggs (EC-96-340) has been submitted by the petitioner. Adequate validation data (recovery, ILV and radiovalidation) were also submitted. The method and ILV have been sent to Beltsville for PMV (Memo, G. Kramer 1/16/97). RAB1 will withhold a final conclusion on the adequacy of this method as an analytical enforcement method pending receipt of the PMV report.

**Deficiency - Conclusion 8g & 8h (from Memo, G. Kramer 8/14/96)**

8g. An HPLC/MS/MS method was used to analyze the tissue samples from the feeding studies. Samples were extracted and cleaned-up by the same procedures used in the HPLC/UV method. Isoxaflutole and metabolites RPA 20704, RPA 205834 and RPA 202248 are then determined with HPLC/MS/MS. Acceptable recoveries were obtained in all tissues. The LOQ was reported to be 0.05 ppm.

8h. As the extraction and cleanup procedures of the LC/MS method closely resemble those of the HPLC/UV method, conclusions related to radiovalidation pertain to both methods. CBTS is thus unable to assess the adequacy of the LC/MS method for data gathering pending satisfactory resolution of the deficiency related to radiovalidation.

**Petitioner's Response:** Three samples of goat liver containing grown-in residues of radiolabeled isoxaflutole, and one untreated control cow liver were extracted using the LC-MS-MS data gathering method of analysis. Aliquots from each of the extracts were analyzed for radioactivity by liquid scintillation counting. Method extraction efficiency was calculated by dividing the activity in the extracts by the activity in the starting samples. The method was shown to extract 53% of the TRR. Therefore, in order to obtain an accurate interpretation of the residue data from the ruminant and poultry magnitude of residue studies, adjustments in the results from the LC-MS-MS data gathering method were made to correct for the lower extraction efficiency (Table 3).

Table 3-Adjustments in the results of the animal feeding studies made to correct for the lower extraction efficiency of the LC-MS-MS data gathering method.

| Tissue | Dose | Isoxaflutole (ppm) | RPA 202248 (ppm) | Total as Isoxaflutole[a] (ppm) | Total Corrected for Extraction Efficiency[b] (ppm) |
|---|---|---|---|---|---|
| Cow Muscle | 10X | <LOQ | <LOQ | 0.050 | 0.10 |
| Cow Fat | 10X | ND | <LOQ | 0.025 | 0.05 |
| Cow Kidney | 10X | ND | 0.503 | 0.503 | 1.01 |
| | 3X | --- | 0.296 | 0.296 | 0.59 |
| | 1X | --- | 0.166 | 0.166 | 0.33 |
| Cow Liver | 10X | ND | 1.840 | 1.840 | 3.68 |
| | 3X | ND | 1.090 | 1.090 | 2.18 |
| | 1X | ND | 0.770 | 0.770 | 1.54 |
| Poultry Liver | 10X | ND | 0.645 | 0.645 | 1.29 |
| | 3X | ND | 0.378 | 0.378 | 0.76 |
| | 1X | --- | 0.159 | 0.159 | 0.32 |

(a) Method LOQ = 0.050 ppm for calculation total residue, 0.0 ppm and 0.025 ppm assigned for ND and <LOQ, respectively.

(b) Correction for 53% extraction efficiency. Total corrected = Total Isoxaflutole x 2.

The above adjusted data necessitate revision of the proposed tolerances for meat and meat byproducts. The LOQs for the 1996 enforcement method are 0.40 ppm for cow and poultry liver and 0.20 ppm for muscle, kidney, fat, and skin+fat. The proposed tolerances need to be reconciled to accommodate these changes in the data. The new proposed tolerances are presented in Section F of this submission.

**RAB1's Conclusion:** The requested information has been provided. This deficiency is now resolved. The revised tolerances are evaluated below.

**Deficiency - Conclusion 9 (from Memo, G. Kramer 8/14/96)**

9. The petitioner has provided adequate storage stability data for corn RACs. The total residues of isoxaflutole and its metabolites are stable during frozen storage in corn RACs for up to 13 months. However, storage stability data are still required for processed corn commodities for a storage interval of 3 months.

**Petitioner's Response:** A storage stability study has been completed and is included with this submission:

Nandihalli, U. B. 1996. Freezer Storage Stability of RPA 201772 in Field Corn Samples. Study CHW 6224-223. Corning Hazelton Inc. Madison, Wisconsin. MRID# 441690-05.

Samples of corn processed commodities were fortified with residues of isoxaflutole, RPA 202248, and RPA 203328 and stored frozen at <-10°C. Samples were maintained frozen and two subsamples were removed and analyzed after 3 months for residues using the proposed enforcement method. Each analysis included two freshly fortified controls. The results demonstrate that the total residues of isoxaflutole and its metabolites are stable during storage in corn processed fractions for 3 months (Tables 4-6).

Table 4-Percent Recovery of Isoxaflutole from Processed Corn Commodities During Storage at <10 C° For Three Months

| RAC | Initial Level, ppm | Storage Interval, months | Fresh Fortification Recovery, % | Apparent Recovery in Stored Sample, % | Corrected Recovery in Stored Sample, % |
|---|---|---|---|---|---|
| Flour | 0.096 | 3 | 69.8 | 81.2 | 116 |
| Meal | 0.096 | 3 | 84.1 | 95.2 | 114 |
| Grits | 0.096 | 3 | 84.4 | 85.7 | 102 |
| Starch | 0.096 | 3 | 88.9 | 101 | 114 |
| Refined Oil | 0.096 | 3 | 84.4 | 89.7 | 106 |

Table 5-Percent Recovery of RPA 202248 from Processed Corn Commodities During Storage at <10 C° For Three Months

| RAC | Initial Level, ppm | Storage Interval, months | Fresh Fortification Recovery, % | Apparent Recovery in Stored Sample, % | Corrected Recovery in Stored Sample, % |
|---|---|---|---|---|---|
| Flour | 0.096 | 3 | 80.1 | 69.5 | 87.1 |
| Meal | 0.096 | 3 | 91.2 | 91.8 | 101 |
| Grits | 0.096 | 3 | 83.6 | 81.7 | 97.2 |
| Starch | 0.096 | 3 | 97.2 | 96.7 | 99.7 |
| Refined Oil | 0.096 | 3 | 83.8 | 91.0 | 109 |

Table 6-Percent Recovery of RPA 203328 from Processed Corn Commodities During Storage at <10 C° For Three Months

| RAC | Initial Level, ppm | Storage Interval, months | Fresh Fortification Recovery, % | Apparent Recovery in Stored Sample, % | Corrected Recovery in Stored Sample, % |
|---|---|---|---|---|---|
| Flour | 0.096 | 3 | 71.7 | 68.3 | 95.2 |
| Meal | 0.096 | 3 | 79.2 | 75.4 | 95.4 |
| Grits | 0.096 | 3 | 78.4 | 67.1 | 85.7 |
| Starch | 0.096 | 3 | 79.6 | 78.4 | 98.8 |
| Refined Oil | 0.096 | 3 | 80.4 | 80.2 | 99.7 |

**RAB1's Conclusion:** The requested information has been provided. The storage stability results indicated that there were no

significant losses of isoxaflutole, RPA 202248, or RPA 203328 in any of the matrices during storage under freezer conditions. This deficiency is now resolved.

**Deficiency - Conclusion 10b (from Memo, G. Kramer 8/14/96)**
10b. Based on these data, the appropriate tolerances for isoxaflutole and its metabolites are 0.2 ppm in grain, 0.5 ppm in stover and 1.0 ppm in forage. Also, tolerances should be proposed for: "the combined residues of the herbicide isoxaflutole and its metabolites 1-(2-methylsulfonyl-4-trifluoromethylphenyl-2-cyano-3-cyclopropyl propane-1,3-dione and 2-methylsulfonyl-4-trifluoromethyl benzoic acid, calculated as the parent compound, in/on Corn, field, grain..." **A revised Section F is thus required for this petition.** Further revisions to Section F will be required if additional metabolites are determined to be of toxicological significance by the HED Metabolism Committee.

**Petitioner's Response:** Section F has been revised to propose the following tolerances for residues of isoxaflutole and its metabolites 1-(2-methylsulfonyl-4-trifluoromethylphenyl-2-cyano-3-cyclopropyl propane-1,3-dione (RPA 202248) and 2-methylsulfonyl-4-trifluoromethyl benzoic acid (RPA 203328), calculated as the parent compound, in/on:

| | | | |
|---|---|---|---|
| Field Corn, Grain | -- 0.20 ppm | Field Corn, Fodder | -- 0.50 ppm |
| Field Corn, Forage | -- 1.0 ppm | | |

**RAB1's Conclusion:** The requested revisions to Section F have been provided. Further revisions to Section F will be required if additional metabolites are determined to be of toxicological significance by the HED Metabolism Committee.

**Deficiency - Conclusion 11 (from Memo, G. Kramer 8/14/96)**
11. Isoxaflutole residues do not appear to concentrate in processed corn commodities. Provided the storage stability of isoxaflutole residues in corn processed commodities can be demonstrated, food/feed additive tolerances for isoxaflutole and its metabolites will not be required.

**Petitioner's Response:** see above

**RAB1's Conclusion:** The submitted storage stability data indicated that there were no significant losses of isoxaflutole, RPA 202248, or RPA 203328 in any processed corn commodity during storage under freezer conditions. Tolerances on these commodities are not required. This deficiency is now resolved.

**Deficiency - Conclusion 12b (from Memo, G. Kramer 8/14/96)**
12b. Storage stability data for ruminant RACs have not been provided. The petitioner stated that a storage stability study is in progress. Also, the analytical methods may not be adequate for data gathering (see above). All conclusions pertaining to the magnitude of the residue in ruminants are contingent on submission of adequate storage stability data and radiovalidation of the analytical methods.

**Petitioner's Response:** A storage stability study has been completed and is included with this submission:

Lowder, J. F. 1996. Isoxaflutole: Storage Stability of Residues in Dairy Cow and Poultry Matrices. Study Number EC-96-338. Rhone-Poulenc Ag Company. MRID# 441690-07.

Samples of animal commodities were fortified with residues of isoxaflutole, RPA 202248, RPA 205834, RPA 207048, and RPA 203328 and stored frozen at <-10 °C. Samples were maintained frozen and two subsamples were removed and analyzed after 0.5, 1, 2, 3 and 4 months for residues using the data gathering method. Each analysis included a freshly fortified control. The results are shown in Tables 7-27. The results for milk indicate that RPA 202248, RPA 205834 and RPA 203328 are stable or show no indication of degradation during the conditions of the study. The parent compound, isoxaflutole appears to degrade with an estimated half life of approximately 111 days. The results for the tissues indicate that RPA 207048 does degrade in some tissue matrices. The other analytes appear to be stable in the kidney, muscle and fat tissues. For liver, isoxaflutole and RPA 202248 appear to be generally stable, whereas RPA 205834 and RPA 207048 appear to degrade with an estimated half life of about 3 months. The results for egg indicate that RPA 202248 is stable in the egg matrix (Table 27). As indicated in the feeding studies, the parent isoxaflutole is immediately converted to RPA 202248 in the egg matrix, so that no fresh recovery of isoxaflutole is possible.

Table 7 Milk Fortified with 0.1μg/g (0.1 ppm) of Isoxaflutole

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 4/29/96 | ---- a | ---- a | ---- a | ---- a |
| 15 Days 5/14/96 | 68 | 0.064<br>0.069 | 64<br>69 | 95<br>102 |
| 29 Days 5/28/96 | 86 | 0.072<br>0.069 | 72<br>69 | 84<br>80 |
| 56 Days 6/24/96 | 91 | 0.067<br>0.075 | 67<br>75 | 74<br>83 |
| 85 Days 7/23/96 | 77 | 0.061<br>0.064 | 61<br>64 | 79<br>83 |
| 127 Days 9/3/96 | 99 | 0.044<br>0.042 | 44<br>42 | 44<br>42 |

a) No apparent recovery in samples.

Table 8- Milk Fortified with 0.1μg/g (0.1 ppm) of RPA 202248

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 4/29/96 | ---- a | ---- a | ---- a | ---- a |
| 15 Days 5/14/96 | 72 | 0.066<br>0.072 | 66<br>72 | 92<br>100 |
| 29 Days 5/28/96 | 81 | 0.086<br>0.080 | 86<br>80 | 105<br>98 |
| 56 Days 6/24/96 | 82 | 0.084<br>0.079 | 84<br>79 | 102<br>96 |
| 85 Days 7/23/96 | 74 | 0.079<br>0.077 | 79<br>77 | 107<br>103 |
| 127 Days 9/3/96 | 88 | 0.109<br>0.090 | 109<br>90 | 123<br>102 |

a) Data not used, Low recovery.

Table 9- Milk Fortified with 0.1μg/g (0.1 ppm) of RPA 205834

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 4/29/96 | 87 | 0.094<br>0.110 | 94<br>110 | 108<br>126 |
| 21 Days 5/20/96 | 100 | 0.101<br>0.097 | 101<br>97 | 100<br>97 |
| 29 Days 5/28/96 | 95 | 0.088<br>0.098 | 88<br>98 | 93<br>103 |
| 56 Days 6/24/96 | 96 | 0.090<br>0.093 | 90<br>93 | 93<br>96 |
| 85 Days 7/23/96 | 90 | 0.092<br>0.092 | 92<br>92 | 102<br>102 |
| 128 Days 9/4/96 | 94 | 0.096<br>0.092 | 96<br>92 | 102<br>97 |

Table 10- Milk Fortified with 0.1μg/g (0.1 ppm) of RPA 203328

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 4/29/96 | 74 | 0.091<br>0.089 | 91<br>89 | 124<br>121 |
| 15 Days 5/14/96 | 72 | 0.084<br>0.088 | 84<br>88 | 117<br>122 |
| 29 Days 5/28/96 | 97 | 0.093<br>0.093 | 93<br>93 | 95<br>95 |
| 56 Days 6/24/96 | 87 | 0.077<br>0.085 | 77<br>85 | 88<br>97 |
| 85 Days 7/23/96 | 84 | 0.105<br>0.096 | 105<br>96 | 125<br>115 |
| 128 Days 9/4/96 | 92 | 0.085<br>0.102 | 85<br>102 | 92<br>111 |

Table 11- Cow Liver Fortified with 0.25μg/g (0.25 ppm) of Isoxaflutole

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 5/3/96 | ---- a | ---- a | ---- a | ---- a |
| 10 Days 5/13/96 | 69 | 0.188<br>0.175 | 75<br>70 | 109<br>101 |
| 40 Days 6/12/96 | 89 | 0.195<br>0.186 | 78<br>74 | 88<br>84 |
| 60 Days 7/2/96 | 75 | 0.163<br>0.176 | 65<br>70 | 87<br>94 |
| 83 Days 7/25/96 | 89 | 0.203<br>0.218 | 81<br>87 | 91<br>98 |
| 130 Days 9/10/96 | 94 | 0.189<br>0.167 | 76<br>67 | 80<br>71 |

Values are a total of Isoxaflutole & RPA 202248 measured in samples.
a) Data not used, low recovery.

Table 12- Cow Liver Fortified with 0.25μg/g (0.25 ppm) of RPA 202248

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 5/3/96 | 91 | 0.263<br>0.271 | 105<br>108 | 116<br>119 |
| 10 Days 5/13/96 | 69 | 0.147<br>0.211 | 59<br>84 | 85<br>122 |
| 40 Days 6/12/96 | 104 | 0.195<br>0.212 | 78<br>85 | 75<br>82 |
| 60 Days 7/2/96 | 89 | 0.188<br>0.230 | 75<br>92 | 84<br>103 |
| 83 Days 7/25/96 | 97 | 0.183<br>0.167 | 73<br>67 | 75<br>69 |
| 130 Days 9/10/96 | 100 | 0.174<br>0.210 | 70<br>84 | 70<br>84 |

Table 13- Cow Liver Fortified with 0.25μg/g (0.25 ppm) of RPA 205834

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 5/3/96 | ---- a | ---- a | ---- a | ---- a |
| 10 Days 5/13/96 | 84 | 0.215<br>0.214 | 86<br>86 | 102<br>102 |
| 40 Days 6/12/96 | 108 | 0.169<br>0.226 | 68<br>90 | 63<br>84 |
| 60 Days 7/2/96 | 96 | 0.186<br>0.183 | 74<br>73 | 78<br>76 |
| 94 Days 8/5/96 | 101 | 0.187<br>0.218 | 75<br>87 | 74<br>86 |
| 130 Days 9/10/96 | 101 | 0.089<br>0.092 | 36<br>37 | 35<br>36 |

a) Data not used, low recovery.

Table 14- Cow Liver Fortified with 0.25μg/g (0.25 ppm) of RPA 207048

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 5/3/96 | ---- a | ---- a | ---- a | ---- a |
| 10 Days 5/13/96 | 63 | 0.136<br>0.151 | 54<br>60 | 86<br>96 |
| 40 Days 6/12/96 | 92 | 0.165<br>0.175 | 66<br>70 | 72<br>76 |
| 60 Days 7/2/96 | 87 | 0.129<br>0.135 | 52<br>54 | 59<br>62 |
| 83 Days 7/25/96 | 77 | 0.110<br>0.113 | 44<br>45 | 57<br>59 |
| 130 Days 9/10/96 | 83 | 0.053<br>0.070 | 21<br>28 | 26<br>34 |

a) Data not used, low recovery.

Table 15- Cow Kidney Fortified with 0.25μg/g (0.25 ppm) of Isoxaflutole

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 5/7/96 | 72 | 0.138<br>0.158 | 55<br>63 | 77<br>88 |
| 13 Days 5/20/96 | ---- a | ---- a | ---- a | ---- a |
| 27 Days 6/3/96 | ---- b | ---- b | ---- b | ---- b |
| 62 Days 7/8/96 | 83 | 0.238<br>0.199 | 95<br>80 | 115<br>96 |
| 84 Days 7/30/96 | 80 | 0.232<br>0.198 | 93<br>79 | 116<br>99 |
| 115 Days 8/30/96 | 100 | 0.208<br>0.232 | 83<br>93 | 83<br>93 |

Values are a total of Isoxaflutole & RPA 202248 measured in samples.
a) Data not used, low recovery.
b) Data not used, poor standard curve (r2 < 0.99) for all analytes.

Table 16- Cow Kidney Fortified with 0.25µg/g (0.25 ppm) of RPA 202248

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 5/7/96 | 94 | 0.227<br>0.217 | 91<br>87 | 97<br>92 |
| 13 Days 5/20/96 | 102 | 0.197<br>0.216 | 79<br>86 | 77<br>85 |
| 27 Days 6/3/96 | ---- a | ---- a | ---- a | ---- a |
| 62 Days 7/8/96 | 97 | 0.203<br>0.227 | 81<br>91 | 84<br>94 |
| 84 Days 7/30/96 | 93 | 0.205<br>0.207 | 82<br>83 | 88<br>89 |
| 115 Days 8/30/96 | 122 | 0.302<br>0.276 | 121<br>110 | 99<br>90 |

a) Data not used, poor standard curve (r2 < 0.99) for all analytes.

Table 17- Cow Kidney Fortified with 0.25µg/g (0.25 ppm) of RPA 205834

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 5/7/96 | ---- a | ---- a | ---- a | ---- a |
| 13 Days 5/20/96 | 96 | 0.263<br>0.221 | 105<br>88 | 110<br>92 |
| 27 Days 6/3/96 | ---- b | ---- b | ---- b | ---- b |
| 62 Days 7/8/96 | 93 | 0.208<br>0.201 | 83<br>80 | 89<br>86 |
| 84 Days 7/30/96 | 95 | 0.192<br>0.198 | 77<br>79 | 81<br>83 |
| 115 Days 8/30/96 | 116 | 0.237<br>0.275 | 95<br>110 | 82<br>95 |

a) Data not used, low recovery.
b) Data not used, poor standard curve (r2 < 0.99) for all analytes.

Table 18- Cow Kidney Fortified with 0.25µg/g (0.25 ppm) of RPA 207048

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 5/7/96 | 88 | 0.190<br>0.195 | 76<br>78 | 86<br>89 |
| 13 Days 5/20/96 | 95 | 0.125<br>0.103 | 50<br>41 | 53<br>43 |
| 27 Days 6/3/96 | ---- a | ---- a | ---- a | ---- a |
| 62 Days 7/8/96 | 77 | 0.102<br>0.122 | 41<br>49 | 53<br>63 |
| 84 Days 7/30/96 | 91 | 0.112<br>0.111 | 45<br>44 | 49<br>49 |
| 115 Days 8/30/96 | 104 | 0.113<br>0.100 | 45<br>40 | 43<br>38 |

a) Data not used, poor standard curve (r2 < 0.99) for all analytes.

Table 19- Cow Muscle Fortified with 0.25μg/g (0.25 ppm) of Isoxaflutole

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 5/9/96 | ---- a | ---- a | ---- a | ---- a |
| 14 Days 5/23/96 | ---- a | ---- a | ---- a | ---- a |
| 28 Days 6/6/96 | ---- a | ---- a | ---- a | ---- a |
| 63 Days 7/11/96 | 94 | 0.224<br>0.201 | 90<br>80 | 95<br>86 |
| 85 Days 8/2/96 | 83 | 0.204<br>0.230 | 82<br>92 | 98<br>111 |
| 131 Days 9/17/96 | ---- a | ---- a | ---- a | ---- a |

Values are a total of Isoxaflutole & RPA 202248 measured in samples.
a) Data not used, low recovery.

Table 20- Cow Muscle Fortified with 0.25μg/g (0.25 ppm) of RPA 202248

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 5/9/96 | 92 | 0.238<br>0.213 | 95<br>85 | 103<br>93 |
| 14 Days 5/23/96 | 101 | 0.341<br>0.303 | 136<br>121 | 135<br>120 |
| 28 Days 6/6/96 | 87 | 0.265<br>0.203 | 106<br>121 | 122<br>93 |
| 63 Days 7/11/96 | 100 | 0.225<br>0.245 | 106<br>81 | 90<br>98 |
| 85 Days 8/2/96 | 79 | 0.226<br>0.225 | 90<br>98 | 114<br>114 |
| 131 Days 9/17/96 | 113 | 0.298<br>0.299 | 119<br>120 | 105<br>106 |

Table 21- Cow Muscle Fortified with 0.25μg/g (0.25 ppm) of RPA 205834

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 5/9/96 | 82 | 0.236<br>0.219 | 94<br>88 | 115<br>107 |
| 14 Days 5/23/96 | ---- a | ---- a | ---- a | ---- a |
| 28 Days 6/6/96 | 90 | 0.226<br>0.206 | 90<br>82 | 100<br>92 |
| 63 Days 7/11/96 | 100 | 0.236<br>0.216 | 94<br>86 | 94<br>86 |
| 85 Days 8/2/96 | 97 | 0.178<br>0.193 | 71<br>77 | 73<br>80 |
| 131 Days 9/17/96 | 99 | 0.220<br>0.213 | 88<br>85 | 89<br>86 |

a) Data not used, low recovery.

Table 22- Cow Muscle Fortified with 0.25μg/g (0.25 ppm) of RPA 207048

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 5/9/96 | 71 | 0.208<br>0.210 | 83<br>84 | 117<br>118 |
| 14 Days 5/23/96 | 81 | 0.209<br>0.186 | 84<br>74 | 103<br>92 |
| 28 Days 6/6/96 | 78 | 0.148<br>0.128 | 59<br>51 | 76<br>66 |
| 63 Days 7/11/96 | 87 | 0.133<br>0.137 | 53<br>55 | 61<br>63 |
| 85 Days 8/2/96 | 74 | 0.120<br>0.108 | 48<br>43 | 65<br>58 |
| 131 Days 9/17/96 | 95 | 0.114<br>0.101 | 46<br>40 | 48<br>43 |

Table 23- Cow Fat Fortified with 0.25μg/g (0.25 ppm) of Isoxaflutole

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 5/8/96 | ---- a | ---- a | ---- a | ---- a |
| 13 Days 5/21/96 | 97 | 0.293<br>0.120 | 117<br>48 | 121<br>49 |
| 28 Days 6/5/96 | 79 | 0.170<br>0.235 | 68<br>94 | 86<br>119 |
| 62 Days 7/9/96 | 80 | 0.215<br>0.207 | 86<br>83 | 108<br>104 |
| 84 Days 7/31/96 | 85 | 0.207<br>0.204 | 83<br>82 | 97<br>96 |
| 113 Days 8/29/96 | 84 | 0.227<br>0.214 | 91<br>86 | 108<br>102 |

Values are a total of Isoxaflutole & RPA 202248 measured in samples.
a) Data not used, low recovery.

Table 24- Cow Fat Fortified with 0.25μg/g (0.25 ppm) of RPA 202248

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 5/8/96 | 70 | 0.216<br>0.210 | 86<br>84 | 123<br>120 |
| 13 Days 5/21/96 | 120 | 0.116<br>0.218 | 46<br>87 | 39<br>73 |
| 28 Days 6/5/96 | 89 | 0.252<br>0.248 | 101<br>99 | 113<br>111 |
| 62 Days 7/9/96 | 82 | 0.196<br>0.243 | 78<br>97 | 96<br>119 |
| 84 Days 7/31/96 | 95 | 0.214<br>0.252 | 86<br>101 | 90<br>106 |
| 113 Days 8/29/96 | 120 | 0.240<br>0.247 | 96<br>99 | 80<br>82 |

Table 25- Cow Fat Fortified with 0.25μg/g (0.25 ppm) of RPA 205834

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 5/8/96 | 73 | 0.219<br>0.232 | 88<br>93 | 120<br>127 |
| 13 Days 5/21/96 | 89 | 0.278<br>0.161 | 111<br>64 | 125<br>72 |
| 28 Days 6/5/96 | 89 | 0.208<br>0.235 | 83<br>94 | 93<br>106 |
| 62 Days 7/9/96 | 92 | 0.217<br>0.251 | 87<br>100 | 94<br>109 |
| 84 Days 7/31/96 | 90 | 0.212<br>0.223 | 85<br>89 | 94<br>99 |
| 113 Days 8/29/96 | 110 | 0.229<br>0.237 | 92<br>95 | 83<br>86 |

Table 26- Cow Fat Fortified with 0.25μg/g (0.25 ppm) of RPA 207048

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 5/8/96 | 69 | 0.171<br>0.170 | 68<br>68 | 99<br>99 |
| 13 Days 5/21/96 | 94 | 0.213<br>0.213 | 85<br>85 | 91<br>91 |
| 28 Days 6/5/96 | 82 | 0.195<br>0.178 | 78<br>71 | 95<br>87 |
| 62 Days 7/9/96 | 80 | 0.162<br>0.117 | 65<br>47 | 81<br>59 |
| 84 Days 7/31/96 | 78 | 0.171<br>0.226 | 68<br>90 | 88<br>116 |
| 113 Days 8/29/96 | 94 | 0.131<br>0.124 | 52<br>50 | 56<br>53 |

Table 27- Egg Fortified with 0.25μg/g (0.25 ppm) of RPA 202248

| Storage Period and Date of Extraction | Percent Fresh Fortification Recovery | Measured Amount in Stored Sample (ppm) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
|---|---|---|---|---|
| 0 Day 4/29/96 | 95 | 0.237<br>0.241 | 95<br>96 | 100<br>101 |
| 15 Days 5/14/96 | 99 | 0.237<br>0.251 | 95<br>100 | 96<br>101 |
| 29 Days 5/28/96 | 99 | 0.238<br>0.244 | 95<br>98 | 96<br>99 |
| 56 Days 6/24/96 | 95 | 0.209<br>0.216 | 84<br>86 | 88<br>91 |
| 88 Days 7/26/96 | 101 | 0.227<br>0.227 | 91<br>91 | 90<br>90 |
| 129 Days 9/5/96 | 95 | 0.215<br>0.242 | 86<br>97 | 91<br>102 |

**RAB1's Conclusion:** The requested information has been provided. This deficiency is now resolved.

**Deficiency - Conclusion 12c (from Memo, G. Kramer 8/14/96)**

12c. Based on the estimated maximum dietary burden of 1.2-1.4 ppm, the dietary feeding levels in this study were ≈3X, ≈10X and ≈35X. The results of this feeding study indicate that the appropriate tolerances are:

| | | | | | |
|---|---|---|---|---|---|
| Milk | -- | 0.02 ppm | Liver* | -- | 0.20 ppm |
| Meat Byproducts (except liver)* | -- | 0.03 ppm | | | |

*of cattle, goat, hogs, horses and sheep

The tolerance expression proposed by the petitioner includes RPA 203328. However, this metabolite is neither found in animals nor is it measured in the proposed enforcement method for animal tissues. Meat and milk tolerances should thus be proposed for: "the combined residues of the herbicide isoxaflutole and its metabolite 1-(2-methylsulfonyl-4-trifluoromethylphenyl-2-cyano-3-cyclopropyl propane-1,3-dione, calculated as the parent compound, in/on..." **A revised Section F is required for this petition.** Further revisions to Section F will be required if additional metabolites are determined to be of toxicological significance by the HED Metabolism Committee.

**Petitioner's Response:** A revised Section F in which the following tolerances were proposed:

Tolerances are also proposed for the combined residues of the herbicide isoxaflutole and its metabolite 1-(2-methylsulfonyl-4-trifluoromethylphenyl-2-cyano-3-cyclopropyl propane-1,3-dione, calculated as the parent compound, in/on:

| | | | | | |
|---|---|---|---|---|---|
| Milk | -- | 0.02 ppm | Liver* | -- | 2.0 ppm |
| Poultry, Liver - 2.0 ppm | | | Kidney* | -- | 0.40 ppm |
| Meat Byproducts (except liver and kidney)* | -- | 0.20 ppm | | | |

*of cattle, goat, hogs, poultry and sheep

**RAB1's Conclusion:** The samples from the feeding studies were stored for a maximum of 3 months. The results of the ruminant feeding study have been recalculated, correcting for the 50% extraction efficiency of the LC-MS-MS data gathering method and the decline of residues observed in some tissue/metabolite combinations (Tables.28 & 29).

Based on the estimated maximum dietary burden of 1.2-1.4 ppm (from Memo, G. Kramer 8/14/96), the dietary feeding levels in this study were ≈3X, ≈10X and ≈35X. The results of this feeding study indicate that the appropriate tolerances are:

| | | | | | |
|---|---|---|---|---|---|
| Milk | -- | 0.02 ppm | Liver* | -- | 0.50 ppm |
| Meat Byproducts (except liver)* | -- | 0.10 ppm | | | |

*of cattle, goat, hogs, horses and sheep

**A revised Section F is required for this petition.** Further revisions to Section F will be required if additional metabolites are determined to be of toxicological significance by the HED Metabolism Committee.

Table 28- Average Residues of Isoxaflutole Found at Indicated Time Interval in Milk (ppm)*

| Dose Level, ppm | | 13.8 | | 46 | | | | |
|---|---|---|---|---|---|---|---|---|
| Sampling Time, days | | 36 | 41 | 4 | 25 | 33 | 36 | 41 |
| Metabolite | Isoxaflutole | <LOQ | <LOQ | <LOQ | LOQ | ND | ND | <LOQ |
| | RPA 202248 | <LOQ | <LOQ | 0.025 | 0.030 | 0.026 | 0.020 | <LOQ |
| | RPA 205834 | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |

*n=4
LOQ = 0.02 ppm
ND=not detected

Table 29- Average Residues of Isoxaflutole Found at 42 Days in Cow Tissue (ppm)* Corrected for Extractability and Storage Stability

| Matrix | | Liver | | | Kidney | | | Muscle | Fat |
|---|---|---|---|---|---|---|---|---|---|
| Dose Level, ppm | | 4.6 | 13.8 | 46 | 4.6 | 13.8 | 46 | 46 | 46 |
| Metabolite | Isoxaflutole | ND | ND | ND | - | - | ND | ND | ND |
| | RPA 202248 | 1.56 | 2.12 | 4.41 | 0.28 | 0.47 | 0.96 | <LOQ | <LOQ |
| | RPA 205834 | 0.35 | 0.99 | 2.92 | <LOQ | <LOQ | <LOQ | <LOQ | 0.14 |
| | RPA 207048 | <LOQ | <LOQ | 0.17 | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |

*n=4
LOQ= 0.05 ppm
ND = Not detected

Table 30- Average residues of Isoxaflutole Found at 42 Days in Poultry Tissues (ppm)* Corrected for Extractability

| Matrix | | Liver | | | Eggs | Muscle | | Skin Plus Fat | |
|---|---|---|---|---|---|---|---|---|---|
| Dose Level, ppm | | 0.18 | 0.54 | 1.8 | 1.8 | 0.54 | 1.8 | 0.54 | 1.8 |
| Metabolite | Isoxaflutole | NA | ND | ND | ND | ND | ND | ND | ND |
| | RPA 202248 | 0.28 | 0.71 | 1.11 | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |

*n=3
NA = not analyzed
ND = not detected; LOQ = 0.05 ppm

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**Deficiency - Conclusion 13b & 13c (from Memo, G. Kramer 8/14/96)**

13b. Storage stability data for poultry RACs have not been provided. The petitioner stated that a storage stability study is in progress. Also, the analytical methods may not be adequate for data gathering (see above). All conclusions pertaining to the magnitude of the residue in poultry are contingent on submission of adequate storage stability data and validation of the analytical methods.

13c. Based on the estimated maximum dietary burden of 0.2 ppm, the dietary feeding levels in this study were 0.9X, 2.7X and 9X. The results of this feeding study indicate that the appropriate tolerances are: Poultry, Liver - 0.20 ppm.

**Petitioner's Response:** see above

**RAB1's Conclusion:** The samples from the feeding studies were stored for a maximum of 3 months. The results of the storage stability study showed that residues were stable during this interval. The results of the poultry feeding study have been recalculated, correcting for the 50% extraction of the LC-MS-MS data gathering method (Table 30).

Based on the estimated maximum dietary burden of 0.2 ppm (from Memo, G. Kramer 8/14/96), the dietary feeding levels in this study were 0.9X, 2.7X and 9X. The results of this feeding study indicate that the appropriate tolerances are: Poultry, Liver - 0.30 ppm. **A revised Section F is required for this petition.** Further revisions to Section F will be required if additional metabolites are determined to be of toxicological significance by the HED Metabolism Committee.

cc: PP#6F04664, G. Kramer (RAB1)
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