8-14-96

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

AUG 14 1996

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP# 6F04664. Isoxaflutole in/on Field Corn and Animal RACs. **Evaluation of Residue Data and Analytical Methods.** MRID#s 439048-01, -02, -27 thru -37 and -39. Chemical 123000. Barcode D224213. CBTS# 17015. Case 287353.

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THRU: E.T. Haeberer, Acting Branch Chief
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Registration Section, RCAB
Health Effects Division (7509C)

Rhône-Poulenc Ag Company has proposed permanent tolerances for the preemergent herbicide 5-cyclopropyl-4-isoxazolyl [2-(methylsulfonyl)-4-trifluoromethyl) phenyl] methanone (isoxaflutole, RPA 201772) and its metabolites, 1-(2-methylsulphonyl-4-trifluoromethylphenyl-2-cyano-3-cyclopropyl propane-1,3-dione (RPA 202248) and 2-methylsulphonyl-4-trifluoromethyl benzoic acid (RPA 203328) in/on the raw agricultural commodities (RACs) as follows:

Field Corn, Grain --	0.10 ppm	Field Corn, Fodder --	0.40 ppm
Field Corn, Forage --	0.40 ppm	Liver* --	0.20 ppm
Hog, Liver --	0.04 ppm	Eggs --	0.05 ppm
Poultry, Fat --	0.05 ppm	Poultry, Meat --	0.05 ppm
Kidney* --	0.03 ppm	Hog, Kidney --	0.01 ppm

*of cattle, goat, poultry and sheep

In review of a request for an EUP and temporary tolerances for

isoxaflutole on corn (PP#5G4484), CBTS identified the deficiencies which must be addressed by the registrant in order for us to recommend in favor of permanent tolerances (Memo, P. Errico 12/7/95; CBTS# 15430). In the Detailed Considerations section of this Memo, the outstanding deficiencies, listed as presented in the Memo of P. Errico (12/7/95), are followed by the petitioner's response and our conclusions.

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

Executive Summary of Chemistry Deficiencies

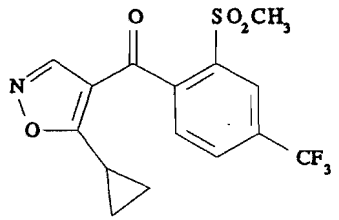
- Revised Section B.
- Additional data for confined crop rotation study.
- Additional data for corn metabolism study.
- Additional data for poultry metabolism study.
- Additional data for method specificity study.
- Agency validation of analytical method for plants.
- Radiovalidation of analytical method for animals.
- Agency validation of analytical method for animals.
- Storage stability data for corn processed commodities.
- Storage stability data for animal RACs.
- Revised Section F.
- HED Metabolism Committee decision

BACKGROUND

The registrant identifies this chemical as one of a new class of benzoylisoxazoles which is taken up by the roots and effects the synthesis of quinone by inhibiting the enzyme 4-hydroxyphenyl-pyruvate dioxygenase. Because quinone is required for the biosynthesis of carotenoids, susceptible grasses and weeds are bleached. Isoxaflutole is formulated as Balance WDG Herbicide (EPA File Symbol No. 264-XX). The structure of isoxaflutole is shown

below:

ISOXAFLUTOLE



CONCLUSIONS

1. The submitted product chemistry studies satisy the data requirements for this tolerance request. CBTS does not anticipate a residue problem in corn and animal RACs from the impurities present in the TGAI at the levels determined from the preliminary analysis when the TGAI is formulated and used as directed. Once full commercial production has started, the registrant should submit the analysis of 5 batches of the technical grade active ingredient (GLN 62-1).

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.

3a. In the submitted confined rotational crop study, [Phenyl(U)-^{14}C]-isoxaflutole was applied to outdoor plots at a rate of 0.18 lbs. ai/A (0.9X) using preplant incorporation (PPI) or preemergence (PRE) application to separate plots. Lettuce, sorghum and radishes were planted 34 days after treatment (DAT); mustard, radishes and wheat were planted 123 DAT; and lettuce, sorghum and radishes were planted 365 DAT. The highest residue levels were seen in 34 DAT sorghum forage (0.13-0.24 ppm).

3b. In 34 DAT crops, RPA 203328 accounted for 9-100% of the TRR (0.01-0.24 ppm); RPA 202248, 0-27% (0-0.005 ppm). In 123 DAT wheat, RPA 203328 accounted for 56-100% of the TRR (0.01-0.03 ppm). In 365 DAT sorghum, RPA 203328 accounted for 0-66% of the TRR (0.0-0.02 ppm).

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.

3e. The petitioner has previously submitted a discussion of their rationale for conducting metabolism studies with isoxaflutole labelled only in the phenyl ring (MRID# 435732-50; Memo, P. Errico 12/7/95). The petitioner stated that they conducted numerous preliminary metabolism studies, with plants, soil, and animals, in which isoxaflutole was labelled in the phenyl ring, in the isoxazole ring, or at the carbonyl carbon. Based on these studies, RPA 203328 is the major metabolite and opening of the isoxazole ring to form RPA 202248 was observed in plants, soil, and rats. Provided that the metabolism of isoxaflutole in rotational crops is demonstrated to proceed via opening of the isoxazole ring, CBTS concludes that a confined study using isoxaflutole labelled in this ring will not be required.

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.

5a. The nature of the residue in ruminants is considered to be understood. RPA 202248, RPA 207048 and RPA 205834 are the primary components of the residue, accounting for 54-98% of the TRR. Metabolism of isoxaflutole in ruminants proceeds via: 1) hydrolysis of the isoxazole ring to form RPA 202248 and RPA 205834; 2) further hydrolysis to produce RPA 207048.

5b. For compounds with multiple rings, CBTS generally requires that metabolism studies be performed with each ring labelled. However, as the metabolism of isoxaflutole in ruminants proceeds via opening of the isoxazole ring, CBTS concludes that a goat metabolism study using isoxaflutole labelled in this ring will not

be required.

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.

7a. The petitioner has submitted method P 93/011 for enforcement of the proposed tolerances in corn RACs. The method involves hydrolysis of isoxaflutole to RPA 202248, conversion of RPA 202248 to RPA 203328, and then derivatization of RPA 203328 to a methyl ester for GC/MSD analysis.

7b. An ILV of this method was performed by ABC Labs. The method and ILV have been sent to Beltsville for PMV (Memo, G. Kramer 2/14/96).

7c. Data pertaining to the recovery of isoxaflutole and its metabolites RPA 202248 and RPA 203328 using FDA multiresidue methods were submitted in conjunction with PP#5G04484 (MRID 43573252). These multiresidue screening data were forwarded to FDA (Memo, P. Errico 10/23/95).

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.

7e. A fodder sample from the plant metabolism study was analyzed with the proposed enforcement method. Of the TRR, 77.5% was detected as RPA 204497 by GC/MS. This value corresponds well with the expected result (68.1%) based on the metabolism study. CBTS concludes that method P/93011 has been adequately radiovalidated.

7f. Provided that deficiencies pertaining to the interference study are resolved, a confirmatory method will not be required.

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).

8a. The petitioner has submitted method EC-95-313 for enforcement of the proposed tolerances in animal RACs. In milk samples, RPA 203328, isoxaflutole, RPA 205834 and RPA 202248 are analyzed on HPLC (C-18 column with UV-Vis detection). Egg samples are analyzed for RPA 202248 by HPLC. Tissue samples are analyzed by a common moiety technique. Isoxaflutole is converted to RPA 202248 by base hydrolysis and analyzed with HPLC. The LOQ is 0.01 ppm for milk and eggs; 0.05 ppm for beef liver, beef and poultry muscle and fat; 0.075 ppm for beef kidney; and 0.10 ppm for poultry liver.

8b. Acceptable recoveries were obtained in all matrices except cow muscle. Some recoveries were slightly less than 70% (cow fat and kidney, and poultry muscle), but are acceptable due to the low standard errors (0-7%). CBTS notes that the actual LOQs in cow and poultry liver appear to be higher than that claimed by the petitioner (0.05 ppm for cow and 0.10 for ppm poultry).

8c. An ILV of this method was performed by Horizon Labs, Columbia, MO. Acceptable recoveries were obtained by the laboratory. The method and ILV have been sent to Beltsville for PMV (Memo, G. Kramer 6/14/96).

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).

8f. 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, see below) is also available as a confirmatory technique.

8g. A 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 clean-up 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.

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.

10a. Between the residue data submitted with this petition and those submitted previously, the petitioner has provided the results of 32 field corn trials, located in Regions 1 (1 trial), 2 (1 trial), 6 (1 trial) and 5 (29 trials). CBTS concludes that these trials were conducted in accordance with the *EPA Guidance on Number and Location of Domestic Crop Field Trials for Establishment of Pesticide Residue Tolerances, 6/2/94*. The maximum residues were 0.88 ppm in forage, 1.1 ppm in silage, 0.40 ppm in fodder, and 0.11 ppm in grain.

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.

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.

12a. Dairy cows were dosed daily for 42 days with isoxaflutole levels of 4.6, 13.8, and 46 ppm in the diet. At the 4.6 ppm dietary burden, quantifiable residues were observed only in liver (up to 0.8 ppm), milk (up to 0.03 ppm), and kidney (up to 0.2 ppm). At the highest dose level, quantifiable residues of isoxaflutole or RPA 202248 were not observed in fat or muscle.

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.

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.

13a. Laying hens were dosed for 42 days with isoxaflutole at levels of 0, 0.18, 0.54 and 1.8 ppm in the diet. At the 1.8 ppm dietary burden, quantifiable residues were observed only in liver (up to 0.6 ppm). At the highest dose level, quantifiable residues of isoxaflutole or RPA 202248 were not observed in eggs, meat, fat or muscle.

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.

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. The poultry liver tolerance should 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 magnitude of the residue data for poultry and revisions to Section F will be required if additional metabolites are determined to be of toxicological significance by the HED Metabolism Committee.

14. There is neither a Codex proposal, nor Canadian or Mexican limits for residues of isoxaflutole and its metabolites in corn. Therefore, a compatibility issue is not relevant to the proposed tolerance. A copy of the IRLS sheet is attached to this memorandum.

RECOMMENDATIONS

CBTS recommends against the proposed tolerances for isoxaflutole and its metabolites in/on field corn and animal RACs for reasons detailed in conclusions 2, 3c, 3d, 4a, 4b, 6a, 6b, 6c, 7d, 7f, 7g, 8d, 8e, 8h, 9, 10b, 11, 12b, 12c, 13b, and 13c.

DETAILED CONSIDERATIONS

Product Chemistry

Deficiency - Conclusion 1 (from Memo, P. Errico 12/7/95)

1. The submitted product chemistry studies satisfies the data requirements for this temporary tolerance request. For the permanent tolerance request and once full commercial production has started, the registrant should submit the analysis of 5 batches of the technical grade active ingredient (GLN 62-1).

Petitioner's Response: Once commercial production is initiated, these data will be submitted.

CBTS' Conclusion: The submitted product chemistry studies satisfy the data requirements for this tolerance request. Once full commercial production has started, the registrant should submit the analysis of 5 batches of the technical grade active ingredient (GLN 62-1). This deficiency is now resolved.

CBTS does not anticipate a residue problem in corn and animal RACs from the impurities present in the TGAI at the levels determined from the preliminary analysis when the TGAI is formulated and used as directed.

Formulation

Isoxaflutole is formulated as Balance WDG Herbicide (EPA File Symbol No. 264-XX), a water-dispersible granule containing 76.5% a.i.

Proposed Use

Balance is proposed for a single early preplant or preemergence broadcast application to field corn grown in either conventional, reduced tillage, or no-till crop management systems. The proposed application rates are dependent on application timing and soil type and are listed below:

	Maximum Rate (lb ai/A) By Soil Type	
Application Timing	Medium and Heavy Soils	Sandy Soils
Early preplant	0.191	0.096
Preemergence	0.120	0.072

Early preplant application may be made up to 30 days prior to planting. For effective weed control when the pesticide is applied early preplant, the label specifies that treated soil should not be moved out of the row; untreated soil should also not be moved to surface during planting. Application is to be made in a minimum of 10 gal/A using ground equipment and may be made alone or as a tank mix with other herbicides.

There are no rotational crop restrictions listed on the label.

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.

Rotational Crop Studies

Deficiency - Conclusion 9 (from Memo, P. Errico 12/7/95)

9. No confined or field rotational crop studies were submitted. The proposed label specifies rotational crops can be planted the season following use in corn. No studies will be necessary for this proposed use. For the permanent tolerance request, confined crop rotations will be necessary, and, depending on these results, field rotational crop studies and proposed tolerances for inadvertent residues may be necessary.

Petitioner's Response: Submission of:

> ^{14}C-RPA201772: Accumulation Study on Confined Rotational Crops. MRID# 439048-39.

In-Life Phase: [Phenyl(U)-^{14}C]-isoxaflutole (18.4 mCi/mmmol) was applied to outdoor plots at a rate of 200 g ai/ha (0.18 lbs. ai/A, 0.9X) using preplant incorporation (PPI) or preemergence (PRE) application to separate plots. Test plots were established in NC (sandy loam soil, pH 6.3). Lettuce, sorghum and radishes were planted 34 days after treatment (DAT); mustard, radishes and wheat were planted 123 DAT; and lettuce, sorghum and radishes were planted 365 DAT. All crops were harvested when mature. Immature samples of wheat and sorghum forage, radish roots and foliage and mustard or lettuce were also taken.

Crop Residue Quantitation: Crop residues as determined by combustion are shown in Table 1. The highest residue levels were seen in 34 DAT sorghum forage (0.13-0.24 ppm).

Extraction and Fractionation: Plant samples containing >0.01 ppm were extracted sequentially in hexane:ethyl acetate (9:1), acetonitrile, water and acetonitrile:0.2N HCl (1:1). Aqueous extracts were partitioned with ethyl acetate. The total extractability of residues was generally >80% of the TRR (Table 2).

Nature of the Residue: The organic-soluble residues were analyzed by HPLC and the retention times compared with that of isoxaflutole *per se* and standards of suspected metabolites (fig. 1). The identities of metabolites was confirmed by MS when possible. Unknown #1 was characterized as a acidic degradate of RPA 203328 with a MW of 192.

Table 1- TRR in rotational grown in soil treated with ^{14}C-labelled isoxaflutole.

DAT	Crop	RAC	TRR (ppm)	
			PRE	PPI
34	Lettuce	Leaf	0.030	0.022
	Radish	Leaf	0.011	0.019
		Root	0.003	0.003
	Sorghum	Forage	0.126	0.241
		Stover	0.037	0.130
		Grain	0.038	0.119
123	Mustard	Leaf	0.002	0.002
	Radish	Leaf	0.002	0.002
		Root	0.000	0.001
	Wheat	Forage	0.009	0.013
		Straw	0.042	0.030
		Grain	0.015	0.017
365	Lettuce	Leaf	0.010	0.005
	Radish	Leaf	0.010	0.010
		Root	0.001	0.001
	Sorghum	Forage	0.030	0.051
		Stover	0.029	0.029
		Grain	0.004	0.007

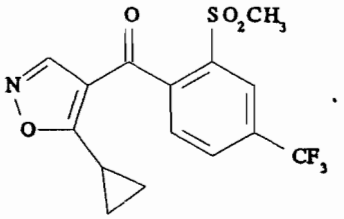
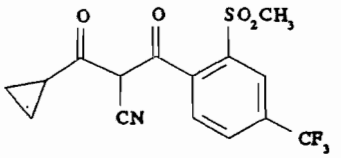
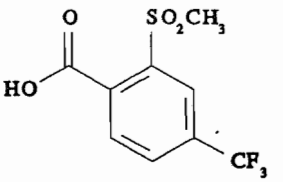
PRE = Preemergence application
PPI = Preplant Incorporation application

Table 2- Fraction of TRR in rotational grown in soil treated with ^{14}C-labelled isoxaflutole.

DAT	Crop	RAC	Application	Extractable		Bound	
				ppm	% TRR	ppm	% TRR
34	Lettuce	Leaf	PRE	0.029	96.7	0.002	5.3
			PPI	0.022	100	0.001	6.3
	Radish	Leaf	PRE	0.012	>100	0.001	5.4
			PPI	0.018	94.7	0.001	5.5
	Sorghum	Forage	PRE	0.109	86.5	0.002	2.3
			PPI	0.240	99.6	0.005	1.9
		Stover	PRE	0.307	83.0	0.026	7.1
			PPI	0.130	1000	0.009	7.3
		Grain	PRE	0.031	81.6	0.005	12.6
			PPI	0.102	85.7	0.014	11.4
123	Wheat	Forage	PRE	0.008	88.9	<0.001	4.6
			PPI	0.013	100	0.001	5.3
		Straw	PRE	0.031	73.8	0.003	7.1
			PPI	0.021	70.0	0.007	21.9
		Grain	PRE	0.015	100	0.001	7.9
			PPI	0.017	100	0.001	6.8
356	Radish	Leaf	PRE	0.010	100	<0.001	4.8
			PPI	0.010	100	<0.001	3.9
	Sorghum	Forage	PRE	0.018	60.0	0.001	2.8
			PPI	0.041	80.4	0.002	3.4
		Stover	PRE	0.024	82.8	0.001	3.6
			PPI	0.037	>100	0.003	10.3

PRE = Preemergence application
PPI = Preplant Incorporation application

Figure 1. Chemical structures of isoxaflutole and its metabolites in rotational crops.

Common Name Chemical Name	Structure	Substrate
Isoxaflutole; RPA 201772 5-cyclopropyl-4-(2-methylsulfonyl-4-fluoromethyl)benzoyl isoxazole		
RPA 202248 1-(2-methylsulfonyl-4-trifluoromethylphenyl)-2-cyano-3-cyclopropyl propane-1,3-dione		30 DAT radish leaf and sorghum grain
RPA 203328 2-methylsulfonyl-4-trifluoromethyl benzoic acid		30 DAT lettuce, radish leaf and sorghum, 123 DAT wheat, 365 DAT sorghum

Nature of the Residue in 34-DAT Lettuce: The results of HPLC fractionation of mature phenyl-labelled lettuce extracts are shown in Table 3. RPA 203328 accounted for 59-63% of the TRR. Unknown #1 accounted for another 27-32% of the TRR.

Nature of the Residue in 34-DAT Radish Leaf: The results of HPLC fractionation of mature phenyl-labelled radish leaf extracts are shown in Table 3. RPA 203328 accounted for 9-37% of the TRR; RPA 202248, 26-27%.

Table 3- Summary of Identification of Radioactive Residues of 34 DAT Samples

Metabolite	Lettuce				Radish Leaf				Sorghum Forage				Sorghum Stover				Sorghum Grain			
	PRE		PPI		PRE		PPI		PRE		PPI		PRE		PPI		PRE		PPI	
	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR
Isoxaflutole	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-
RPA 202248	0.0	-	0.0	-	0.003	27.3	0.005	26.4	0.0	-	0.0	-	0.0	-	0.0	-	0.002	5.3	0.001	0.8
RPA 203328	0.019	63.3	0.013	59.1	0.009	9.1	0.007	36.8	0.109	86.5	0.241	100.0	0.009	24.3	0.097	74.6	0.024	63.2	0.080	67.2
Unknown #1	0.008	26.7	0.007	31.8	0.0	-	0.0	-	0.0	-	0.0	-	0.016	43.2	0.0	-	0.006	15.8	0.012	10.1
Total Identified	0.019	63.3	0.013	59.1	0.008	36.4	0.012	63.2	0.109	86.5	0.241	100.0	0.009	24.3	0.097	74.6	0.026	68.5	0.081	68.0

PRE = Preemergence application
PPI = Preplant Incorporation application

Table 4- Summary of Identification of Radioactive Residues of 123 DAT Samples

Metabolite	Wheat Forage				Wheat Straw				Wheat Grain			
	PRE		PPI		PRE		PPI		PRE		PPI	
	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR
Isoxaflutole	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-
RPA 202248	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-
RPA 203328	0.005	55.6	0.013	100.0	0.031	73.8	0.021	70.0	0.015	100.0	0.018	105.8
Unknown #1	0.003	33.3	0.00		0.00	-	0.0	0	0.0	-	0.0	-
Total Identified	0.005	55.6	0.013	100.0	0.031	73.8	0.021	70.0	0.015	100.0	0.018	105.8

PRE = Preemergence application
PPI = Preplant Incorporation application

Nature of the Residue in 34-DAT Sorghum: The results of HPLC fractionation of phenyl-labelled sorghum extracts are shown in Table 3. RPA 203328 was observed in forage, stover and grain, accounting for 24-100% of the TRR. RPA 202248 was observed in grain, accounting for 1-5% of the TRR. Unknown #1 accounted for another 10-42% of the TRR in stover and grain.

Nature of the Residue in 123-DAT Wheat: The results of HPLC fractionation of phenyl-labelled wheat extracts are shown in Table 4. RPA 203328 was observed in forage, straw and grain, accounting for 56-100% of the TRR. Unknown #1 accounted for up to 33% of the TRR in forage.

Nature of the Residue in 365-DAT Radish Leaf: The results of HPLC fractionation of mature phenyl-labelled radish leaf extracts are shown in Table 5. RPA 203328 accounted for up to 20% of the TRR. Unknown #1 accounted for another 90-100% of the TRR.

Table 5- Summary of Identification of Radioactive Residues of 365 DAT Samples

	Radish Leaf				Sorghum Forage				Sorghum Stover			
	PRE		PPI		PRE		PPI		PRE		PPI	
Metabolite	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR
Isoxaflutole	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-
RPA 202248	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-
RPA 203328	0.0	-	0.002	20.0	0.004	13.3	0.016	31.4	0.002	6.9	0.019	65.5
Unknown #1	0.010	100.0	0.009	90.0	0.014	46.7	0.017	33.3	0.022	75.9	0.019	65.5
Total Identified	0.0	-	0.002	20.0	0.004	13.3	0.016	31.4	0.002	6.9	0.019	65.5

PRE = Preemergence application
PPI = Preplant Incorporation application

Nature of the Residue in 365-DAT Sorghum: The results of HPLC fractionation of phenyl-labelled sorghum extracts are shown in Table 5. RPA 203328 was observed in forage and stover, accounting for 7-66% of the TRR. Unknown #1 accounted for another 33-76% of the TRR in stover and forage.

Bound Residues: Since the levels of bound residues did not exceed 0.05 ppm and 10% of the TRR in any sample, CBTS will require no further attempts to characterize these residues.

Storage Stability: Samples were stored for up to 630 days prior to extraction and extracts were stored for up 275 days prior to analysis. As evidence of storage stability, the petitioner

fortified samples with a solution of labelled isoxaflutole, RPA 202248 and RPA 203328. These data have not yet been submitted. However, in order to demonstrate storage stability, the petitioner should submit data which shows that the metabolite profile remains unchanged during 630 days of storage in the intact matrices and for 275 days in extracts.

CBTS' Conclusion: One major deficiency in this study was noted: storage stability was not demonstrated as described above. Such information is needed in order for the confined study to be acceptable. 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 will be withheld pending resolution of deficiencies regarding storage stability.

The petitioner has previously submitted a discussion of their rationale for conducting metabolism studies with isoxaflutole labelled only in the phenyl ring (MRID# 435732-50; Memo, P. Errico 12/7/95). The petitioner stated that they conducted numerous preliminary metabolism studies, with plants, soil, and animals, in which isoxaflutole was labelled in the phenyl ring, in the isoxazole ring, or at the carbonyl carbon. Based on these studies, the petitioner observed that RPA 203328 is the major metabolite, that the isoxazole ring is highly unstable and hydrolyses rapidly to form RPA 202248, and that the cyclopropyl moiety metabolizes/degrades to cyclopropane carboxylic acid. The petitioner noted that the opening of the isoxazole ring to form RPA 202248 was observed in plants, soil, and rats, and that the half-life for isoxaflutole in both clay and sandy soils is less than 24 hours. The petitioner also stated that when plant studies were conducted with the ^{14}C-label in the isoxazole ring, $^{14}CO_2$ was released, indicating loss of the cyano group. Provided that the metabolism of isoxaflutole in rotational crops is demonstrated to proceed via opening of the isoxazole ring, CBTS concludes that a confined study using isoxaflutole labelled in this ring will not be required.

Nature of Residue- Plants

Deficiency - Conclusion 7b (from Memo, P. Errico 12/7/95)

7b. No dates of sample extraction and analysis were submitted for the samples isolated in the plant metabolism study. For the permanent tolerance request, the

registrant should submit the dates of sample extraction and analysis.

Petitioner's Response: Submission of the requested dates. The samples were stored for up to 7 months prior to extraction and the extracts were stored for up to 3 months prior to analysis.

CBTS' Conclusion: The petitioner must submit data which demonstrates that the metabolite profile of these samples remained unchanged during the storage conditions employed in this study.

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.

Nature of Residue- Animals

Deficiency - Conclusion 3b (from Memo, P. Errico 12/7/95)

3b. For a permanent tolerance request, ruminant and poultry metabolism studies will be necessary. Depending on the terminal residues and mass balance results, metabolism studies of separately C-14 labeled benzene and isoxazole rings may be necessary.

Petitioner's Response: Submission of the requested studies.

Nature of the Residue-Ruminants: Submitted with this petition:

> (^{14}C)-RPA201772: Absorption, Distribution, Metabolism and Excretion Following Repeat Oral Administration to the Dairy Goat. Performing Laboratory: Hazleton Europe. MRID# 439048-27

In-Life Phase: [Phenyl(U)-^{14}C]-isoxaflutole (18.4 mCi/mmol) was isotopically diluted, placed in a gelatin capsule and administered orally to lactating goats (weight of 63-87 kg, age <8 years) with the aid of a balling gun. The goats were dosed at a total rate of 1 ppm, 10 ppm or 50 ppm per day. Doses were administered twice daily for 7 consecutive days. The animals were sacrificed approximately 24 hours after administration of the final dose.

Quantitation of Total Radioactivity: Milk was collected twice daily. Tissues were obtained after sacrifice. The distribution of the radioactivity is shown in Table 6. Of the administered

radioactivity, 28-31% was recovered in feces, <1% in the milk and 9-11% in the tissues. The total recovery was 72-97%. The TRR in tissues and milk is shown in Table 7. The greatest tissue residues were observed in liver (3.9 ppm at 50 ppm dose).

Table 6- Total recovery of radioactivity from lactating goats treated with ^{14}C-labelled isoxaflutole at a dietary burden of 1, 10 or 50 ppm for 7 consecutive days.

Fraction	% of Total Radioactivity Administered		
	1 ppm	10 ppm	50 ppm
Urine	54.3	27.4	27.1
Feces	31.0	28.3	29.4
Milk	ND	0.6	0.5
Cage Wash/Debris	ND	11.8	5.8
Tissues	11.3	9.7	8.8
Total	96.6	77.7	71.6

ND = Not Detected

Table 7- TRR in goat milk and tissues following treatment with ^{14}C-labelled isoxaflutole at a dietary burden of 1, 10 or 50 ppm for 7 consecutive days.

Fraction	TRR (ppm)		
	1 ppm	10 ppm	50 ppm
Omental Fat	0.011	0.062	0.235
Renal Fat	0.015	0.069	0.230
Skeletal Muscle	0.037	0.263	0.927
Liver	0.536	2.100	3.946
Kidney	0.164	0.905	2.123
GI Tract	NA	0.374	1.872
Milk*	ND	0.093	0.350

*Day 5 sample
ND = Not Detected; NA = Not Analyzed

Extraction: Tissue samples (from the 10 ppm dose) were extracted with 0.1 M phosphate. Fat samples were homogenized in hexane prior to extraction. The feces were extracted with methanol and partitioned with ethyl acetate. Muscle extracts were treated with protease. Milk was extracted with chloroform/methanol (2/1). Approximately 84-98% of the TRR in all samples was extractable.

Metabolite Identification: Extractable residues were analyzed by HPLC and the retention times compared with those of possible metabolites. The identity of metabolites was confirmed by LC-MS. The results presented below are for the 10 ppm dose level.

Nature of the Residue in Feces: RPA 202248 was the major component of the residue, accounting for 65% of the TRR (Table 8). RPA 207048 and RPA 205834 were also identified, accounting for 20% and 4% of the TRR, respectively. A total of 7 unknown peaks were also observed, accounting for a total of 10% of the TRR.

Table 8- Summary of radioactive residues characterized / identified in ruminant following administration of ^{14}C - isoxaflutole at 10 ppm in the diet.

Metabolite Fraction	Liver		Kidney		Muscle		Renal Fat		Omental Fat		Milk		Urine		Feces	
	%TRR	PPM	%TRR	PPM	%TTR	PPM	%TTR	PPM	%TTR	PPM	%TTR	PPM	%TTR	PPM	%TTR	PPM
Identified																
Isoxaflutole	-	ND	-	ND	-	ND	-	ND	-	ND	-	ND	-	-	-	-
RPA 202248	85.8	1.801	82.0	0.742	41.4	0.109	24.6	0.017	24.2	0.015	41.7	0.025	82.3	NA	65.1	NA
RPA 205834	-	ND	-	ND	-	ND	14.5	0.010	8.1	0.005	18.3	0.011	0.4	NA	3.7	NA
RPA 207048	12.4	0.261	11.6	0.105	12.6	0.033	18.8	0.013	25.8	0.016	15.0	0.009	9.5	NA	20.3	NA
Total Identified	98.2	2.062	93.6	0.847	54.0	0.142	57.9	0.040	58.1	0.036	75.0	0.045	92.2		89.1	
Characterized																
Extractable Unknowns	-	NA	-	NA	6.5	0.017	34.8	0.024	40.3	0.025	20.0	0.012	6.9	NA	9.9	NA
Total Characterized / Identified	98.2	2.062	93.6	0.847	60.5	0.159	92.7	0.064	98.4	0.061	95.0	0.057	99.1	NA	99.0	NA
Non-Extractable	-	NA	5.6	0.050	15.6	0.041	-	NA	-	NA	-	NA	-	-	-	-

Nature of the Residue in Liver: RPA 202248 was the major component of the residue, accounting for 86% of the TRR (Table 8). RPA 207048 was also identified, accounting for 12% of the TRR. A total of 98% of the TRR was identified.

Nature of the Residue in Kidney: RPA 202248 was the major component of the residue, accounting for 82% of the TRR (Table 8). RPA 207048 was also identified, accounting for 12% of the TRR. A total of 94% of the TRR was identified.

Nature of the Residue in Muscle: RPA 202248 was the major component of the residue, accounting for 41% of the TRR (Table 8). RPA 207048 was also identified, accounting for 13% of the TRR. A total of 54% of the TRR was identified. A total of 2 aqueous-soluble unknown peaks were also observed, accounting for a total of 7% of the TRR.

Nature of the Residue in Renal Fat: RPA 202248 was the major component of the residue, accounting for 25% of the TRR (Table 8). RPA 207048 and RPA 205834 were also identified, accounting for 19% and 15% of the TRR, respectively. A total of 2 aqueous-soluble unknown peaks were also observed, accounting for a total of 16% of the TRR. The hexane extract, containing 19% of the TRR, was not subjected to chromatographic analysis. A total of 58% of the TRR was identified.

Nature of the Residue in Omental Fat: RPA 207048 was the major component of the residue, accounting for 26% of the TRR (Table 8). RPA 202248 and RPA 205834 were also identified, accounting for 24% and 8% of the TRR, respectively. A total of 3 aqueous-soluble unknown peaks were also observed, accounting for a total of 16% of the TRR. The hexane extract, containing 24% of the TRR, was not subjected to chromatographic analysis. A total of 58% of the TRR was identified.

Nature of the Residue in Renal Fat: RPA 202248 was the major component of the residue, accounting for 25% of the TRR (Table 8). RPA 207048 and RPA 205834 were also identified, accounting for 19% and 15% of the TRR, respectively. A total of 2 aqueous-soluble unknown peaks were also observed, accounting for a total of 16% of the TRR. The hexane extract, containing 19% of the TRR, was not subjected to chromatographic analysis. A total of 58% of the TRR was identified.

Nature of the Residue in Milk: RPA 202248 was the major component of the residue, accounting for 42% of the TRR (Table 8). RPA 207048 and RPA 205834 were also identified, accounting for 15% and 18% of the TRR, respectively. A total of 75% of the TRR was identified. A total of 6 unknown peaks were also observed, accounting for a total of 20% of the TRR.

Bound Residues: The levels of bound residues exceeded 0.05 ppm

and 10% of the TRR only in muscle. Post-extraction solids (PES) of muscle were treated with protease. A total of 2 unknown compounds were released, accounting for a total of 16% of the TRR.

Storage Stability: As the samples were analyzed within 6 months of collection, storage stability is not an issue for these matrices.

CBTS' Conclusion: The nature of the residue in ruminants is considered to be understood. RPA 202248, RPA 207048 and RPA 205834 are the primary components of the residue, accounting for 54-98% of the TRR. Metabolism of isoxaflutole proceeds via: 1) hydrolysis of the isoxazole ring to form RPA 202248 and RPA 205834; 2) further hydrolysis to produce RPA 207048 (figure 2). Hydrolytic pathways were also observed in corn, producing RPA 202248, RPA 203328.

For compounds with multiple rings, CBTS generally requires that metabolism studies be performed with each ring labelled. However, as the metabolism of isoxaflutole in ruminants proceeds via opening of the isoxazole ring, CBTS concludes that a goat metabolism study using isoxaflutole labelled in this ring will not be required.

Nature of the Residue- Poultry: Submitted with this petition:

> (^{14}C)-RPA201772: Absorption, Distribution, Metabolism and Excretion Following Repeat Oral Administration to the Laying Hen. Performing Laboratory: Hazleton Europe. MRID# 439048-27

In-Life Phase: [Phenyl(U)-^{14}C]-isoxaflutole (18.4 mCi/mmol) was isotopically diluted, placed in a gelatin capsule and administered orally to laying hens (weight of 1.5-2.1 kg, age 22 weeks). The hens were dosed at a rate of 1 ppm or 10 ppm. There were five birds in each dosing group. Doses were administered daily for 14 consecutive days. The animals were sacrificed approximately 24 hours after administration of the final dose.

Quantitation of Total Radioactivity: Eggs were collected twice daily. Tissues were obtained after sacrifice. The results presented are the average of the values for the individual birds of each dosing group. The distribution of the radioactivity is shown in Table 9. Of the administered radioactivity, 88-100% was recovered in excreta, 0.2% in the eggs and <2% was recovered in the tissues. The total recovery was 92-100%. The TRR in tissues and eggs is shown in Table 10. The greatest tissue residues were observed in liver (1 ppm at 10 ppm dose).

Table 9- Total recovery of radioactivity from laying hens treated with ^{14}C-labelled isoxaflutole at a dietary burden of 1 or 10 for 14 consecutive days.

Fraction	% of Total Radioactivity Administered	
	1 ppm	10 ppm
Excreta	112.4	88.4
Cage Wash/Debris	2.8	3.2
Egg White	ND	0.1
Egg Yolk	0.2	<0.1
Tissues	1.7	0.2
Total	117.1	92.0

ND = Not Detected

Table 10- TRR in hen eggs and tissues following treatment with ^{14}C-labelled isoxaflutole at a dietary burden of 1 or 10 ppm for 14 consecutive days.

Fraction	TRR (ppm)	
	1 ppm	10 ppm
Skin	0.008	0.068
Fat	ND	0.028
Skeletal Muscle	ND	0.035
Liver	0.845	0.953
Kidney	0.055	0.155
Egg Yolk*	0.024	0.152
Egg White**	ND	0.015

*Day 12 sample; **Day 10 sample
ND = Not Detected

Extraction: Samples of excreta, liver, kidney, skin, and fat were extracted in methanol. Some of these extracts were washed with hexane. Muscle was homogenized in 2 mM ammonium acetate and extracted sequentially in hexane, ethyl acetate, acetonitrile and acidified methanol. The extracts were combined and washed with hexane. Egg yolk was extracted sequentially in hexane, methanol, acetonitrile, acidified methanol and water. The extracts were combined and washed with hexane. Egg white was extracted sequentially in acetonitrile, methanol, ethyl acetate, acidified methanol and water. Approximately 54-93% of the TRR in all tissue and egg samples was extractable. In tissues with significant

levels of bound residues, the PES were treated with protease and 6 N HCl.

Metabolite Identification: Extractable residues were analyzed by HPLC and the retention times compared with those of possible metabolites. The identity of metabolites was confirmed by LC-MS. The results presented below are for the 10 ppm dose level.

Nature of the Residue in Liver: RPA 202248 was the only component of the residue identified, accounting for 93% of the TRR (Table 11). A single unknown peak was also observed, accounting for 3% of the TRR.

Nature of the Residue in Kidney: RPA 202248 was the only component of the residue identified, accounting for 74% of the TRR (Table 11). A single unknown peak was also observed, accounting for 0.7% of the TRR. This compound was tentatively identified as RPA 203328. All of the bound residues were released by protease treatment (16% of the TRR, 0.025 ppm) and hydrolysis (2% of the TRR, 0.003 ppm). HPLC analysis of the protease extract revealed the presence of 3 unidentified components.

Nature of the Residue in Muscle: RPA 207048 was the only component of the initial extract identified, accounting for 31% of the TRR. All of the bound residues were released by protease treatment (29% of the TRR, 0.010 ppm) and hydrolysis (43% of the TRR, 0.015 ppm). HPLC analysis of the protease extract revealed the presence of a single unidentified component. HPLC analysis of the acid hydrolysate revealed the presence of 3 metabolites and 2 unidentified components. A summary of the metabolites identified in the initial extract and the acid hydrolysate is shown in Table 11. RPA 207048 was the major component of the residue, accounting for 49% of the TRR. RPA 202248 and RPA 203328 were also identified, each accounting for 6% of the TRR. A total of 60% of the TRR was identified.

Nature of the Residue in Fat: RPA 202248 was the major component of the residue, accounting for 29% of the TRR (Table 11). RPA 207048 was also identified, accounting for 21% of the TRR. A total of 50% of the TRR was identified. A total of 2 unidentified peaks were also observed, accounting for a total of 43% of the TRR.

Table 11- Summary of radioactive residues characterized / identified in poultry following administration of ^{14}C - isoxaflutole at 10 ppm in the diet.

Metabolite Fraction	Liver		Kidney		Muscle		Fat		Skin		Egg Yolk		Egg White		Excreta	
	%TRR	PPM	%TRR	PPM	%TTR	PPM	%TTR	PPM	%TTR	PPM	%TTR	PPM	%TTR	PPM	%TTR	PPM
Identified																
Isoxaflutole	-	ND	-	ND	-	ND	-	ND	-	ND	-	ND	-	ND	-	-
RPA 202248	93.1	0.887	73.6	0.114	5.7	0.002	28.6	0.008	54.4	0.037	26.3	0.036	-	ND	85.1	NA
RPA 205834	-	ND	-	ND	-	ND	-	ND	-	ND	27.7	0.038	-	ND	-	-
RPA 207048	-	ND	-	ND	48.6	0.017	21.4	0.006	-	ND	-	ND	-	ND		-
RPA 203328	-	ND	-	ND	5.7	0.002	-	ND		ND	-	ND	-	ND	-	
Total Identified	93.1	0.887	73.6	0.114	60.0	0.021	50.0	0.014	54.4	0.037	54.0	0.074	0.0	0.0	85.1	NA
Characterized																
Extractable Unknowns	3.4	0.033	18.7	0.029	65.7	0.023	42.9	0.012	16.1	0.011	58.4	0.080	90.0	0.009	1.9	NA
Total Characterized /Identified	96.5	0.887	92.3	0.143	125.7	0.044	92.9	0.026	70.5	0.048	112.4	0.154	90.0	0.009	87.0	NA
Non-Extractable	3.5	0.034	-	ND	-	ND	-	ND	9.9	0.007	-	ND	-	ND	2.0	NA

ND = Not detected; NA = Not Applicable

Nature of the Residue in Skin: RPA 202248 was the only component of the initial extract identified, accounting for 37% of the TRR. Most of the bound residues were released by protease treatment (20% of the TRR, 0.014 ppm) and hydrolysis (7% of the TRR, 0.005 ppm), with 9.9% of the TRR remaining bound. HPLC analysis of the protease extract revealed the presence of one metabolite and a single unidentified component (3% of the TRR). A summary of the metabolites identified in the initial extract and the protease digesate is shown in Table 11. RPA 202248 was the only metabolite identified, accounting for 54% of the TRR.

Nature of the Residue in Egg Yolk: RPA 205834 was the major component of the residue, accounting for 28% of the TRR (Table 11). RPA 202248 was also identified, accounting for 26% of the TRR. A total of 54% of the TRR was identified. A total of 2 unidentified peaks were also observed, accounting for a total of 12% of the TRR. All of the bound residues were released by hydrolysis (46% of the TRR, 0.063 ppm). HPLC analysis of the hydrolysate revealed the presence of 3 unidentified components, none of which exceeded 0.05 ppm. One of these compounds was tentatively identified as RPA 202248.

Nature of the Residue in Egg White: The initial extract contained only 0.005 ppm and was thus not further characterized (Table 11). All of the bound residues were released by protease treatment (20% of the TRR, 0.002 ppm) and hydrolysis (20% of the TRR, 0.002 ppm).

Nature of the Residue in Excreta: RPA 202248 was the only component of the residue identified, accounting for 85% of the TRR (Table 11). A single unknown peak was also observed, accounting for 2% of the TRR.

Storage Stability: The actual dates of sample collection, extraction and analysis were not provided in this report so that the sample storage interval could not be calculated. As evidence of storage stability, the petitioner presented HPLC chromatograms of excreta run 4, 12 and 18 months after collection. Some evidence of instability was observed as the area percent of RPA 202248 decreased from 100% to 87%, concomitant with the appearance of two unknown compounds (accounting for 3% and 10%, respectively).

CBTS' Conclusion: 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.

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 via: 1) hydrolysis of the isoxazole ring to form RPA 202248 and RPA 205834; 2) further hydrolysis to produce RPA 207048 and RPA 203328 (figure 2).

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.

Analytical Methodology- Plants

Submitted with this petition:

> Analytical Method for the Determination of Residues of RPA 201772, RPA 202248, and RPA 203328 in Maize Grain and Fodder. P94/110. MRID# 439048-29.

PROPOSED ENFORCEMENT METHOD

Procedure: The method involves hydrolysis of of isoxaflutole to RPA 202248, conversion of RPA 202248 to RPA 203328, and then derivatization of RPA 203328 to a methyl ester for GC analysis. Briefly, field corn commodity samples are ground and extracted by homogenizing three times with methanol. Crude and refined oil samples are mixed with hexane prior to methanol extraction. A 2% sodium hydroxide solution is added to the combined extracts to hydrolyze isoxaflutole to RPA 202248. The methanol is removed by rotary evaporation, and the extract is salinized with a saturated sodium chloride solution and sequentially washed with dichloromethane (twice) and petroleum ether. The aqueous extract is acidified with concentrated hydrochloric acid to ~pH 1.0, partitioned into dichloromethane, and drained through anhydrous sodium sulfate. The dichloromethane phase is then evaporated to dryness and hydrolyzed with 1 M methanolic sodium hydroxide solution at 100 °C for one hour to convert RPA 202248 residues to RPA 203328. Water is added, and the pH is lowered to ~1.0 using concentrated hydrochloric acid. The hydrolysate is partitioned into dichloromethane, and the dichloromethane phase is evaporated to dryness. The residue is re-dissolved in dichloromethane and

derivatized to the methyl ester RPA 204497 with a diazomethane solution at 30 °C for one hour. Acetic acid is added to destroy any excess diazomethane, and the derivatized extract is brought to volume with dichloromethane and analyzed by GC/MSD in the selective ion mode. Residues are reported as ppm isoxaflutole equivalents. The limit of quantitation is 0.01 ppm.

Results: This method was previously reviewed by CBTS and determined to be adequate for data-gathering purposes (Memo, P. Errico 12/7/95).

ILV: An ILV of this method was performed by ABC Labs. The method and ILV have been sent to Beltsville for PMV (Memo, G. Kramer 2/14/96). CBTS will withhold a final conclusion on the adequacy of this method as an analytical enforcement method pending receipt of the PMV report.

Multiresidue Method Testing: Data pertaining to the recovery of isoxaflutole and its metabolites RPA 202248 and RPA 203328 using FDA multiresidue methods were submitted in conjunction with PP#5G04484 (MRID 43573252). These multiresidue screening data were forwarded to FDA (Memo, P. Errico 10/23/95).

Specificity: Submitted with this petition:

> Interference Study on Pesticides Used on Corn Using the "Analytical Method for the Determination of Residues of RPA 201772, RPA 202248, and RPA 203328 in Maize Grain and Fodder." MRID# 439048-30.

The specificity of the proposed analytical enforcement method was investigated by performing an interference study with 115 different pesticides. The pesticides were analyzed both with and without methylation. 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.

Radiovalidation: A fodder sample from the plant metabolism study was analyzed with the proposed enforcement method. Of the TRR, 77.5% was detected by GC/MS. This value corresponds well with the expected result (68.1%) based on the metabolism study. CBTS concludes that method P/93011 has been adequately radiovalidated.

Confirmatory Method: Provided that deficiencies pertaining to the interference study are resolved, a confirmatory method will not be required.

Conclusions: 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).

Analytical Methodology- Animals

Deficiency - Conclusion 6b (from Memo, P. Errico 12/7/95)

6b. No proposed enforcement methodology was submitted for meat, milk, and eggs. Depending on the results of the metabolism and feeding studies as stated in conclusions 3b and 5b a proposed enforcement method for meat, milk and eggs may be needed. Any required proposed enforcement method must be validated successfully by the Agency before a positive recommendation for a permanent tolerance can be made. A second laboratory validation of the proposed enforcement method should also be provided.

Petitioner's Response: Submission of:

> Method of Analysis for the Determination of Isoxaflutole (RPA 201772) and Its Metabolites (RPA 203328, RPA 202248, and RPA 205834) in Milk, Eggs, Liver, Kidney, Muscle and Fat Tissues. EC-95-313. Appendix B of MRID# 439048-32.
>
> Independent Method Validation of the Rhone-Poulenc Methods Entitled, "Method of Analysis for the Determination of ..." Horizon Labs. MRID# 439048-33.

PROPOSED ENFORCEMENT METHOD

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 acetonitrile or in acidified acetonitrile. The extracts are partitioned against hexane and isoxaflutole is converted to RPA 202248 by base hydrolysis. After dichloromethane partitioning, 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. The LOQ is 0.01 ppm

for milk and eggs; 0.05 ppm for beef liver, beef and poultry muscle and fat; 0.075 ppm for beef kidney; and 0.10 ppm for poultry liver.

Results: Acceptable recoveries were obtained in all matrices except cow muscle (Table 12). Some recoveries were slightly less than 70% (cow fat and kidney and poultry muscle), but are acceptable due to the low standard errors (0-7%). Note that the actual LOQs in cow and poultry liver appear to be higher than that claimed by the petitioner (0.05 ppm for cow and 0.10 for ppm poultry).

Table 12- Results of validation of proposed enforcement method for meat, milk and eggs.

Animal	RAC	Fortification Level (ppm)	Average Recovery ± s.d. (n)
Cow	Fat	0.05	66 ± 2% (2)
		0.15	67 ± 7% (2)
	Kidney	0.07	68 ± 7% (4)
		0.40	81 ± 7% (5)
	Liver	0.05	58 ± 41% (2)
		0.72	76 ± 0% (2)
	Muscle	0.05	41 ± 12% (2)
		0.15	37 ± 9% (2)
	Milk	0.01	99 ± 18% (20)
		0.05	96 ± 16% (20)
Poultry	Eggs	0.01	74 ± 10% (5)
		0.05	93 ± 16% (5)
	Liver	0.10	145 ± 1% (2)
		0.72	78 ± 1% (2)
	Fat + Skin	0.05	83 ± 10% (2)
		0.15	78 ± 4% (2)
	Muscle	0.05	69 ± 0% (2)
		0.15	73 ± 5% (2)

ILV: An ILV of this method was performed by Horizon Labs, Columbia, MO. Acceptable recoveries were obtained by the laboratory. The method and ILV have been sent to Beltsville for PMV (Memo, G. Kramer 6/14/96). CBTS will withhold a final

conclusion on the adequacy of this method as an analytical enforcement method pending receipt of the PMV report.

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: Two samples from the ruminant metabolism study were 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%. In milk, 87% of the TRR was extractable. No further analysis of this sample was performed. 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.

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.

METHOD UTILIZED FOR DATA GATHERING

Procedure: A 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.

Results: Acceptable recoveries were obtained in all tissues. The LOQ was reported to be 0.05 ppm.

Conclusions: As the LC/MS method closely resembles 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.

Storage Stability Studies

Deficiency - Conclusion 7c (from Memo, P. Errico 12/7/95)

7c. No storage stability studies have been submitted for field trial residue samples. For this proposed temporary tolerance request, we will translate the results of the storage stability studies for the radiolabelled metabolites. For the proposed permanent tolerance and future submissions, the registrant should submit a storage stability study for corn grain, forage, fodder, and processed commodities to support the submitted field residue data (a minimum of 15 months).

Petitioner's Response: Submission of:

> Freezer Stability of RPA 201772 in Field Corn Samples. MRID# 439048-34.

Samples of corn grain, forage, fodder, and silage with field-incurred residues stored frozen at <-10 °C. Samples were maintained frozen and two subsamples were removed and analyzed at various intervals for residues using the proposed enforcement method over the course of 13 months. 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 RACs up to 13 months (Table 15).

CBTS' Conclusion: 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 mohths. However, storage stability data are still required for processed corn commodities for a storage interval of 3 months.

Table 15- % Recovery of isoxaflutole from RACs with field-incurred residues during storage at <-10 °C (average values, n=2)

RAC	Initial Level (ppm)	Storage Interval (months)	Fresh Fortification Recovery (%)	Apparent Recovery in Stored Sample (%)	Corrected Recovery in Stored Sample (%)
Forage	0.132	9	96.7	86.7	89.8
		11	109	86.0	78.9
		13	99.4	83.7	84.2
Forage	0.170	9	96.7	98.2	102
		11	109	93.2	85.6
		13	99.4	92.6	93.2
Silage	0.078	9	102	108	106
		11	117	107	91.5
		13	80.7	98.7	122
Silage	0.068	9	102	121	119
		11	117	114	97.4
		13	80.7	101	126
Fodder	0.044	9	97.4	90.9	93.3
		11	97.2	89.8	92.4
		13	85.8	77.3	90.1
Fodder	0.042	9	97.4	88.9	90.4
		11	97.2	86.9	89.4
		13	85.8	81.0	94.4
Grain	0.051	9	96.1	61.8	64.3
		11	105	54.9	52.3
		13	85.3	56.9	66.7
Grain	0.057	9	96.1	77.2	80.3
		11	105	76.3	72.7
		13	85.3	78.1	91.5

Magnitude of Residue- Plants

Submitted with this petition:

EXP 31130A/Field Corn/Magnitude of the Residue (USA94701R). MRID# 439048-37.

A total of 22 field residue trials were conducted in 1994 in 13 different states. These trials were located in Regions 1 (1 trial), 2 (1 trial), 6 (1 trial) and 5 (19 trials). A single preemergence broadcast application of isoxaflutole (75 WG) was performed at a rate of 0.223 lbs. ai/A (1.2X). The spray volume was 15-20 gal/A. Three replicate samples were harvested from each treated plot 55-61 (forage), 93-145 (silage) and 114-183 (fodder and grain) days after application. The samples were frozen and shipped to Hazelton, Inc. for analysis. All samples were analyzed within 319 days of harvest. Sample analysis for isoxaflutole and its metabolites was performed using the proposed enforcement method. The method was validated over a range of 0.01-1.4 ppm. The average recovery was 111 ± 4.9% in forage; 104 ± 9.8% in silage; 99.7 ± 6.9% in fodder; 96.1 ± 10.4% in grain. Analysis of the treated samples showed that the maximum residues were 0.88 ppm in forage, 1.1 ppm in silage, 0.40 ppm in fodder, and 0.11 ppm in grain.

Conclusions: Between the residue data submitted previously and those submitted with this petition, the petitioner has provided the results of 32 field corn trials, located in Regions 1 (1 trial), 2 (1 trial), 6 (1 trial) and 5 (29 trials). CBTS concludes that these trials were conducted in accordance with the *EPA Guidance on Number and Location of Domestic Crop Field Trials for Establishment of Pesticide Residue Tolerances, 6/2/94*. The maximum residues were 0.88 ppm in forage, 1.1 ppm in silage, 0.40 in fodder, and 0.11 ppm in grain. 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.**

CBTS notes that one value (1.1 ppm) in excess of the proposed forage tolerance was observed in silage (NE-2). However, as the residues other two silage samples from this site were well below 1.0 ppm and the residues in the other 191 forage and silage samples were below 1.0 ppm, the appropriate tolerance for forage appears to be 1.0 ppm.

Table 16- Residues of Isoxaflutole Equivalents in/on Field Corn Forage.

Crop Matrix	Formulation	Application Rate, lbs. ai/A	Location	PHI, days	Residues, ppm*
Forage	31130A (75% WG)	0.223	IL - 1	56	0.040, 0.067,0.086
			IL - 2	57	0.028, 0.032, 0.053
			IL - 3	56	0.087, 0.092, 0.10
			IN - 1	56	0.055, 0.075, 0.086
			IN - 2	55	0.030, 0.035, 0.059
			IN - 3	56	0.049, 0.072, 0.088
			IA - 1	52	0.035, 0.054, 0.069
			IA - 2	61	0.058, 0.058, 0.090
			IA - 3	61	0.021, 0.029, 0.032
			MI	56	0.037, 0.051, 0.052
			MN -1	61	0.040, 0.045, 0.10
			MN - 2	61	0.044, 0.050, 0.057
			MN - 3	60	0.069, 0.073, 0.098
			MO	60	0.099, 0.12, 0.14
			NE - 1	55	0.058, 0.094, 0.094
			NE - 2	59	0.58, 0.64, 0.88
			NY	59	0.063, 0.073, 0.077
			NC	60	0.083, 0.14, 0.14
			OH	57	0.016, 0.027, 0.036
			OK	54	0.20, 0.21, 0.22
			SD	55	0.057, 0.062, 0.066
			WI	55	0.092, 0.11, 0.11

Table 17- Residues of Isoxaflutole Equivalents in/or Field Corn Silage.

Crop Matrix	Formulation	Application Rate, lbs. ai/A	Location	PHI, Days	Residues, ppm*
Silage	31130A (75% WG)	0.223	IL - 1	113	0.021, 0.025, 0.038
			IL - 2	106	0.093, 0.11, 0.13
			IL - 3	104	0.10, 0.13, 0.16
			IN - 1	125	0.058, 0.064, 0.10
			IN - 2	117	0.11, 0.11, 0.20
			IN - 3	118	0.12, 0.13, 0.17
			IA - 1	112	0.13, 0.13, 0.17
			IA - 2	133	0.10, 0.13, 0.19
			IA - 3	133	0.017, 0.021, 0.029
			MI	119	0.011, 0.032, 0.078
			MN -1	145	0.020, 0.035, 0.051
			MN - 2	138	0.031, 0.037, 0.057
			MN - 3	133	0.014, 0.026, 0.027
			MO	103	0.19, 0.25, 0.28
			NE - 1	126	0.030, 0.065, 0.14
			NE - 2	112	0.46, 0.65, 1.1
			NY	93	0.13, 0.14, 0.14
			NC	98	0.21, 0.22, 0.32
			OH	112	0.018, 0.044, 0.080
			OK	102	0.20, 0.25, 0.32
			SD	116	0.028, 0.029, 0.035
			WI	107	0.17, 0.18, 0.22

*n = 3

Table 18- Residues of Isoxaflutole Equivalents in/or Field Corn Fodder.

Crop Matrix	Formulation	Application Rate, lbs. ai/A	Location	PHI, days	Residues, ppm*
Fodder	31130A (75% WG)	0.223	IL - 1	148	0.012, 0.016, 0.024
			IL - 2	138	0.12, 0.15, 0.17
			IL - 3	124	0.17, 0.17, 0.18
			IN - 1	168	0.036, 0.063, 0.064
			IN - 2	139	0.073, 0.12, 0.17
			IN - 3	146	0.026, 0.035, 0.037
			IA - 1	143	0.043, 0.051, 0.056
			IA - 2	166	0.012, 0.018, 0.018
			IA - 3	163	<0.010(3)
			MI	149	0.014, 0.020, 0.028
			MN -1	183	<0.010, <0.010, 0.012
			MN - 2	184	<0.010(3)
			MN - 3	171	<0.010(3)
			MO	132	0.092, 0.11, 0.15
			NE - 1	161	<0.010, 0.022, 0.029
			NE - 2	149	0.10 0.12, 0.13
			NY	136	0.20, 0.21, 0.31
			NC	137	0.068, 0.077, 0.11
			OH	147	0.011, 0.030, 0.043
			OK	114	0.21, 0.30, 0.40
			SD	145	0.012, 0.013, 0.017
			WI	142	0.099, 0.10, 0.10

Table 19- Residues of Isoxaflutole Equivalents in/or Field Corn Grain.

Crop Matrix	Formulation	Application Rate, lbs. ai/A	Location	PHI, days	Residues, ppm*
Grain	31130A (75% WG)	0.223	IL - 1	148	<0.010(3)
			IL - 2	138	<0.010(3)
			IL - 3	124	0.021, 0.022, 0.028
			IN - 1	168	<0.010(3)
			IN - 2	139	<0.010(3)
			IN - 3	146	0.014, 0.014, 0.022
			IA - 1	143	0.018, 0.018, 0.020
			IA - 2	166	0.012, 0.012, 0.017
			IA - 3	163	<0.010(3)
			MI	149	<0.010, 0.010, 0.011
			MN -1	183	<0.010(3)
			MN - 2	184	<0.010(3)
			MN - 3	171	<0.010(3)
			MO	132	0.033, 0.033, 0.041
			NE - 1	161	<0.010, <0.010, 0.011
			NE - 2	149	0.051, 0.097, 0.11
			NY	136	0.010, 0.015, 0.018
			NC	137	0.011, 0.013, 0.013
			OH	147	<0.010(3)
			OK	114	0.028, 0.031, 0.033
			SD	145	<0.010, 0.013, 0.014
			WI	142	0.032, 0.032, 0.034

*n = 3

Magnitude of the Residue- Processed Fractions

Deficiency - Conclusion 8c (from Memo, P. Errico 12/7/95)

8c. Corn grain processing studies were submitted using both the wet and dry-milling methods. No concentration of residues was indicated from this proposed use in corn meal, grits, flour, starch, crude oil and refined oil. With submission of the storage stability studies noted in conclusion 7c above, the results of these studies would be satisfactory for any subsequent proposed permanent tolerance request.

Petitioner's Response: none

Conclusions: Isoxaflutole residues do not appear to concentrate in processed 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.

Magnitude of the Residue- Ruminants

Submitted with this petition:

> Isoxaflutole: Magnitude of the Residues in Milk and Tissues of Lactating Dairy Cows. MRID# 439048-35.

Holstein dairy cows were dosed daily with isoxaflutole levels of 0, 4.6, 13.8, and 46 ppm in the diet. Each treatment group had four cows; the control group, 2. Milk samples were taken for analysis twice weekly. The cows were sacrificed on day 42. The maximum sample storage interval was 87 days. Samples of tissues were analyzed with LC/MS method described above; milk, with the proposed enforcement method. The method was validated in milk over a range of 0.02-0.10 ppm. The average recoveries were 87 ± 11%, 98 ± 11, and 94 ± 8% for isoxaflutole, RPA 202248, and RPA 205834, respectively. The method was validated in tissues over a range of 0.05-2.0 ppm. The average recoveries were 84 ± 10%, 93 ± 9%, 95 ± 11%, and 92 ± 17% for isoxaflutole, RPA 202248, RPA 205834, and RPA 207048, respectively The maximum residues observed in milk and tissues are shown in Tables 20 & 21. At the 4.6 ppm dietary burden, quantifiable residues were observed only in liver (up to 0.8 ppm), milk (up to 0.03 ppm), and kidney (up to 0.2 ppm). At the highest dose level, quantifiable residues of isoxaflutole or RPA 202248 were not observed in fat or muscle.

Table 20- 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, ND(3)	ND, LOQ(3)	<LOQ(4)	ND, <LOQ(3)	ND(4)	<LOQ, ND(3)	<LOQ(4)
	RPA 202248	<LOQ(4)	<LOQ(4)	0.020, 0.023, 0.027, 0.030	0.023, 0.024. 0.035, 0.036	0.020, 0.024, 0.028, 0.030	<LOQ, 0.020, 0.022, 0.027	<LOQ(3), 0.023
	RPA 205834	<LOQ(4)	<LOQ(4)	<LOQ(4)	<LOQ(3), 0.027	<LOQ(3), 0.029	<LOQ(3), 0.028	<LOQ(3), 0.022

n = 4
LOQ = 0.02 ppm
ND = not detected

Table 21- Residues of Isoxaflutole Found at 42 Days in Cow Tissue (ppm)*

Matrix		Liver			Kidney			Muscle	Fat
Dose Level, ppm		4.6	13.8	46	4.6	13.8	46	46	46
Metabolite	Isoxaflutole	ND(4)	ND(4)	ND(4)	-	-	ND(4)	ND(2)	ND(4)
	RPA 202248	0.499, 0.534, 0.696, 0.770	0.475, 0.879, 0.941, 1.09	1.70, 1.72, 1.79, 1.84	0.114, 0.128, 0.160, 0.166	0.173, 0.223, 0.248, 0.296	0.447, 0.468, 0.495, 0.503	<LOQ(4)	<LOQ(4)
	RPA 205834	0.071, 0.082, 0.094, 0.105	0.210, 0.231, 0.246, 0.299	0.560, 0.750, 0.800, 0.810	ND, <LOQ(3)	<LOQ(4)	<LOQ(3), 0.06	<LOQ(4)	<LOQ, 0.060, 0.065, 0.090
	RPA 207048	<LOQ(4)	<LOQ(4)	<LOQ(3), 0.068	<LOQ(4)	<LOQ(4)	<LOQ(4)	<LOQ(4)	ND, <LOQ(3)

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

Dietary Burden: The maximum dietary burden in dairy cows results from diet comprised of corn RACs:

Feed Item	% Diet	Needed Tolerance*	% DM	ppm in Diet
Forage	50	1.0 ppm	40	1.25
Stover	15	0.5 ppm	83	0.09
Grain	35	0.2 ppm	88	0.08
Total	100			**1.42**

*As determined by CBTS (see above)

The maximum dietary burden in beef cows results from diet comprised of corn RACs:

Feed Item	% Diet	Needed Tolerance*	% DM	ppm in Diet
Forage	40	1.0 ppm	40	1.00
Stover	25	0.5 ppm	83	0.15
Grain	35	0.2 ppm	88	0.08
Total	100			**1.23**

*As determined by CBTS (see above)

Conclusions: 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 ruminant RACs are contingent on submission of adequate storage stability data and validation of the analytical methods.

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 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.

Magnitude of the Residue- Poultry

Submitted with this petition:

> Isoxaflutole: Magnitude of the Residues in Tissues and Eggs of Laying Hens. MRID# 439048-36.

White Leghorn laying hens were dosed daily with isoxaflutole at levels of 0, 0.18, 0.54 and 1.8 ppm in the diet. Each group had 15 hens. Egg samples were taken daily. The animals were sacrificed on day 42. The maximum sample storage interval was 83 days. Samples of tissues were analyzed with LC/MS method described above; eggs, with the proposed enforcement method. The method was validated in eggs at 0.05 ppm. The average recovery was 92 ± 3%. The method was validated in tissues over a range of 0.05-1.0 ppm. The average recoveries were 83 ± 18%, and 84 ± 9% for isoxaflutole and RPA 202248, respectively. The maximum residues observed in eggs and tissues are shown in Tables 22 & 23. At the 1.8 ppm dietary burden, quantifiable residues were observed only in liver (up to 0.6 ppm). At the highest dose level, quantifiable residues of isoxaflutole or RPA 202248 were not observed in eggs, meat, fat or muscle.

Table 22- Residues of Isoxaflutole Found at Indicated Time Interval in Eggs (ppm)*

Dose Level, ppm		1.8					
Sample Time, days		0	31	33	36	39	41
Metabolite	Isoxaflutole	-	-	-	-	-	-
	RPA 202248	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

*n = 3

Table 23- Residues of Isoxaflutole Found at 42 Days in Poultry Tissues (ppm)*

Matrix		Liver			Muscle		Skin Plus Fat	
Dose Level, ppm		0.18	0.54	1.8	0.54	1.8	0.54	1.8
Metabolite	Isoxaflutole	NA	ND	ND	ND	ND	ND	ND
	RPA 202248	0.123, 0.133, 0.159	0.327, 0.353, 0.379	0.438, 0.588, 0.645	<0.05	<0.05	<0.05	<0.05

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

Dietary Burden: The maximum dietary burden results from a poultry diet comprised of corn grain and milled by-products:

Feed Item	% Diet	Recommended Tolerance	ppm in Diet
Grain	80	0.2 ppm	0.16
Milled Bypdts	20	0.2 ppm*	0.04
Total	100		**0.20**

*Covered by RAC tolerance

Conclusions: 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.

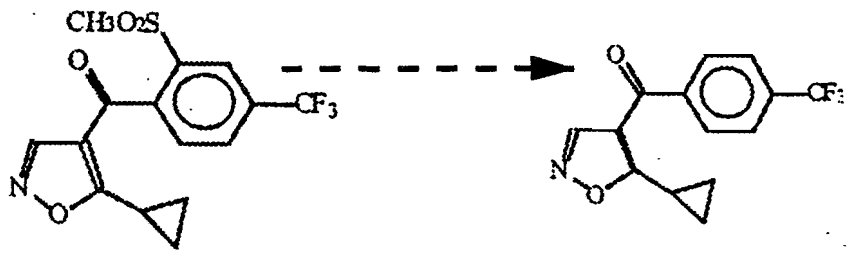
Based on the estimated maximum dietary burden of 0.2 ppm, the dietary feeding levels in this study were 0.9X, 2.7X and 9X. The results of this feeding study indicate that the appropriate tolerances are: Poultry, Liver - 0.20 ppm.

The tolerance expression proposed by the petitioner includes RPA 203328. However, this metabolite is not found in animals nor is it measured in the proposed enforcement method for animal tissues. The poultry liver tolerance should 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 magnitude of the residue data for poultry and revisions to Section F will be required if additional metabolites are determined to be of toxicological significance by the HED Metabolism Committee.

Attachment 1- IRLS Sheet

cc: J. Miller/D. Kenny (RD, 7505C), PP#6F04664, Kramer, R.F., Circ.
RDI: TPT1 (7/30/96), E.T. Haeberer (8/12/96), R.A. Loranger (8/7/96)
G.F. Kramer:804V:CM#2:(703)305-5079:7509C

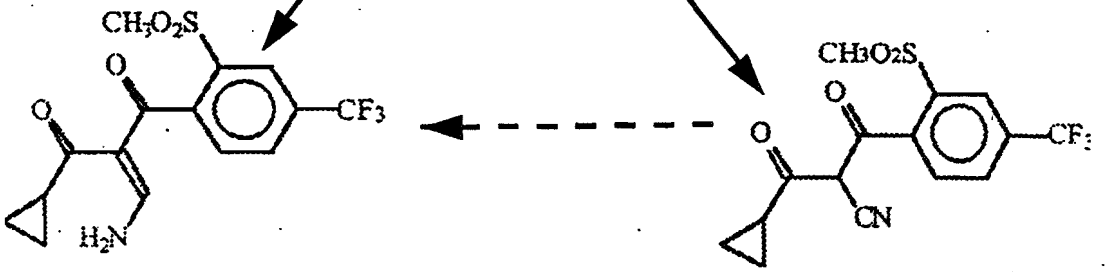
Proposed General Metabolic Pathway for Isoxaflutole in Animals



RPA 201772: ISOXAFLUTOLE

RPA 205568

Rat: Minor metabolite in urine and faeces



RPA 205834

Rat: Minor metabolite detected in urine and faeces.

Hen: Minor metabolite detected in egg yolk.

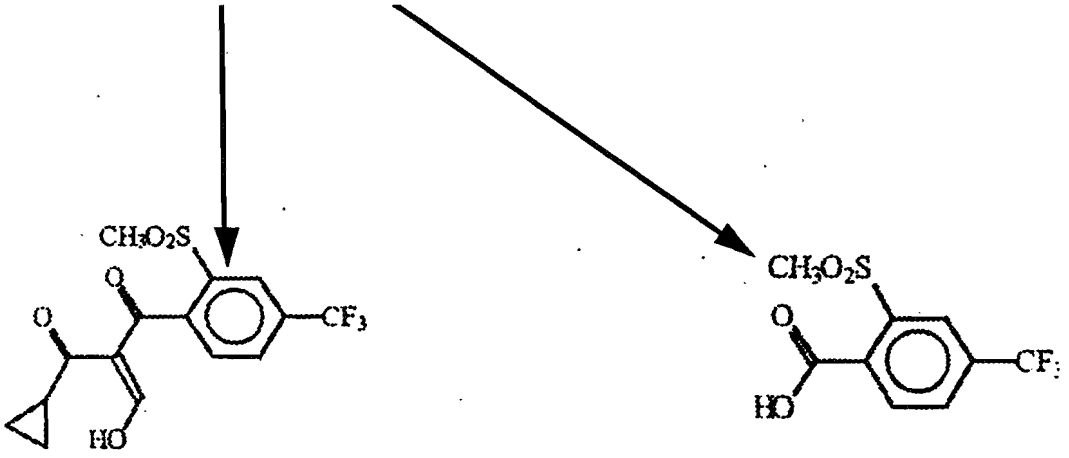
Goat: Minor metabolite detected in faeces, milk, renal and omental fat.

RPA 202248

Rat: Major metabolite detected in urine, faeces and liver.

Hen: Major metabolite detected in excreta, egg yolk, muscle fat, skin, liver and kidney.

Goat: Major metabolite in urine, faeces, milk, liver, kidney, muscle, renal and omental fat.



RPA 207048

Rat: Minor metabolite detected in faeces.

Hen: Minor metabolite detected in muscle and fat.

Goat: Minor metabolite detected in urine, faeces, milk, liver, kidney, muscle, renal and omental fat.

RPA 203328

Rat: Minor metabolite detected in urine and faeces.

Hen: Minor metabolite detected in muscle and possibly kidney.

F. Hoes 7/7/96

(45)

Attachment:

INTERNATIONAL RESIDUE LIMIT STATUS

CHEMICAL Isoxaflutole*

CODEX NO. _____

CODEX STATUS:

[✓] No Codex Proposal Step 6 or Above

Residue (if Step 8): _____

_____

PROPOSED U.S. TOLERANCES:

Petition No. 6F04664

CBTS Reviewer G.F. Kramer

Residue: Parent plus metabolites**

Crop(s)	Limit (mg/KG)
Field Corn, Grain	0.10
Field Corn, Fodder	0.40
Field Corn, Forage	0.40
Liver*	0.20
Hog, Liver	0.04
Eggs	0.05
Poultry, Fat	0.05
Poultry, Meat	0.05
Kidney*	0.03
Hog, Kidney	0.01

*of cattle, goat, poultry and sheep

CANADIAN LIMITS:

[✓] No Canadian Limits

Residue: _____

_____

Crop(s)	Limit (mg/KG)

MEXICAN LIMITS:

[✓] No Mexican Limits

Residue: _____

_____

Crop(s)	Limit (mg/KG)

NOTES

*5-cyclopropyl-4-isoxazolyl [2-(methylsulfonyl)-4-trifluoromethyl) phenyl] methanone

**1-(2-methylsulphonyl-4-trifluoromethylphenyl-2-cyano-3-cyclopropyl propane-1,3-dione and 2-methylsulphonyl-4-trifluoromethyl benzoic acid