I/II/C-E

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

DATE: 3/12/2001

SUBJECT: PP#s 7F4841 and 0F6171. Tolerance Petitions for the Use of Flumioxazin on Peanuts, Soybeans, and Sugarcane. Evaluation of Residue Chemistry and Analytical Methodology

Chemical Number:	129034	
40CFR:	Not yet established	
Chemical Class:	Herbicide	
Trade Names:	Valor WP	EPA Reg. No. 59639-01
	Valor WDG	EPA Reg. No. 59639-00
DP Barcodes:	D259493 and D268181	
MRID Numbers:	44013001, 44013002, 44295049 - 44295057	

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SUMMARY OF RESIDUE CHEMISTRY DEFICIENCIES

The registrant has submitted all required studies relevant to the establishment of the proposed tolerances for flumioxazin residues in/on soybean and peanut commodities. However, a revised Section F is needed for PP#7F4841 proposing 0.02 ppm tolerances for soybean seed and peanut nutmeat. Revised labels are also needed specifying a 30-day restriction on the planting of rotational crops other than peanuts or soybeans. HED recommends that a more specific confirmatory analytical method be required as a condition of registration. Alternatively, the registrant could submit an interference study showing whether other pesticides registered on soybeans and peanuts interfere with the analysis of flumioxazin. HED recommends against the establishment of the proposed tolerance on sugarcane because of the following data deficiencies: an appropriate plant metabolism study, radiovalidation data for the analytical method, and field rotational crop studies.

INTRODUCTION

Valent U.S.A. Corporation has submitted two petitions for the establishment of permanent tolerances for residues of flumioxazin in/on soybean, peanut, and sugarcane commodities. The petitioner has proposed tolerances for residues of flumioxazin [2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propynyl)-2*H*-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3(2H)-dione] in or on:

Soybean, seed	0.01 ppm
Peanut, nutmeat	.0.01 ppm
Sugarcane	0.20 ppm

Flumioxazin is a new N-phenylphthalimide herbicide proposed for preemergence application to peanuts and soybeans and pre- and postemergence applications to sugarcane for the selective control of susceptible broadleaf weeds. Flumioxazin will be marketed as 2 formulations: Valor WP Herbicide (EPA Reg. No. 59639-01), a 51% wettable powder (WP) marketed in water soluble bags, and Valor WDG Herbicide (EPA Reg. No. 59639-00), a 51% water-dispersible granule (dry flowable; DF).

Valent has previously submitted an EUP request and temporary tolerance petition (PP#3G4250) for the use of flumioxazin on soybeans, along with supporting residue chemistry data (DP Barcodes D194594 and D222275, 9/21/94 and 4/25/96, J. Garbus). Because of data deficiencies, no temporary tolerances for flumioxazin were established.

No Codex Maximum Residue Limits (MRLs) are established for residues of flumioxazin in/on plant or animal commodities.

Product Properties

1. All product chemistry data requirements have been fulfilled for the technical grade of the active ingredient (TGAI).

Proposed Uses

2a. Flumioxazin (51% WP or DF/WDG) is proposed for a single preemergence application to peanuts or soybeans at a maximum of 1.53 oz ai/A (0.096 lb ai/A) using ground equipment. The proposed labels should be amended to specify a 30-day restriction on the planting of rotational crops other than peanuts or soybeans.

2b. The 51% WDG formulation is proposed for use on sugarcane with preemergence and postemergence applications (maximum 0.383 and 0.13 lb ai/A, respectively). The maximum seasonal rate is 0.383 lb ai/A and the PHI is 90 days. Until a metabolism study on sugarcane or grass is submitted (Conclusion 6), residue data will not be reviewed.

Nature of the Residue - Plants

3. In conjunction with an EUP request (PP#3G4250; DP Barcode D194594, 9/21/94, J. Garbus), Valent has previously submitted an acceptable study depicting the metabolism of [^{14}C]flumioxazin in/on soybeans following preemergence application with separate labels in the phenyl and tetrahydrophthalimide (THP) rings. Total radioactivities were always higher with the THP label (up to 7x in the case of soybean seeds). Both labels indicate that hydrolysis of the imide moiety is the major metabolic route. The only identified metabolites greater than 0.01 ppm from a 1x rate were 3,4,5,6-tetrahydrophthalmic acid (THPA) and 1-hydroxy-trans-1,2-cyclohexanedicarboxylic acid (1-OH-HPA).

4a. The submitted peanut metabolism study used both phenyl and THP labeled [^{14}C]flumioxazin which was applied at rates of up to 3x the proposed maximum label rate. Agency guidance for plant metabolism studies (OPPTS GLN 860.1300) requires the use of application(s) at up to 10x the maximum labeled rate in order to achieve sufficient ^{14}C-residues for analysis. Although flumioxazin is a broadleaf herbicide, data from the field trials indicate that no phytotoxicity was observed on peanuts treated at 0.48 lb ai/A (5x the maximum rate). In addition, the planting-to-harvest intervals for peanuts in the present study were unusually long (197-245 days), and no immature samples were collected to aid in the identification of ^{14}C-residues.

Despite the above questions about these data, the present peanut metabolism study indicates that total radioactive residues (TRR) are low in nutmeats, hulls, and vines (0.009-0.031 ppm) harvested 194 days following a single preemergence application of [^{14}C]flumioxazin at 0.1 lb ai/A (~1x). Following an application at 0.3 lb ai/A (3x), TRRs were 0.044-0.085 ppm in nutmeats, 0.097-0.166 ppm in hulls, and 0.023-0.027 ppm in vines. Radioactive residues in each commodity were generally comprised of unknown polar components, each accounting for <0.01 ppm. HPLC and TLC analyses tentatively detected trace amounts of parent (<1% TRR, <0.001 ppm), 1-OH-HPA (≤4% TRR, ≤0.006 ppm) and THPA (≤2% TRR, ≤0.003 ppm) in vines and hulls. No parent or known metabolites were detected in nutmeats. The structure and chemical name of flumioxazin and its metabolites are presented in Attachment 1.

5. Taken together, the soybean and peanut metabolism studies adequately define the nature of the residue in legume crops following preemergence application. The major metabolic pathway is hydrolysis of the imide moiety to produce the metabolites THPA and APF. The THPA is then hydrated to produce 1-OH-HPA. The Metabolism Assessment Review Committee (MARC) has concluded that the residue of concern in soybeans and peanuts for both tolerance enforcement and risk assessment purposes is the parent compound only.

6. No metabolism data have been submitted reflecting application of flumioxazin to sugarcane or a grass crop. HED does not consider the data for a preemergent application to legume crops appropriate to define the metabolism by sugarcane, especially when postemergence use is proposed for the latter. A metabolism study showing the fate of phenyl labeled flumioxazin in sugarcane following the latest proposed application (90-day PHI) should be submitted for further consideration of the use on this crop.

Nature of the Residue in Animals

7. In conjunction with an EUP request (PP#3G4250; DP Barcode D194594, 9/21/94, J. Garbus), Valent has previously submitted acceptable animal metabolism studies reflecting the dosing of goats and poultry with [Phenyl-^{14}C]flumioxazin. The major metabolic pathways observed in goats were hydrolysis of the imide moiety, hydroxylation of the cyclohexene ring, and the equivalent of addition of sulfonic acid to the alkene function. The same basic pathways occur in poultry, although metabolism is less extensive with the parent being the highest level identified residue in most tissues.

8a. **Ruminants.** The submitted goat metabolism study is acceptable. Following oral administration of [THP-^{14}C]flumioxazin to lactating goats for 5 consecutive days at a feeding level of 7.2 ppm (2060x the maximum theoretical dietary burden for

beef and dairy cattle), the TRR (expressed as flumioxazin equivalents) were 0.0045-0.0553 ppm in milk, 0.286-0.330 ppm in liver, 0.189-0.238 ppm in kidney, 0.022-0.028 ppm in muscle, and 0.006-0.010 ppm in fat. Residues in milk appeared to plateau within one day of the first dose.

8b. Approximately 66-90% of the TRR were characterized/identified in milk and tissues; residues in fat were not characterized because TRR were ≤0.01 ppm. The parent, flumioxazin, was identified at low levels only in liver and muscle (1.4-1.7% TRR, 0.0003-0.0039 ppm). The major metabolites identified in milk and tissues included: 3-OH-flumioxazin (4.1-8.1% TRR, 0.008-0.023 ppm), 4-OH-flumioxazin (7.2-12.4% TRR, 0.002-0.025 ppm), Metabolite B (1.5-18.9% TRR, 0.004-0.024 ppm), Metabolite C (7.9-21.7% TRR, 0.003-0.015 ppm), and Metabolite F (10.8% TRR, 0.031, only in liver). All other identified metabolites were present at <7% TRR. Several unknown metabolites were resolved by HPLC chromatography; each of these was present at <0.023 ppm.

9a. **Poultry**. The submitted hen metabolism study is acceptable. Following oral administration of [THP-^{14}C]flumioxazin to laying hens for 14 consecutive days at a feeding level of 9.0 ppm (2000x the maximum theoretical dietary burden for poultry), the TRR were 0.025-0.041 ppm in egg white, 0.009-0.760 ppm in egg yolk, 1.137 ppm in liver, 0.138-0.175 ppm in muscle, 0.226 ppm in fat, and 0.667 ppm in skin with fat. Residues in egg white appeared to remain constant over the dosing period while residues in egg yolks appeared to plateau on Day 11 of the study at 0.638 ppm.

9b. Approximately 50->100% TRR were characterized/identified in eggs and tissues. The parent, flumioxazin, was the major metabolite identified in fat, at 56.3% TRR (0.127 ppm), but was present at lower levels in all other matrices (0.7-12.3% TRR, <0.001-0.079 ppm). 4-OH-S-53482-SA was the major metabolite identified in egg yolk, at 33.6-47.5% TRR (0.139-0.299 ppm) and was also identified in liver (as a mixture with the 3-OH analog; 6.0% TRR, 0.068 ppm). THPA, its anhydride (TPA), and its 3-OH and 4-OH derivatives were the major metabolites identified in egg whites, each at 13.7-27.1% TRR (0.003-0.010 ppm); THPA and 4-OH-THPA were also identified in other hen matrices at 2.6-14.3% TRR (0.005-0.163 ppm). Other identified metabolites were hydroxy derivatives of flumioxazin (3.1-13.2% TRR, <0.001-0.121 ppm).

10. The results of the ruminant and poultry metabolism studies with both phenyl and THP labeled flumioxazin show that the major metabolic pathways are hydrolysis of the imide, hydroxylation of the cyclohexene ring, and the equivalent of addition of sulfonic acid to the alkene function. The MARC has determined that the residues of concern in ruminants are the parent, 3-OH-flumioxazin, 4-OH-flumioxazin, and metabolites B, C, and F. In poultry, the residues of concern are

parent, 3-OH-flumioxazin, 4-OH-flumioxazin, and 4-OH-S-53482-SA. Although feeding studies are not required for the proposed uses on peanuts and soybeans (see Conclusion 15), the above analytes should be determined in any such studies which are needed in future petitions.

Analytical Methods

11a. **Plant Commodities.** The GC/nitrogen-phosphorus detection (NPD) methods, Valent Methods RM-30A, RM-30A-1, RM-30B, and RM-30C are adequate for collecting data on residues of flumioxazin *per se* in/on peanut and soybean commodities. These methods were validated by the petitioner down to an LOQ of 0.02 ppm and have reported LODs of 0.01 ppm.

11b. The proposed enforcement method, Valent Method RM-30A, has undergone both a successful ILV trial and has been successfully validated down to an LOQ of 0.01 ppm by the Agency in a petition method validation (PMV) trial using soybean seed, forage, and hay. A revised copy of the method (RM-30A-1), including changes suggested by ACB, has been submitted. Because of the low residue levels of flumioxazin in peanuts and soybeans, samples from these metabolism studies are of limited value for method validation purposes. Therefore, GC/NPD Method RM-30A-1 should be radiovalidated in conjunction with the requested sugarcane metabolism study using mature cane samples with quantifiable residues of flumioxazin.

11c. The confirmatory procedure in Method RM-30A-1 involves a change in the GC column, but not the detector. Because of the relative lack of specificity in these methods, HED recommends that a more specific confirmatory method (e.g., MS detection) be required as a condition of registration. Alternatively, the petitioner could submit an interference study showing whether other pesticides registered on soybeans and peanuts interfere with the analysis of flumioxazin.

11d. The GC/MS methods, Valent Methods RM-30M and RM-30P, are adequate for collecting data on residues of the metabolite 1-OH-HPA (free and conjugated) in/on peanuts and soybeans and their processed commodities. The LOQ for these methods is 0.02 ppm, and the LOD is reported to be 0.01 ppm.

12. **Animal Commodities.** The proposed GC/thermionic specific detection method is not adequate for determining flumioxazin residues in animal commodities. Although recoveries of flumioxazin were generally acceptable from control samples of goat and poultry commodities fortified at 0.01 and 1.0 ppm, recoveries from fortified samples of poultry muscle (85 ± 22%) and liver (97 ± 28%) were quite variable. In addition, radiovalidation data using ^{14}C-samples from the poultry metabolism study indicate that the method does not adequately

recover endogenous flumioxazin residues from animal commodities. However, for purposes of this petition, tolerance enforcement and data collection methods for flumioxazin residues in animal commodities are not required as neither livestock feeding studies nor animal tolerances are required at the present time (See Conclusion 15).

Multiresidue Method

13. Data depicting the analysis of flumioxazin through FDA Multiresidue Protocols were submitted and will be forwarded to FDA for review. The multiresidue method testing data indicate that flumioxazin is not recovered through Sections 304 and 402 of PAM, Vol I.

Storage Stability Data

14a. The submitted storage stability data are adequate and indicate that flumioxazin is stable at -20°C in peanut nutmeats, forage, hay, and hulls for at least 297-300 days and in peanut oil and meal for at least 31 days. Although residues of flumioxazin declined in frozen soapstock by 40-60% after 30 days of storage, this commodity is not considered a significant food or feed. Previously submitted data on soybeans (PP#3G4250, DP Barcode D194594, 9/21/94, J. Garbus) also indicated that flumioxazin is stable at -20°C in soybean seed, forage and hay for at least 12 months. Residues of the metabolite 1-OH-HPA are stable at -20°C in soybean seed for at least 290 days.

14b. The available storage stability data adequately support the peanut and soybean field trial and processing studies. Prior to analysis for flumioxazin, maximum frozen sample storage intervals were 114-382 days for peanut and soybean RACs and 30-57 days for peanut and soybean processed fractions. Prior to analysis of 1-OH-HPA, the maximum frozen storage interval was 245 days for soybean seed, and 29-39 days for peanut and soybean processed fractions.

Meat/Milk/Poultry/Eggs

15. Livestock feeding studies and tolerances for flumioxazin residues in animal commodities are not required for the current petition. Based on the results of the poultry and goat metabolism studies, which were conducted at >2,000x the maximum theoretical dietary burden (MTDB), there is no reasonable expectation that quantifiable residues of flumioxazin will occur in livestock commodities [40 CFR 180.6(a)(3)] from the proposed uses on soybeans and peanuts. The impact of the sugarcane use on livestock residues will be evaluated after an appropriate plant metabolism study is submitted (see Conclusion 6).

Crop Field Trials

16. **Peanuts.** The submitted field trial data on peanuts are adequate. However, as the lowest fortification level in the peanut RACs was 0.02 ppm, HED recommends that the nutmeat tolerance be set at 0.02 ppm. A revised Section F should be submitted. As the proposed label prohibits the feeding of treated forage or hay to livestock, a tolerance for peanut hay is not required. Following a preemergence or pre-plant incorporated application of flumioxazin (51% DF or WP) at 0.091-0.099 lb ai/A (1x the maximum proposed rate), residues of flumioxazin were <0.01 ppm in/on 18 samples each of peanut vines and hay from 9 tests harvested at normal crop maturity (97-152 DAT), and in/on 12 samples of vines collected at 14-28 DAT from 2 tests. Flumioxazin residues were also <0.01 ppm in/on 26 samples of nutmeats from 13 tests and in/on 18 samples of hulls from 9 tests. In four tests conducted at 0.047-0.049 lb ai/A(5x rate), residues of flumioxazin were <0.01 ppm in/on 8 samples of nutmeats and 6 samples of hulls; two hull samples from one 5x test in TX had flumioxazin residues of 0.04 ppm.

17a. **Soybeans.** The submitted soybean field trial data are adequate. However, as the lowest fortification level in the soybean RACs was 0.02 ppm, HED recommends that the seed tolerance be set at 0.02 ppm. A revised Section F should be submitted. As the proposed label prohibits the feeding of treated forage or hay to livestock, tolerances for soybean forage and hay are not required. Following a single pre-plant incorporated (PPI) or pre-emergence application of flumioxazin (51% DF or WP) at 0.09 lb ai/A (1x the proposed rate), residues of flumioxazin were <0.01 ppm in/on 106 samples of forage harvested 13-42 DAT, 36 samples of hay harvested 80-123 DAT, and 36 samples of seed harvested at maturity (112-160 DAT). Detectable residues of flumioxazin (0.02-0.03 ppm) were found only in/on four samples of forage harvested at 28 DAT from two tests. Of the 26 samples of seeds (13 tests) analyzed for residues of 1-OH-HPA, residues were <0.01 ppm in/on 25 seed samples and 0.01 ppm in/on one sample. Duplicate reanalysis of the one sample with detectable residues found 1-OH-HPA residues of <0.01 ppm.

17b. Data on flumioxazin residues in aspirated grain fractions derived from soybeans are not required because (i) the proposed use is for a single preemergence application; (ii) residues of flumioxazin and its metabolite 1-OH-HPA were each <0.01 ppm in/on all samples of soybean seeds harvested at normal maturity following a 1x or 5x application; and (iii) and there was no evidence from the processing study that residues concentrate in hulls.

Processed Food/Feed

18. **Peanut.** The submitted peanut processing studies are adequate and indicate that tolerances are not required for flumioxazin residues in peanut processed commodities. In two processing studies conducted at 5x, residues of flumioxazin were <0.01 ppm in nutmeats and regulated processed fractions. In one of these studies, residues of 1-OH-HPA were also <0.01 ppm in nutmeats and regulated processed fractions.

19. **Soybeans.** The submitted soybean processing study is adequate and indicates that tolerances are not required for flumioxazin residues in soybean processed commodities. Following a 5x application to soybeans, residues of flumioxazin and 1-OH-HPA were <0.01 ppm in whole seeds and all regulated processed commodities.

Confined/Field Accumulation in Rotational Crops

20a. The submitted confined rotational crop study is adequate for the proposed uses on soybeans and peanuts. Although TRR values for all RACs were slightly higher in the present study, results from this study are consistent with results from the previous confined rotational crop study using [^{14}C-Phenyl]flumioxazin (PP#3G4250, DP Barcode D194594, 9/21/94, J. Garbus). Following a 1x application of [THP-^{14}C]flumioxazin to a sandy loam soil, TRRs were 0.004-0.057 ppm in RACs from representative rotational crops planted 30 days post-treatment (60 days for lettuce, due to phytotoxicity). Extraction and analysis of plant samples with TRRs >0.01 ppm indicated that the majority of extractable ^{14}C-residues were comprised of unknown polar components each present at ≤0.015 ppm. Low levels of flumioxazin (≤0.003 ppm) were detected in carrot tops and wheat chaff and straw, along with trace amounts (<0.001 ppm) of the metabolites 482-HA, IMOXA, and 482-CA. The MARC has concluded that the residue of concern in rotational crops is the parent compound only.

As TRR levels in rotational crops grown in soil treated at 1x were >0.01 ppm at the 30-day plant-back interval (PBI), the 51% WP and DF product labels should be amended to specify a 30-day restriction on the planting of rotational crops other than peanuts or soybeans.

20b. With respect to the proposed use on sugarcane, the highest rate used in the confined study was only 0.5x the maximum proposed rate. Taking into account that 0.03 ppm parent flumioxazin was found at the confined study's higher rate, limited rotational crop field studies reflecting the 0.383 lb ai/A rate will be needed to support the use on sugarcane. The petitioner may wish to consult with HED as to which crops to include in these studies.

Other Considerations:

As there are no Codex MRLs established for residues of flumioxazin in/on peanuts and soybeans (see Attachment 2), a discussion of compatibility with U.S. tolerances is not relevant at this time.

RECOMMENDATIONS

Provided the labels are revised to have a 30-day plantback interval (Conclusions 2a and 20a), and a revised Section F is submitted (Conclusions 16 and 17a), there are no residue chemistry data gaps precluding the establishment of 0.02 ppm tolerances for residues of flumioxazin in soybean seeds and peanut nutmeats. HED recommends the registration be made conditional based upon submission of a more specific confirmatory method (Conclusion 11c). HED recommends against the establishment of the proposed tolerance on sugarcane because of the lack of an appropriate plant metabolism study (Conclusion 6), radiovalidation data for the analytical method (Conclusion 11b), and field rotational crop studies (Conclusion 20b).

A separate memorandum will be issued with a human health risk assessment for the proposed uses on soybeans and peanuts.

DETAILED CONSIDERATIONS

OPPTS 830 Series GLNs: Product Properties

Valent has submitted product chemistry data (1997; MRIDs 44295001 and 44295004) for a 97.9% flumioxazin technical product in support of a petition for the establishment of permanent tolerances in/on peanuts and soybeans (PP#7F4841). The detailed review of these product chemistry submissions, which contains CBI information, is included as Attachment 3. Product chemistry data were previously submitted in conjunction with a non-food use registration of flumioxazin (RD Memorandum, 4/16/93, A. Smith), and were found to fulfill all product chemistry data requirements for the technical grade of the active ingredient (TGAI). In the administrative materials supporting the current submissions, the petitioner stated that new preliminary analysis data and certified limits were being submitted because the purity of the TGAI has improved. Although no changes have been made to the manufacturing process or starting materials, the technical product is now being commercially produced under tightly controlled conditions. The new preliminary analysis and certified limits reflect current commercial production. In addition, the petitioner has submitted data pertaining to UV/visible absorption of the PAI. Two additional submissions concerning the storage stability (OPPTS 830.6317) of the 51% WDG (DF) and WP formulations (1997; MRIDs 44295002 and 44295003), are deferred to the Registration Division for review.

OPPTS GLN 860.1200: Proposed Uses

The petitioner provided specimen labels for a 51% WP (Valor WP Herbicide; EPA Reg. No. 59639-01) and a 51% DF (Valor WDG Herbicide; EPA Reg. No. 59639-00) formulation that include the proposed uses on peanuts and soybeans. Flumioxazin is proposed for a single preemergence application to soybeans or peanuts at 1.02-1.53 oz ai/A (0.063-0.096 lb ai/A). Flumioxazin may be applied using ground equipment as either a broadcast or banded application using a minimum volume of 10 gal/A. For a banded application, proportionately less product should be applied per acre. The labels specify a maximum seasonal application rate of 1.53 oz ai/A (0.096 lb ai/A), and prohibit the grazing of livestock in treated areas and the feeding of treated forage or hay to livestock. Applications through irrigation systems or using aerial equipment are also prohibited. No preharvest intervals or rotational crop restrictions are specified on either label. A restricted entry interval of 12 hours is also listed on the proposed labels.

The 51.0% WDG formulation is also proposed for use on sugarcane. The maximum rate for preemergence applications is 12 oz product per acre (0.383 lb ai/A). Postemergence applications (post directed, layby) may be made at 3-4 oz product per acre (0.096-0.13 lb ai/A). The maximum seasonal rate is 0.383 lb ai/A and the preharvest interval is 90 days.

Based on the results of the confined rotational crop study, the proposed labels should be amended to specify a 30-day restriction on the planting of rotational crops other than peanuts or soybeans.

Conclusions: Other than the need for a rotational crop restriction, the proposed use directions are adequate for soybeans and peanuts. The sugarcane directions (and residue data) will be reviewed upon submission of a sugarcane or grass metabolism study.

OPPTS GLN 860.1300: Nature of the Residue in Plants

In conjunction with the EUP for use of flumioxazin on soybeans (PP#3G4250, DP Barcode D194594, J. Garbus, 9/21/94), the petitioner previously submitted an acceptable soybean metabolism study in which [^{14}C]flumioxazin, ^{14}C-labeled in either the phenyl or tetrahydrophthaloyl (THP) ring, was applied to soybeans as a preemergence application at 0.088 or 0.18 lb ai/A (0.8x and 1.9x rates).

Following the ~1x application to soybeans, ^{14}C-residues were 0.055-0.069 ppm in forage, 0.155-0.257 ppm in hay, 0.033-0.245 ppm in seeds, and 0.152-0.207 ppm in straw. Following the ~2x application, ^{14}C-residues were 0.108-0.196 ppm in forage, 0.348-0.617 ppm in hay, 0.055-0.177 ppm in seeds, and 0.176-0.254 ppm in straw. At both application rates, ^{14}C-residues resulting from application of [THP-^{14}C]flumioxazin

were 1.3-1.8x as high in forage, hay, and straw and 3.2-7.4x as high in seeds as they were in the same commodities treated with [phenyl-^{14}C]flumioxazin.

In addition to parent, metabolites identified in soybeans were the result of hydrolysis of the imide moiety and included: 482-HA, APF, THPA, and 1-OH-HPA. Parent accounted for 4.4-6.1% of the TRR in forage and hay, but was not detected in seeds. In [Phenyl-^{14}C]flumioxazin-treated plants, trace amounts of 482-HA and APF were detected in forage and hay. The major metabolite detected in soybeans was free and bound 1-OH-HPA in [THP-^{14}C]flumioxazin-treated plants. Metabolite 1-OH-HPA which results from hydroxylation of the cyclohexene ring, accounted for 15.3-25.2% of the TRR in forage, 25.8-31.5% of the TRR in hay, and 37.9-42.2% of the TRR in seeds. Minor amounts of THPA (2.6-8.6% TRR) were also detected in forage, hay, and seeds of [THP-^{14}C]flumioxazin-treated plants. Only 1-OH-HPA and THPA were found at levels greater than 0.01 ppm.

Peanut

With the current petition, Valent submitted a study depicting the metabolism of [^{14}C]flumioxazin in peanuts (MRID No. 44013001). The in-life phase of the study was conducted by Plant Science, Inc (PSI), Watsonville, CA, and the analytical phase of the study was performed by XenoBiotic Laboratories Inc. (XBL), Plainsboro, NJ.

The two [^{14}C]flumioxazin test substances used in this study were either uniformly ^{14}C-labeled in the phenyl ring **(phenyl-^{14}C)** or ^{14}C-labeled at two carbons of the tetrahydrophthaloyl ring **(THP-^{14}C)**. The [phenyl-^{14}C]flumioxazin had a radiochemical purity ≥93.8% and a specific activity of 365 μCi/mg (810,300 dpm/μg), and the [THP-^{14}C]flumioxazin phenyl-^{14}C]flumioxazin had a radiochemical purity ≥94.6% and a specific activity of 300 μCi/mg (666,000 dpm/μg). For application, each test substance was diluted in acetonitrile (ACN).

Each ^{14}C-test substance was applied to peanuts as a single preemergence soil incorporated application at rates equivalent to 0.099 and 0.30 lb ai/A (1x and 3x the maximum proposed rate). For each treatment, four plots (2'×2'×1.5') of sandy loam soil were established in greenhouses and the top ½ inch of soil was removed from each plot for treatment. The test substances were applied to the soil, and the soil was mixed and added back to the tops of the respective test plots. Peanut seeds were planted in each plot 3 days prior to soil treatment (10/8/92).

Because of poor germination, the 3x-treated plots were replanted with fungicide-treated seeds approximately 1 month after the initial application (11/9/92) and new test plots were established for the 1x treatments the following spring (3/11/93) using new soil, fungicide-treated seeds, and the same application procedures.

No immature crop samples were collected for detailed analysis. Samples of mature foliage and whole peanuts were harvested from the 1x test plots 194 days after treatment (DAT) and from the 3x test plots 245 days after replanting (277 DAT). [*HED notes that the planting to harvest intervals for peanuts in this study were extremely long; peanuts typically mature within 3-5 months of planting.*] Samples of foliage (vines) were frozen immediately after sampling. Whole peanuts were washed to remove adhering soil and separated into hulls, seed coats, and nutmeats. Samples were frozen and shipped on dry ice by overnight courier to the analytical laboratory (XBL) where samples were stored at <-10 C prior to analysis. Initial extractions were conducted within 1-2 months of sample harvest.

Total radioactive residues (TRR)

Frozen samples of vines, hulls, nutmeats, and seed coats were homogenized and the TRRs in subsamples were determined in triplicate by liquid scintillation counting (LSC) following combustion. TRR in peanut matrices are presented in Table 1.

Following a 1x application, ^{14}C-residues in mature peanut samples were low (<0.04 ppm), with ^{14}C-residues being lower in the [phenyl-^{14}C] samples (0.009-0.019 ppm) than in the [THP-^{14}C] samples (0.020-0.036 ppm). With the exception of hulls and vines ([^{14}C-THP] only), TRR were ~3x higher in the 3x treated samples, [phenyl-^{14}C] samples (0.027-0.166 ppm) and [THP-^{14}C] samples (0.023-0.097 ppm). For each treatment, ^{14}C-residues were lowest in vines (0.009-0.027 ppm) and were generally highest in hulls (0.019-0.166 ppm). ^{14}C-Residues in control samples of each matrix were <0.001 ppm.

Table 1. Total radioactive residues found in/on peanuts harvested at maturity following a single soil application of [phenyl-^{14}C] or [THP-^{14}C]flumioxazin

Matrix	Sampling Interval (days) [a]	Total radioactive residues (ppm) [b]			
		[Phenyl-^{14}C]-label		[THP-^{14}C]-label	
		1x	3x	1x	3x
Vines	194/245	0.009	0.027	0.021	0.023
Hulls	194/245	0.019	0.166	0.020	0.097
Nutmeats	194/245	0.012	0.044	0.031	0.085
Seed coats	194/245	0.013	0.045	0.036	0.093

[a] Sampling intervals (1x/3x)are expressed as days after treatment for the 1x samples and as days after replanting for the 3x samples

[b] Data are expressed in [^{14}C]flumioxazin equivalents.

Extraction and hydrolysis of residues

The extraction and distribution of ^{14}C-residues in 1x- and 3x-treated samples of vines, hulls, and nutmeats are summarized in Table 2. All samples were initially extracted with acetone:water (4:1, v/v) and filtered. Each extract was concentrated to an aqueous solution and partitioned with hexane, resulting in Aqueous-1 and Hexane-1 fractions. Hexane-1 fractions from vines and hulls were not further analyzed as ^{14}C-residues in these fractions were low (≤0.002 ppm). However, the Hexane-1 fraction from selected nutmeats samples (0.001-0.015 ppm) were concentrated and partitioned between hexane:acetonitrile (ACN; 1:1, v/v), with essentially all the ^{14}C-residues remaining in the Hexane-2 fraction. Using the Hexane-2 fraction from the 3x-[phenyl-^{14}C] nutmeats, the analytical laboratory attempted further purification of the ^{14}C-residues by freezing to precipitate fats or by chromatography using a silica gel, C_{18}, or gel permeation columns, but was unable to separate the ^{14}C-residues from the oil fraction. However, data from extraction of control nutmeat samples fortified with flumioxazin indicate that parent is unlikely to be present in the Hexane-2 fraction.

The Aqueous-1 fractions from all 1x-treated samples and from 3x-treated hulls and nutmeats ([THP-^{14}C] sample only) were acidified to pH 2-3 and partitioned with ethyl acetate (EtOAc) resulting in Aqueous-2 and EtOAc-1 fractions. Selected Aqueous-1, Aqueous-2 and EtOAc-1 fractions from various samples were then analyzed by reverse-phase (RP)-HPLC.

Following solvent extraction, the majority of ^{14}C-residues in nutmeats (67.2-83.1% TRR), hulls (61.8-69.1% TRR) and vines (50.5-58.9% TRR) remained in the post-extraction solids (PES-1). To further characterize unextracted ^{14}C-residues, PES-1 fractions from all 1x-treated samples of nutmeats, hulls, and vines (both [^{14}C]-labels) were subjected to sequential enzymatic and acid/base hydrolyses (Table 3). The PES-1 fractions were initially incubated with cellulase at 37°C in an acetate buffer (pH 5) for 24 hours and filtered, and the resulting filtrates were analyzed by RP-HPLC. The PES-2 fraction was then acid hydrolyzed by refluxing for ~2 hours in 2 N HCl. The hydrolysate was cooled and filtered, and the resulting filtrate was partitioned with EtOAc. The remaining aqueous fraction (AQ-3) was purified using a C_{18} SPE column eluted with water and methanol, and analyzed by RP-HPLC. The residual solids (PES-3) were then base hydrolyzed by refluxing for ~2 hours in 2 N NaOH. The hydrolysate was cooled and filtered, and the resulting filtrate was partitioned with EtOAc. ^{14}C-Residues remaining in the final PES-4 fraction accounted for 23.2-35.3% of the TRR (0.003-0.010 ppm) in nutmeats and hulls and 6.4-8.6% of the TRR (0.001-0.002 ppm) in vines.

Table 2. Distribution and fractionation of radioactivity in 1x- and 3x-treated peanut nutmeats, hulls, and vines.

Fraction	[Phenyl-^{14}C]-1x		[Phenyl-^{14}C]-3x		[THP-^{14}C]-1x		[THP-^{14}C]-3x	
	% TRR	ppm [a]	% TRR	ppm	%TRR	ppm	%TRR	ppm
Nutmeats								
Acetone/water	24.2	0.002	32.8	0.014	16.9	0.005	29.3	0.027
Hexane-1	12.2	0.001	23.0	0.010	6.4	0.002	16.5	0.015
Hexane-2	12.0	0.001	22.7	0.010	NA	--	16.4	0.015
ACN	0.14	<0.001	0.33	<0.001	NA	--	0.07	<0.001
Aqueous-1	12.0	0.001	**9.8** [b]	**0.004**	10.6	0.003	12.9	0.012
Aqueous-2	8.4	0.001	NA [c]	--	7.1	0.002	**5.7**	**0.005**
EtOAc-1	3.5	<0.001	NA	--	3.5	0.001	7.2	0.007
PES	75.86	0.009	67.2	0.030	83.1	0.026	70.7	0.066
Hulls								
Acetone/water	34.5	0.006	30.9	0.051	31.2	0.006	38.2	0.037
Hexane-1	1.0	<0.001	0.98	0.001	1.2	<0.001	1.1	0.001
Aqueous-1	33.5	0.006	30.2	0.050	30.0	0.006	37.1	0.036
Aqueous-2	**18.5**	**0.004**	**10.8**	**0.018**	**17.0**	**0.003**	**13.1**	**0.013**
EtOAc-1	**15.0**	**0.003**	**19.4**	**0.032**	**13.1**	**0.003**	**24.0**	**0.023**
PES	65.5	0.012	69.1	0.115	68.8	0.014	61.8	0.060
Vines								
Acetone/water	47.0	0.004	49.5	0.013	41.1	0.009	47.2	0.011
Hexane-1	0.25	<0.001	4.5	0.001	3.5	0.001	8.8	0.002
Aqueous-1	46.7	0.004	**45.1**	**0.012**	37.6	0.008	**38.4**	**0.009**
Aqueous-2	**25.4**	**0.002**	NA	--	**23.8**	**0.005**	NA	--
EtOAc-1	**21.3**	**0.002**	NA	--	**13.8**	**0.003**	NA	--
PES	53.0	0.005	50.5	0.014	58.9	0.012	52.8	0.012

[a] Expressed in [^{14}C]flumioxazin equivalents.
[b] Fractions indicated in **Bold** were analyzed by HPLC.
[c] NA = not applicable.

Table 3. Hydrolysis and fractionation of ^{14}C-residues remaining in post-extraction solids from 1x-treated samples of nutmeats, hulls, and vines.

Fraction	Nutmeats				Hulls				Vines			
	1x-[Phenyl^{14}C]		1x-[THP-^{14}C]		1x-[Phenyl^{14}C]		1x-[THP-^{14}C]		1x-[Phenyl-^{14}C]		1x-[THP-^{14}C]	
	% TRR	ppm [a]	% TRR	ppm	% TRR	ppm	% TRR	ppm	%TRR	ppm	%TRR	ppm
PES-1	75.9	0.009	83.1	0.026	65.5	0.012	68.8	0.014	53.0	0.005	58.9	0.012
Cellulase hydrolysate	**13.9** [b]	**0.002**	**15.6**	**0.005**	**10.3**	**0.002**	7.7	0.002	**11.2**	**0.001**	**13.9**	**0.003**
PES-2	61.9	0.007	67.4	0.021	55.2	0.010	61.2	0.012	41.9	0.004	45.0	0.009
2N HCL hydrolysis AQ-3	**22.9**	**0.003**	**23.2**	**0.007**	**14.8**	0.003	**11.9**	**0.002**	**17.8**	**0.002**	**18.1**	**0.004**
EtOAc-2	5.8	0.001	7.5	0.002	7.0	0.001	4.6	0.001	5.7	0.001	8.9	0.002
PES-3	33.2	0.004	36.8	0.011	33.4	0.006	44.7	0.009	18.3	0.002	18.0	0.004
2N NaOH hydrolysis AQ-4	8.9	0.001	3.8	0.001	5.0	0.001	4.0	0.001	6.9	0.001	5.5	0.001
EtOAc-3	NA [c]	--	NA	--	5.2	0.001	5.5	0.001	5.0	0.001	3.9	0.001
PES-4	24.3	0.003	32.9	0.010	23.2	0.004	35.3	0.007	6.4	0.001	8.6	0.002

[a] Expressed in [^{14}C]flumioxazin equivalents.
[b] Fractions indicated in **Bold** were analyzed by RP-HPLC.
[c] NA = not applicable.

Characterization of residues

Radioactive residues in selected aqueous and organic fractions containing ≥10% of the TRR were analyzed by RP-HPLC using a C_{18} column with a mobile phase gradient of aqueous 0.01% trifluoroacetic acid to ACN. ^{14}C-Residues were detected and quantified by LSC of collected eluate fractions, and reference standards were detected using a UV absorbance detector (220 nm). Peak retention times for ^{14}C-residues were compared to retention times (R_t) of reference standards. HPLC peaks containing significant amounts of radioactivity were also analyzed by TLC using silica gel plates with a variety of solvent systems. ^{14}C-Residues on the plates were detected using a radioimaging system. Residues and reference standards were visualized under UV light (254 nm). Reference standards used in this study included: parent, IMOXA, 482-CA, THPA, 482-HA, 1-OH-HPA, TPA, APF, and PNF. The chemical names and structures of flumioxazin and its metabolites detected in plants and animals are shown in Attachment 1.

Because of the low TRR levels in samples and the use of the 1x-treated samples for the majority of the characterization work, identification of ^{14}C-residues in peanuts was limited. HPLC analyses of the EtOAc-1 fractions from hull and vines revealed trace amounts of parent (<1% TRR, <0.001 ppm), but the majority of ^{14}C-residues in solvent and hydrolysate fractions was generally comprised of four regions designated Regions A, B, C, and D. Regions A and B were polar in nature with R_ts of ~4 and ~12 minutes, respectively, and did not correspond to any of the reference standards used in this study. Region C was typically a broad peak, suggesting multiple components, with a R_t (~16-18 minutes) that was similar to R_ts for 1-OH-HPA, THPA, APF, and 482-HA. Region D was a minor peak with a R_t (~35 minutes) similar to the standards IMOXA, PNF, and 482-CA.

In both 1x and 3x treated nutmeats and vines, each of these general regions accounted for ≤0.01 ppm in each fraction analyzed by HPLC. These regions also each accounted for ≤0.005 ppm in fractions from hulls, with the exception of Region C which accounted for 0.025-0.038 ppm in solvent extracts from 3x-treated hulls. Subsequent TLC analyses of Region C from EtOAc-1 fractions of hulls and vines suggested that this region contained minor levels of 1-OH-HPA (≤4% TRR, ≤0.006 ppm) and THPA (≤2% TRR, ≤0.003 ppm); however, the majority of ^{14}C-residues in Region C were multiple unknown components.

The detailed distribution and HPLC analysis of ^{14}C-residues in 1x-[Phenyl-^{14}C]-treated nutmeats, hulls, and vines is presented in Table 4. The distribution among extracts and HPLC fractions was similar for the 1x-treated [^{14}C-THP] samples and for 3x-treated samples from both ^{14}C-labels.

Table 4. Characterization of ^{14}C-residues in/on peanut nutmeat, hulls, and vines harvested 194 days after a preemergence application of [phenyl-^{14}C]flumioxazin at 0.10 lb ai/A (1x proposed rate)

Fraction	% TRR	ppm [a]	Characterization/Identification
1x-[Phenyl- ^{14}C] Nutmeats (TRR = 0.012 ppm)			
Acetone/water	24.2	0.002	concentrated and partitioned with hexane
Hexane-1	12.2	0.001	concentrated and partitioned with ACN
Hexane-2	12.0	0.001	Not further analyzed.
ACN	0.14	<0.001	
Aqueous-1	12.0	0.001	Acidified and partitioned with EtOAc.
Aqueous-2	8.4	0.001	Not further analyzed.
EtOAc-1	3.5	<0.001	
PES-1	75.86	0.009	Sequentially extracted by enzymatic and acid/base hydrolysis
Cellulase hydrolysate	13.9	0.002	RP-HPLC analysis. Region A 5.4% TRR <0.001 ppm Region C 7.7% TRR 0.001 ppm 4 other peaks 0.9% TRR <0.001 ppm
PES-2	61.9	0.007	Acid hydrolyzed by refluxing in 2N HCl, followed by partitioning with EtOAc.
2N HCL hydrolysis AQ-3	22.9	0.003	Purified AQ-3 fraction was analyzed by RP-HPLC. Region A 14.4% TRR 0.002 ppm Region B 0.5% TRR <0.001 ppm Region C 1.2% TRR <0.001 ppm 5 other peaks 0.4% TRR <0.001 ppm
EtOAc-2	5.8	0.001	Not further analyzed.
PES-3	33.2	0.004	Base hydrolyzed by refluxing in 2N NaOH, followed by partitioning with EtOAc.
2N NaOH hydrolysis AQ-4	8.9	0.001	Not further analyzed.
PES-4	24.3	0.003	

Table 4. Characterization of ^{14}C-residues in/on peanut nutmeat, hulls, and vines harvested 194 days after a preemergence application of [phenyl-^{14}C]flumioxazin at 0.10 lb ai/A (1x proposed rate)

Fraction	% TRR	ppm [a]	Characterization/Identification
1x-[Phenyl- ^{14}C] Hulls (TRR = 0.019 ppm)			
Acetone/water	34.5	0.006	Partitioned with hexane.
Hexane-1	1.0	<0.001	Not further analyzed.
Aqueous-1	33.5	0.006	Acidified and partitioned with EtOAc.
Aqueous-2	18.5	0.004	RP-HPLC analysis. Region A 5.2% TRR <0.001 ppm Region C 13.3% TRR 0.003 ppm
EtOAc-1	15.0	0.003	RP-HPLC analysis. Region A 0.5% TRR <0.001 ppm Region C 12.1% TRR 0.002 ppm Region D 1.0% TRR <0.001 ppm Flumioxazin 0.6% TRR <0.001 ppm 3 other peaks 0.9% TRR <0.001 ppm
PES-1	65.5	0.012	Sequentially extracted by enzymatic and acid/base hydrolyses.
Cellulase hydrolysate	10.3	0.002	RP-HPLC analysis. Region A 4.5% TRR <0.001 ppm Region B 2.3% TRR <0.001 ppm Region C 3.2% TRR <0.001 ppm 2 other peaks 0.3% TRR <0.001 ppm
PES-2	55.2	0.010	Acid hydrolyzed by refluxing in 2N HCl, followed by partitioning with EtOAc.
2N HCL hydrolysis AQ-3	14.8	0.003	Purified AQ-3 fraction was analyzed by RP-HPLC. Region A 7.7% TRR 0.001 ppm Region C 1.0% TRR <0.001 ppm 3 other peaks 2.7% TRR <0.001 ppm
EtOAc-2	7.0	0.001	Not further analyzed.
PES-3	33.4	0.006	Base hydrolyzed by refluxing in 2N NaOH, followed by partitioning with EtOAc.
2N NaOH hydrolysis AQ-4	5.0	0.001	Not further analyzed.
EtOAc-3	5.2	0.001	
PES-4	23.2	0.004	

Table 4. Characterization of ^{14}C-residues in/on peanut nutmeat, hulls, and vines harvested 194 days after a preemergence application of [phenyl-^{14}C]flumioxazin at 0.10 lb ai/A (1x proposed rate)

Fraction	% TRR	ppm [a]	Characterization/Identification
1x-[Phenyl- ^{14}C] Vines (TRR = 0.009 ppm)			
Acetone/water	47.0	0.004	Partitioned with hexane
Hexane-1	0.25	<0.001	Not further analyzed.
Aqueous-1	46.7	0.004	Acidified and partitioned with EtOAc.
Aqueous-2	25.4	0.002	RP-HPLC analysis. Region A 9.9% TRR <0.001 ppm Region B 0.8% TRR <0.001 ppm Region C 14.6% TRR 0.001 ppm one other peak 0.1% TRR <0.001 ppm
EtOAc-1	21.3	0.002	RP-HPLC analysis. Region A 0.3% TRR <0.001 ppm Region B 2.0% TRR <0.001 ppm Region C 14.9% TRR 0.001 ppm Region D 0.6% TRR <0.001 ppm Flumioxazin 0.2% TRR <0.001 ppm 3 other peaks 0.1% TRR <0.001 ppm
PES-1	53.0	0.005	Sequentially extracted by enzymatic and acid/base hydrolysis
Cellulase hydrolysate	11.2	0.001	RP-HPLC analysis. Region B 2.8% TRR <0.001 ppm Region C 7.5% TRR <0.001 ppm Region D 0.3% TRR <0.001 ppm one other peak 0.6% TRR <0.001 ppm
PES-2	41.9	0.004	Acid hydrolyzed by refluxing in 2N HCl, followed by partitioning with EtOAc.
2N HCL hydrolysis AQ-3	17.8	0.002	Purified AQ-3 fraction was analyzed by RP-HPLC. Region A 9.8% TRR <0.001 ppm Region C 1.1% TRR <0.001 ppm one other peak 0.2% TRR <0.001 ppm
EtOAc-2	5.7	0.001	Not further analyzed.
PES-3	18.3	0.002	Base hydrolyzed by refluxing in 2N NaOH, followed by partitioning with EtOAc.
2N NaOH hydrolysis AQ-4	6.9	0.001	Not further analyzed.
EtOAc-3	5.0	0.001	
PES-4	6.4	0.001	

[a] Expressed in [^{14}C]flumioxazin equivalents.

Storage Stability

Samples of peanut matrices were stored frozen for 1-2 months prior to the initial extractions for analysis, and the analytical phase of the study was completed within 7-10 months.

To demonstrate the frozen storage stability of ^{14}C-residues in peanut fractions, the petitioner provided data on the extraction and chromatographic analysis of ^{14}C-residues in 3x-treated [^{14}C-THP] hulls (TRR= 0.097 ppm). This sample was initially extracted and analyzed within 1 month of harvest and was later reextracted and reanalyzed at 10 months after harvest. This hull sample was solvent extracted and fractionated as described above (Table 2), and the EtOAc-1 fractions from each extraction were analyzed by RP-HPLC and 1D-TLC. The distribution of ^{14}C-residues among the various fractions was essentially the same for the two extractions, and the chromatographic data showed a similar metabolic profile for ^{14}C-residues in the EtOAc-1 fraction from the two extractions. These data indicate that ^{14}C-residues were stable in frozen storage for the duration of the study; no additional storage stability data are needed to support this peanut metabolism study.

Conclusions: Metabolism in Legume Crops

In the present study, [^{14}C]flumioxazin was applied at rates of up to 3x the proposed maximum label rate. Agency guidance for plant metabolism studies (OPPTS GLN 860.1300) requires the use of application(s) at up to 10x the maximum labeled rate in order to achieve sufficient ^{14}C-residues for analysis. Although flumioxazin is a broadleaf herbicide, data from the field trials indicate that no phytotoxicity was observed on peanuts treated at 0.3 lb ai/A (5x the maximum rate). In addition, the planting to harvest intervals for peanuts in the present study were unusually long (197-245 days), and no immature samples were collected to aid in the identification of ^{14}C-residues.

Despite the above questions about these data, the available peanut metabolism study indicates that TRRs are low in mature nutmeats, hulls, and vines (0.009-0.031 ppm) harvested 194 days following a single preemergence application of [^{14}C]flumioxazin at 0.097 lb ai/A (1x). Following an application at 0.3 lb ai/A (3x), TRRs were 0.044-0.085 ppm in nutmeats, 0.097-0.166 ppm in hulls, and 0.023-0.027 ppm in vines. Radioactive residues in each commodity were generally comprised of unknown polar components, each accounting for <0.01 ppm. HPLC and TLC analyses detected trace amounts of parent (<1% TRR, <0.001 ppm), 1-OH-HPA (≤4% TRR, ≤0.006 ppm) and THPA (≤2% TRR, ≤0.003 ppm) in vines and hulls. Parent and no known metabolites were detected in nutmeats.

Taken together, the soybean and peanut metabolism studies adequately define the nature of the residue in legume crops following preemergence application. The major metabolic pathway is hydrolysis of the imide moiety to produce the metabolites THPA and APF. The THPA is then hydrated to produce 1-OH-HPA. The Metabolism Assessment Review Committee (MARC) has concluded that the residue of concern in soybeans and peanuts for both tolerance enforcement and risk assessment purposes is the parent compound only (Memo, D272652, R. Loranger, 3/12/2001).

Conclusions: Metabolism in Sugarcane

No metabolism data have been submitted reflecting application of flumioxazin to sugarcane or a grass crop. HED does not consider the data for a preemergent application to legume crops appropriate to define the metabolism by sugarcane, especially when postemergence use is proposed for the latter. A metabolism study showing the fate of phenyl labeled flumioxazin in sugarcane following the latest proposed application (90-day PHI) should be submitted for further consideration of the use on this crop.

OPPTS GLN 860.1300: Nature of the Residue in Animals

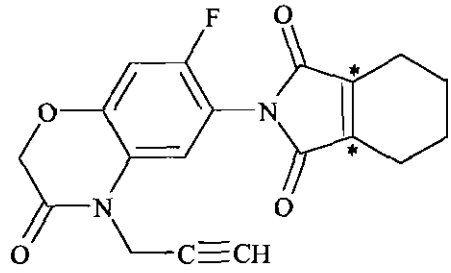
The petitioner previously submitted livestock metabolism studies in conjunction with an EUP request (PP#3G4250; DP Barcode D194594, 9/21/94, J. Garbus). Following dosing of lactating goats with phenyl-labeled [^{14}C]flumioxazin at 11.8 ppm for 5 days, TRR ranged from 0.004 ppm in fat to 0.209 ppm in liver. Approximately 35% of radioactivity in milk and tissues was identified. The only metabolites present at >10% of the TRR in milk and tissues were 482-HA (4.2-14.4% TRR, <0.001-0.020 ppm) and 4-OH-flumioxazin (1.5-13.7% TRR, <0.001-0.025 ppm). Flumioxazin was identified at low levels (<5% TRR, ≤0.01 ppm), along with APF (≤5.8% TRR), 3-OH-flumioxazin (≤6.2% TRR), and 3-OH or 4-OH SA (≤6.5% TRR). Following dosing of laying hens with phenyl-labeled [^{14}C]flumioxazin at 9.9 ppm for 14 days, TRR ranged from 0.018 ppm in egg white to 0.272 ppm in kidney. The predominant residue was found to be flumioxazin at 3.8-48.8% TRR (0.005-0.046 ppm); 482-HA was a minor residue (<7% TRR) except in egg white, where it was detected at 20.0% TRR (0.004 ppm). From these studies it can be concluded that the major metabolic pathways in goats and poultry are hydrolysis of the imide moiety, hydroxylation of the cyclohexene ring, and the equivalent of addition of sulfonic acid to the alkene function. Metabolism occurs more extensively in goats than poultry.

With this petition, Valent submitted two additional livestock metabolism studies, reflecting dosing with flumioxazin labeled in the 3,4,5,6-tetrahydrophthalimide portion of the molecule.

Ruminants

Valent submitted a study (MRID No. 44295051) pertaining to the metabolism of [^{14}C]flumioxazin in lactating goats. The in-life and analytical phases of the study were conducted at Ricerca, Inc. (Painesville, OH).

The test substance, [tetrahydrophthaloyl-1,2-^{14}C]flumioxazin (specific activity 98.6 mCi/mmol and radiochemical purity 98.7%; see structure below for position of radiolabels), was mixed with nonlabeled flumioxazin and ACN to a final specific activity of 213,120 dpm/µg and placed into gelatin capsules. The capsules were administered orally once per day for five consecutive days. Two goats were dosed, and dosing was conducted after feeding. The dosing level, based on the food consumption during the acclimation period (5 days prior to initiation of dosing), was 10 ppm. However, because feed consumption during the dosing period increased, the actual dosing level was 7.2 ppm. This dosing level is 2060x the MTDB for dairy cattle and 1800x the MTDB for swine (see Table 18). Two additional goats received placebo doses to serve as controls.



Position of radiolabels

During the study, the animals were fed commercial goat ration; the ration and water were provided *ad libitum*. The petitioner provided sufficient descriptions of preparation of dose capsules and animal husbandry practices, as well as data concerning daily feed intake, body weights, and milk production.

Milk was collected twice daily. The goats were sacrificed ~6 hours after the final dose, and the following samples were collected: kidneys, heart, liver, muscle (loin and rear leg), fat (omental and perirenal), and GI tract and contents. All milk and tissue samples were stored frozen (temperature unspecified) until analysis.

Total radioactive residues (TRR)

Triplicate aliquots of milk were analyzed directly by LSC. Subsamples of liver, kidney, muscle, and fat were homogenized in dry ice, and then subjected to combustion/LSC. The TRR in goat milk and tissues are presented in Table 5. The LOD for TRR determinations was 0.001 ppm.

Samples of urine, feces, and cage washings were collected and analyzed for TRR. The data indicated that most of the administered radioactivity (91.4-93.2% dose) was either excreted in the urine (27.1- 33.8% dose) and feces (44.6-45.6% dose) or remained in the GI tract (14.9-18.8% dose) at sacrifice. An additional 0.20-0.22% of the dose was excreted in the milk, and 0.55-0.69% of the dose was recovered in the tissues, with liver accounting for 0.40-0.44% of the dose.

Table 5. TRRs in samples of milk and edible tissues from lactating goats following administration of [^{14}C]flumioxazin at a feeding level of 7.2 ppm for 5 consecutive days

Matrix	TRR, ppm [^{14}C]flumioxazin equivalents	
	Goat 1	Goat 2
Milk		
Day 1 am	0.0	0.0
Day 1 pm	0.0326	0.0489
Day 2 am	0.0045	0.0103
Day 2 pm	0.0409	0.0527
Day 3 am	00068	0.0093
Day 3 pm	0.0421	0.0456
Day 4 am	0.0069	0.0119
Day 4 pm	0.0455	0.0553
Day 5 am	0.0056	0.0111
Sacrifice	0.0431	0.0496
Liver	0.286	0.330
Kidney	0.189	0.238
Muscle, loin	0.022	0.025
Muscle, rear leg muscle	0.023	0.028
Fat, omental	0.006	0.010
Fat, perirenal	0.008	0.008

Extraction and hydrolysis of residues

Samples of milk and tissues, except fat, were subjected to extraction and/or hydrolysis procedures in order to characterize and identify residues. Residues in fat were not characterized as TRR were ≤0.01 ppm. The petitioner provided adequate descriptions of the fractionation procedures for each matrix. During the extraction and fractionation procedures, aliquots of extracts, hydrolysates, and nonextractable residues were analyzed for radioactivity by LSC or combustion/LSC. Fractions containing significant residues were concentrated and reserved for chromatographic analysis. Concentrations were conducted using rotary evaporation under vacuum. The general extraction and fractionation procedures are summarized below.

Milk: Milk samples were mixed with ethanol, stirred for 30 min, and filtered. The extract was concentrated and partitioned three times with hexane; the hexane fractions were combined. The ethanol extract was concentrated, additional ethanol was added, and the mixture was centrifuged; the resulting pellet was washed with ethanol:water (1:1, v:v) and centrifuged. All supernatants were combined and concentrated for TLC and HPLC analysis.

Tissues: Subsamples of tissues were extracted two times with ACN and filtered; the filtered solids were rinsed with ACN. The filtrates and rinses were separately combined. The remaining solids were extracted (two times) with ACN:water (1:1, v:v, the water contained 1% acetic acid) and centrifuged; the supernatants were combined. The solids were rinsed with ACN and the rinse was combined with the ACN:water extract.

The distribution of ^{14}C-activity in the extracts of goat milk and tissues is presented in Table 6.

Characterization and identification of residues

The milk and tissue extracts were analyzed by TLC and HPLC. HPLC analyses were conducted using a Spherisorb ODS, Selectosil ODS, or Zorbax ODS column and a gradient mobile phase of ACN and 1% acetic acid. Metabolites were identified by comparison of retention times and/or co-chromatography with the following reference standards: ^{14}C-3-OH-flumioxazin; ^{14}C-4-OH-flumioxazin; ^{14}C-3-OH-flumioxazin-SA; ^{14}C-4-OH-flumioxazin-SA; ^{14}C-3-OH-S-53482A-SA; 482-HA; 482-CA; IMOXA; SAT-482; SAT-482-HA-2; THPA; 3-OH-THPA-DME; and 4-OH-THPA-DME.
^{14}C-Reference standards were labeled in the phenyl ring. Non-labeled standards were detected by UV, and radioactivity was detected and quantitated using a radioactivity flow detector.
TLC analyses were conducted for confirmation of metabolite identities. Analyses were conducted using silica gel plates with one of the following solvent systems:

toluene:EtOAc:acetic acid (5:5:1, v:v:v); or EtOAc:isopropanol:acetic acid (8:2:1, v:v:v). Samples were co-chromatographed with reference standards. Radioactivity was quantitated using an imaging scanner. Non-labeled standards were detected by UV or in an iodine chamber. TLC analyses were used to confirm the identification of 4-OH-flumioxazin in all tissues and 3-OH-flumioxazin in liver and kidney.

The identification of 4-OH-THPA in tissues and milk was based on comparison of retention time with that metabolite in urine. 4-OH-THPA was isolated from urine, methylated (using dimethyl sulfate or diazomethane), and identified as the dimethyl ester based on MS analyses and comparison of chromatographic properties with the reference standard (4-OH-THPA-DME). The petitioner noted that 4-OH-THPA eluted in a broad area as multiple peaks. The major peak was confirmed to be 4-OH-THPA and the multiple peaks are assumed to be due to interconversion of the 4-OH-THPA diacid to the anhydride (TPA).

Metabolites B, C, and F were identified using MS. The metabolites were isolated from urine (coinjection of the isolated metabolites from tissues and urine confirmed coelution) and analyzed by LC/MS (either positive-ion or negative-ion mode). MS analysis indicated that Metabolites B and F are 3- or 4-OH-SAT-482 (hydroxylation position could not be determined for each). MS analysis indicated one of two possible structures for Metabolite C (see Attachment 1); authentic standards of either compound are not available to confirm the structure of Metabolite C. Metabolites D and E could not be identified, although chromatographic analyses indicate that they have similar polarity.

Nonextractable residues of liver were subjected to acid and base hydrolysis procedures, by suspending the nonextractable residues in 1 M HCl or 1 M NaOH at 36-38°C for 16 hours. Acid hydrolysis was found to release about 20% of the radioactivity. Base hydrolysis completely solubilized the residues, interfering with further analysis.

Liver and kidney nonextractable residues were subjected to protease digestion at 37°C for 16 hours (using K_2HPO_4 buffer). Following centrifugation, the solids were washed with water and ACN:water (1:1, v:v); the rinses were combined with the supernatant. The hydrolysate was adjusted to pH 3 (1 M HCl) and partitioned twice with EtOAc. Approximately 66% and 76% of the radioactivity was released during hydrolysis of the kidney and liver nonextractable residues, respectively, and approximately 30% was found to be organosoluble. TLC analysis of the organosoluble fraction did not resolve discrete peaks, and the radioactivity in the aqueous fraction remained at the origin, indicating that radioactivity released during hydrolysis was minor polar components.

The petitioner stated that the unknown metabolites in tissues and milk could not be identified because of low radioactivity levels. These unknowns were not present in

urine or feces, preventing isolation of the compounds from those matrices for identification purposes.

A summary of the characterized and identified ^{14}C-residues in goat matrices is presented in Table 7.

Table 6. Distribution of total radioactive residues in milk and tissues from a lactating goat dosed with [^{14}C]flumioxazin at a feeding level of 7.2 ppm in the diet for 5 consecutive days.

Fraction	% TRR	ppm	Characterization/Identification
Milk (Goat 2, Day 4 pooled; TRR = 0.0268 ppm)			
Ethanol	89.1	0.0226	Partitioned with hexane
Hexane	0.0	0.0	Not further analyzed (N/A).
Aqueous	94.1	0.0239	Concentrated, ethanol added, and centrifuged.
Ethanol	94.5	0.0240	HPLC analysis resolved: 4-OH-THPA 6.3% TRR 0.0016 ppm 4-OH-53482 9.1% TRR 0.0023 ppm Metabolite B 18.9% TRR 0.0048 ppm Metabolite C 13.0% TRR 0.0033 ppm Metabolite D 1.6% TRR 0.0004 ppm Plus an additional 8 unknowns (each ≤11.9% TRR, ≤0.003 ppm), totaling 41.3% TRR.
Solids	Not reported (N/R)	N/R	N/A.
Nonextractable	11.4	0.0029	N/A.
Liver (Goat 1; TRR = 0.286 ppm)			
ACN	55.7	0.159	HPLC analysis resolved: Flumioxazin 1.4% TRR 0.0039 ppm 3-OH-Flumioxazin 6.5% TRR 0.0186 ppm 4-OH-Flumioxazin 7.2% TRR 0.0205 ppm Metabolite D 4.7% TRR 0.0133 ppm Metabolite E 1.4% TRR 0.0041 ppm Metabolite F 10.2% TRR 0.0292 ppm Plus an additional 4 unknowns (each ≤7.0% TRR, ≤0.020 ppm), totaling 19.5% TRR.

Table 6. Distribution of total radioactive residues in milk and tissues from a lactating goat dosed with [^{14}C]flumioxazin at a feeding level of 7.2 ppm in the diet for 5 consecutive days.

Fraction	% TRR	ppm	Characterization/Identification
ACN:water	31.1	0.089	HPLC analysis resolved: 4-OH-THPA 0.9% TRR 0.0025 ppm SAT-482 4.4% TRR 0.0126 ppm 3-OH-Flumioxazin 1.6% TRR 0.0045 ppm 4-OH-Flumioxazin 1.7% TRR 0.0049 ppm THPA 3.1% TRR 0.0087 ppm Metabolite B 1.5% TRR 0.0043 ppm Metabolite F 0.6% TRR 0.0016 ppm Plus an additional 6 unknowns (each ≤5.2% TRR, ≤0.015 ppm), totaling 16.6% TRR.
Nonextractable	7.6	0.022	Subjected to acid and base hydrolysis (1 M HCl or 1 M NaOH, 36-38 C, 16 hours). Acid hydrolysis released about 20% of the radioactivity. Base hydrolysis completely solubilized the residues, interfering with further analysis. Separately subjected to protease digestion (37 °C, 16 hours). Hydrolysate adjusted to pH 3 and partitioned with EtOAc. Approximately 76% of the radioactivity was released, and approximately 30% was found to be organosoluble. TLC analysis of the organosoluble fraction did not resolve discrete peaks, and the radioactivity in the aqueous fraction remained at the origin.
Kidney (Goat 1; TRR = 0.189 ppm)			
ACN	49.9	0.095	HPLC analysis resolved: 3-OH-Flumioxazin 2.9% TRR 0.0055 ppm 4-OH-Flumioxazin 5.2% TRR 0.0098 ppm Metabolite B 6.7% TRR 0.0127 ppm Metabolite C 7.9% TRR 0.0149 ppm Plus an additional 11 unknowns (each ≤7.8% TRR, ≤0.015 ppm), totaling 25.1% TRR.
ACN:water	29.7	0.056	HPLC analysis resolved: 4-OH-THPA 3.5% TRR 0.0066 ppm SAT-482 5.0% TRR 0.0095 ppm 3-OH-Flumioxazin 1.2% TRR 0.0022 ppm 4-OH-Flumioxazin 2.1% TRR 0.0039 ppm THPA 1.1% TRR 0.0020 ppm Metabolite B 6.0% TRR 0.0114 ppm Metabolite D 3.4% TRR 0.0064 ppm Metabolite E 0.9% TRR 0.0016 ppm Plus an additional 4 unknowns (each ≤1.6% TRR, ≤0.003 ppm), totaling 4.8% TRR.

Table 6. Distribution of total radioactive residues in milk and tissues from a lactating goat dosed with [^{14}C]flumioxazin at a feeding level of 7.2 ppm in the diet for 5 consecutive days.

Fraction	% TRR	ppm	Characterization/Identification
Nonextractable	11.7	0.022	Separately subjected to protease digestion (37°C, 16 hours). Hydrolysate adjusted to pH 3 and partitioned with EtOAc. Approximately 66% of the radioactivity was released, and approximately 30% was found to be organosoluble. TLC analysis of the organosoluble fraction did not resolve discrete peaks, and the radioactivity in the aqueous fraction remained at the origin.
Muscle, rear leg (Goat 1; TRR = 0.023 ppm)			
ACN	45.5	0.011	HPLC analysis resolved: Flumioxazin 1.7% TRR 0.0004 ppm 4-OH-Flumioxazin 9.4% TRR 0.0022 ppm Metabolite C 12.8% TRR 0.0030 ppm Plus an additional 2 unknowns (each ≤12.8% TRR, ≤0.003 ppm), totaling 18.0% TRR.
ACN:water	27.0	0.006	HPLC analysis resolved: 4-OH-THPA 6.4% TRR 0.0015 ppm 4-OH-Flumioxazin 3.0% TRR 0.0007 ppm Metabolite C 5.6% TRR 0.0013 ppm Plus an additional 3 unknowns (each ≤5.6% TRR, ≤0.0013 ppm), totaling 10.7% TRR.
Nonextractable	21.4	0.005	N/A.
Muscle, loin (Goat 1; TRR = 0.022 ppm)			
ACN	57.0	0.012	HPLC analysis resolved: Flumioxazin 1.4% TRR 0.0003 ppm 4-OH-Flumioxazin 9.5% TRR 0.0021 ppm Metabolite C 18.1% TRR 0.0040 ppm Plus an additional 2 unknowns (each ≤14.9% TRR, ≤0.0033 ppm), totaling 18.6% TRR.
ACN:water	20.3	0.004	HPLC analysis resolved: 4-OH-THPA 6.3% TRR 0.0014 ppm 4-OH-Flumioxazin 1.4% TRR 0.0003 ppm Metabolite C 3.2% TRR 0.0007 ppm Plus an additional 3 unknowns (each ≤2.7% TRR, ≤0.0006 ppm), totaling 7.2% TRR.
Nonextractable	19.5	0.004	N/A.

Storage stability

The petitioner verified the stability of the parent compound in milk and tissues by fortifying milk, liver, kidney, and muscle samples from a control goat with [^{14}C]flumioxazin. At least 90% of the fortified residues were found to be unchanged parent after 2-3 months of frozen storage. In addition, the petitioner compared the extraction profile of milk residues following initial analysis and repeat analysis (extraction of a stored milk sample) 5 months later; the HPLC chromatograms indicated that the metabolite profile was stable during 5 months of storage. Similar comparisons were made for kidney and liver, indicating that the metabolite profile in these tissues was stable during 5 months of storage.

The petitioner provided the dates of sample extraction and analysis to allow the Agency to determine whether the submitted storage stability data are sufficient to support the metabolism study (electronic transmission to D. Kenny, 1/9/2001). These dates are provided in Table 8.

All analyses were completed in less than 6 months from the sampling dates. The data indicate that significant changes in the metabolic profiles did not occur during the study's analytical phase.

Table 7. Summary of radioactive residues characterized/identified in milk and tissues of a lactating goat orally dosed with [^{14}C]flumioxazin at a feeding level of 7.2 ppm for 5 consecutive days.										
	Milk, Day 4 (TRR = 0.0268)		**Liver (TRR = 0.286 ppm)**		**Kidney (TRR = 0.189 ppm)**		**Muscle, rear leg (TRR = 0.023 ppm)**		**Muscle, loin (TRR = 0.022 ppm)**	
Fraction	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]										
Flumioxazin	–	–	1.4	0.0039	–	–	1.7	0.0004	1.4	0.0003
3-OH-Flumioxazin	–	–	8.1	0.0231	4.1	0.0077	–	–	–	–
4-OH-Flumioxazin	9.1	0.0023	8.9	0.0254	7.2	0.0137	12.4	0.0029	10.9	0.0024
4-OH-THPA	6.3	0.0016	0.9	0.0025	3.5	0.066	6.4	0.0015	6.3	0.0014
SAT-482	–	–	4.4	0.0126	5.0	0.0095	–	–	–	–
THPA	–	–	3.1	0.0087	1.1	0.0020	–	–	–	–
Metabolite B	18.9	0.0048	1.5	0.0043	12.7	0.0241	–	–	–	–
Metabolite C	13.0	0.0033	–	–	7.9	0.0149	18.4	0.0043	21.7	0.0048
Metabolite F	–	–	10.8	0.0308	–	–	–	–	–	–
Total identified	**47.2**	**0.012**	**39.0**	**0.1113**	**41.5**	**0.0785**	**38.9**	**0.0091**	**40.3**	**0.0089**
Characterized										
Metabolite D	1.6	0.0004	4.7	0.0133	3.4	0.0064	–	–	–	–
Metabolite E	–	–	1.4	0.0041	0.9	0.0016	–	–	–	–
Unknowns [b]	41.3 (11.8)	0.0105 (0.003)	36.2 (8.0)	0.1032 (0.0229)	29.9 (7.8)	0.0565 (0.0147)	28.6 (18.4)	0.0067 (0.0043)	25.8 (17.7)	0.0057 (0.0039)
Total characterized/identified	**90.2**	**0.0230**	**81.2**	**0.2319**	**75.6**	**0.1430**	**67.5**	**0.0158**	**66.1**	**0.0148**
Nonextractable	11.4	0.0029	7.6 [c]	0.0217	11.7 [c]	0.0221	21.7	0.0050	19.5	0.0043

[a] See Attachment 1 for chemical structure of identified metabolites.
[b] Maximum value for individual unknown in parentheses.
[c] Hydrolysis procedures were conducted on this fraction; however, chromatographic analyses were unsuccessful.

Table 8. Metabolism of [^{14}C(3,4,5,6-Tetrahydro)Phthalimide]S-53482 in Lactating Goats					
Sample	Sampling Date	Date of Initial Extraction	Date of Initial HPLC	Date of Final Extraction	Date of Final HPLC
Milk (Day 2)	10-8-93	10-14-93	11-18-93	3-21-94	3-28-94
Milk (Day 4)	10-10-93	10-19-93	11-20-93	NA	NA
Milk (Day 2)	11-5-93	11-24-93	12-3-93	NA	NA
Kidney	11-8-93	12-13-93	12/22-28/93	3-23-94	3-25-94
Liver	11-8-93	12-14-93	12/20-28/93	3-22-94	3-24-94
Loin Muscle	10-11-93	11-22-93	12/6-7/93	NA	NA
Leg Muscle	10-11-93	11-17-93	12/3-6/93	NA	NA

NA: Not Analyzed

Proposed metabolic pathway in ruminants

Based on the results of the goat metabolism study, the petitioner proposes that flumioxazin is metabolized by hydroxylation of the cyclohexene ring in the THP moiety, cleavage of the imide linkage, cleavage of the amide linkage, reduction of the C-C double bond in the THP moiety, and incorporation of sulfonic acid in the THP moiety (based on the results for urine, and the results of the previously submitted metabolism study).

Conclusions:

The submitted goat metabolism study is acceptable. Following oral administration of [THP-^{14}C]flumioxazin to lactating goats for 5 consecutive days at a feeding level of 7.2 ppm (2060x the maximum theoretical dietary burden for beef and dairy cattle), the TRR (expressed as flumioxazin equivalents) were 0.0045-0.0553 ppm in milk, 0.286-0.330 ppm in liver, 0.189-0.238 ppm in kidney, 0.022-0.028 ppm in muscle, and 0.006-0.010 ppm in fat. Residues in milk appeared to plateau within one day of the first dose.

Approximately 66-90% of the TRR were characterized/identified in milk and tissues; residues in fat were not characterized because TRR were ≤0.01 ppm. The parent, flumioxazin, was identified at low levels only in liver and muscle (1.4-1.7% TRR, 0.0003-0.0039 ppm). The major metabolites identified in milk and tissues included: 3-OH-flumioxazin (4.1-8.1% TRR, 0.008-0.023 ppm), 4-OH-flumioxazin (7.2-12.4% TRR, 0.002-0.025 ppm), Metabolite B (1.5-18.9% TRR, 0.004-0.024 ppm), Metabolite

C (7.9-21.7% TRR, 0.003-0.015 ppm), and Metabolite F (10.8% TRR, 0.031, only in liver). All other identified metabolites were present at <7% TRR. Several unknown metabolites were resolved by HPLC chromatography; each of these were present at <0.023 ppm.

Poultry

Valent submitted a study pertaining to the metabolism of [^{14}C]flumioxazin in laying hens (MRID No. 44295050). The in-life and analytical phases of the study were conducted at Ricerca, Inc. (Painesville, OH).

The test substance, [tetrahydrophthaloyl-1,2-^{14}C]flumioxazin (specific activity 98.6 mCi/mmol and 106 mCi/mmol; radiochemical purity 98.7%), was mixed with nonlabeled flumioxazin and ACN to a final specific activity of 160,774 dpm/µg and placed into gelatin capsules. The capsules were administered orally once per day for 14 consecutive days to 10 hens. The dosing level was 10 ppm, based on the food consumption during the acclimation period (12 days prior to initiation of dosing). However, because feed consumption during the dosing period increased, the actual dosing level was 9.01 ppm (2000x the MTDB for poultry; Table 20). Four additional hens received placebo doses to serve as controls.

During the study, the animals were fed commercial hen ration; the ration and water were provided *ad libitum*. The petitioner provided sufficient descriptions of preparation of dose capsules and animal husbandry practices, as well as data concerning daily feed intake, body weights, and egg production.

Eggs were collected twice daily. Eggs collected on the same day were pooled and then separated into yolks and whites. The hens were sacrificed ~4 hours after the final dose, and the following samples were collected: kidney, heart, liver, muscle (breast and thigh), abdominal fat, skin with fat, gizzard, reproductive organs, and GI tract and contents. All egg and tissue samples were stored frozen (temperature unspecified) until analysis.

Total radioactive residues (TRR)

Subsamples of kidney, liver, muscle, fat, and skin with fat were homogenized in dry ice, and then subjected to combustion/LSC. Egg yolk and white samples were blended and then subjected to combustion/LSC. The TRR in hen matrices are presented in Table 9. The LOD for TRR determinations was 0.001 ppm.

Samples of excreta and cage washings were collected and were analyzed for TRR. The data indicated that most of the administered radioactivity was recovered in the excreta (78.3% of dose, including cage wash) and in the GI tract (5.8% of dose, including

gizzard). Over the entire dosing period, eggs accounted for 0.43% of the dose, and radioactivity in tissues accounted for 3.53% of the dose at sacrifice.

Table 9. Total radioactive residues in eggs and edible tissues from laying hens following administration of [THP-^{14}C]flumioxazin at a feeding level equivalent to 9.01 ppm for 14 consecutive days

Matrix	TRR, ppm [^{14}C]flumioxazin equivalents	
Eggs:	Egg white	Egg yolk
Day 1	0.029	0.009
Day 2	0.033	0.034
Day 3	0.025	0.119
Day 4	0.041	0.154
Day 5	0.037	0.240
Day 6	0.030	0.338
Day 7	0.036	0.414
Day 8	0.034	0.467
Day 9	0.030	0.531
Day 10	0.036	0.570
Day 11	0.027	0.638
Day 12	0.025	0.640
Day 13	0.024	0.630
Day 14 [a]	0.032	0.760
Kidney	0.887	
Liver	1.137	
Muscle, breast	0.138	
Muscle, thigh	0.175	
Fat, abdominal	0.226	
Skin with fat	0.667	

[a] Eggs collected after the final dose and before termination.

Extraction and hydrolysis of residues

Samples of poultry matrices were subjected to extraction and/or hydrolysis procedures in order to characterize and identify residues. The petitioner provided adequate descriptions of the fractionation procedures for each matrix. During the extraction and fractionation procedures, aliquots of extracts, hydrolysates, and nonextractable residues were analyzed for radioactivity by LSC or combustion/LSC. Fractions containing

significant residues were concentrated and reserved for chromatographic analysis. Concentrations were conducted using rotary evaporation under vacuum. The general extraction and fractionation procedures are summarized below.

Subsamples of tissues, egg yolks, and egg whites were extracted twice with ACN and filtered; the filtered solids were rinsed with ACN. The filtrates and rinses were separately combined. The remaining solids were extracted twice with ACN:water (1:1, v:v, the water contained 1% acetic acid) and centrifuged; the supernatants were combined.

Nonextractable residues of liver were subjected to acid and base hydrolysis procedures, by suspending the nonextractable residues in 1 M HCl or 1 M NaOH at 36-38°C for 16 hours. Acid hydrolysis was found to release about 44% of the radioactivity. Base hydrolysis completely solubilized the residues, interfering with further analysis.

Nonextractable residues of liver were then subjected to protease digestion at 37°C for 16 hours (using K_2HPO_4 buffer). Following centrifugation, the solids were washed with water and the rinses were combined with the supernatant. This was found to release approximately 60% of the radioactivity. Because the protease digestion was more successful than acid or base hydrolysis, the nonextractable residues of egg yolk, kidney, muscle (thigh and breast), and skin with fat were subjected to protease digestion only, using the method described for liver. The supernatants were reserved for HPLC analysis.

The distribution of ^{14}C-activity in the extracts of poultry matrices is presented in Table 10.

Characterization and identification of residues

The extracts were analyzed by TLC and HPLC. HPLC analyses were conducted using a Spherisorb ODS or Selectosil ODS column and a gradient mobile phase of ACN and 1% acetic acid. Metabolites were identified by comparison of retention times and/or co-chromatography with reference standards; the reference standards used were the same as those for the goat metabolism study. Non-labeled standards were detected by UV, and radioactivity was detected and quantitated using a radioactivity flow detector.

TLC analyses were conducted for confirmation of metabolite identities; analyses were conducted in the same manner as for the goat metabolism study. TLC analyses were used to confirm the identification of 3-OH-flumioxazin and 4-OH-flumioxazin in egg yolk, liver, kidney, muscle, and skin with fat, and 3-OH-S-53482-SA and 4-OH-S-53482-SA in egg yolk.

The identification of 3-OH-THPA and 4-OH-THPA in egg white and liver was based on identification of the methylated derivative; the metabolites were isolated, methylated (using dimethyl sulfate or diazomethane), and identified as the dimethyl ester based on HPLC chromatography using methylated reference standards (3-OH-THPA-DME and 4-OH-THPA-DME). THPA identification in egg white was conducted in the same manner. The identification of these metabolites in all other matrices was based on comparison of HPLC retention times.

TPA was identified in egg white as THPA, following isolation and purification; these metabolites are known to interconvert. The petitioner also noted that based on the HPLC elution profile, metabolites 3-OH-THPA and 4-OH-THPA, which were identified after isolation and reanalysis, may have resulted in egg white from hydrolysis of a hydroxy metabolite of flumioxazin (the elution profile changed after isolation).

The metabolite OH-flumioxazin was identified in eggs and tissues based on comparison of retention time with the same metabolite in excreta. This metabolite was isolated from excreta and subjected to MS analysis. The analysis indicated that the compound is a hydroxy metabolite of flumioxazin; however, the position of hydroxylation could not be determined.

The petitioner stated that the unknown metabolites in tissues and eggs could not be identified because of low radioactivity levels.

A summary of the characterized and identified ^{14}C-residues in hen matrices is presented in Table 11.

Table 10. Distribution of total radioactive residues in eggs and tissues from laying hens dosed with [^{14}C]flumioxazin at a feeding level of 9.01 ppm in the diet for 14 consecutive days

Fraction	% TRR	ppm	Characterization/Identification [a]
Egg yolk (Day 7; TRR = 0.414 ppm)			
ACN	32.7	0.136	HPLC analysis resolved: 4-OH-THPA 0.4% TRR 0.002 ppm THPA 1.8% TRR 0.007 ppm 3-OH-S-53482-SA 1.1% TRR 0.005 ppm 4-OH-S-53482-SA 1.5% TRR 0.006 ppm 4-OH-S-53482 5.7% TRR 0.024 ppm 3-OH-S-53482 3.8% TRR 0.016 ppm OH-Flumioxazin [b] 2.5% TRR 0.010 ppm Flumioxazin 7.7% TRR 0.032 ppm Plus additional unknowns totaling 7.3% TRR (each <5% TRR).

Table 10. Distribution of total radioactive residues in eggs and tissues from laying hens dosed with [^{14}C]flumioxazin at a feeding level of 9.01 ppm in the diet for 14 consecutive days

Fraction	% TRR	ppm	Characterization/Identification [a]
ACN:water	63.6	0.264	HPLC analysis resolved: 4-OH-THPA 7.8% TRR 0.032 ppm THPA 5.4% TRR 0.022 ppm 4-OH-S-53482-SA 32.2% TRR 0.133 ppm OH-Flumioxazin [b] 2.7% TRR 0.011 ppm Flumioxazin 1.7% TRR 0.007 ppm Plus additional unknowns totaling 13.2% TRR (each <5% TRR).
Nonextractable	9.4	0.039	Not further analyzed (N/A).
Egg yolk (Day 13; TRR = 0.630 ppm)			
ACN	29.2	0.184	HPLC analysis resolved: 4-OH-THPA 0.3% TRR 0.002 ppm THPA 2.2% TRR 0.014 ppm 3-OH-S-53482-SA 1.1% TRR 0.007 ppm 4-OH-S-53482-SA 1.5% TRR 0.010 ppm 4-OH-S-53482 5.9% TRR 0.037 ppm 3-OH-S-53482 3.9% TRR 0.025 ppm OH-Flumioxazin [b] 1.8% TRR 0.011 ppm Flumioxazin 5.0% TRR 0.031 ppm Plus additional unknowns totaling 6.8% TRR (each <5% TRR).
ACN:water	81.8	0.515	HPLC analysis resolved: 4-OH-THPA 3.2% TRR 0.020 ppm THPA 6.9% TRR 0.043 ppm 4-OH-S-53482-SA 46.0% TRR 0.289 ppm OH-Flumioxazin [b] 3.0% TRR 0.019 ppm Flumioxazin 2.6% TRR 0.017 ppm Plus additional unknowns totaling 19.4% TRR (each <5% TRR).
Nonextractable	15.9	0.100	Subjected to protease digestion (36-38 C for 16 hours).
Hydrolysate	11.2	0.070	HPLC analysis resolved: 4-OH-THPA 3.2% TRR 0.020 ppm THPA 1.6% TRR 0.010 ppm 3-/4-OH-Flumioxazin [c] 1.7% TRR 0.011 ppm OH-Flumioxazin [b] 0.4% TRR 0.003 ppm Flumioxazin 0.9% TRR 0.005 ppm Plus additional unknowns totaling 2.7% TRR.
Nonextractable	Not reported (N/R)	N/R	N/A.
Egg white (Day 7; TRR = 0.036 ppm)			

Table 10. Distribution of total radioactive residues in eggs and tissues from laying hens dosed with [^{14}C]flumioxazin at a feeding level of 9.01 ppm in the diet for 14 consecutive days

Fraction	% TRR	ppm	Characterization/Identification [a]
ACN	86.5	0.031	HPLC analysis resolved: THPA 19.0% TRR 0.007 ppm TPA 16.1% TRR 0.006 ppm 4-OH-THPA 23.1% TRR 0.008 ppm 3-OH-THPA 17.0% TRR 0.006 ppm OH-Flumioxazin [b] 0.2% TRR <0.001 ppm Flumioxazin 0.4% TRR <0.001 ppm Plus additional unknowns totaling 10.8% TRR (each <1% TRR).
ACN:water	11.8	0.004	HPLC analysis resolved: THPA 4.8% TRR 0.002 ppm TPA 1.0% TRR <0.001 ppm 4-OH-THPA 4.0% TRR 0.001 ppm 3-OH-THPA 0.5% TRR <0.001 ppm OH-Flumioxazin [b] 0.3% TRR <0.001 ppm Flumioxazin 0.3% TRR <0.001 ppm Plus additional unknowns totaling 0.9% TRR.
Nonextractable	6.5	0.002	N/A.
Egg white (Day 13; TRR = 0.024 ppm)			
ACN	69.5	0.024	HPLC analysis resolved: THPA 12.6% TRR 0.003 ppm TPA 11.8% TRR 0.003 ppm 4-OH-THPA 19.8% TRR 0.005 ppm 3-OH-THPA 13.9% TRR 0.003 ppm OH-Flumioxazin [b] 0.6% TRR <0.001 ppm Flumioxazin 0.8% TRR <0.001 ppm Plus additional unknowns totaling 9.5% TRR (each <1% TRR).
ACN:water	11.6	0.003	HPLC analysis resolved: THPA 2.8% TRR 0.001 ppm TPA 1.8% TRR <0.001 ppm 4-OH-THPA 4.0% TRR 0.001 ppm 3-OH-THPA 0.7% TRR <0.001 ppm OH-Flumioxazin [c] 0.7% TRR <0.001 ppm Flumioxazin 0.7% TRR <0.001 ppm Plus additional unknowns totaling 0.8% TRR.
Nonextractable	6.5	0.003	N/A.
Liver (TRR = 1.137 ppm)			

Table 10. Distribution of total radioactive residues in eggs and tissues from laying hens dosed with [^{14}C]flumioxazin at a feeding level of 9.01 ppm in the diet for 14 consecutive days

Fraction	% TRR	ppm	Characterization/Identification [a]
ACN	48.4	0.551	HPLC analysis resolved: 4-OH-THPA 1.7% TRR 0.019 ppm THPA 4.2% TRR 0.047 ppm 3-/4-OH-S-53482-SA [d] 4.6% TRR 0.052 ppm 4-OH-Flumioxazin 9.3% TRR 0.106 ppm 3-OH-Flumioxazin 5.7% TRR 0.065 ppm OH-Flumioxazin [b] 2.2% TRR 0.025 ppm Flumioxazin 6.3% TRR 0.072 ppm Plus additional unknowns totaling 14.0% TRR (each <5% TRR).
ACN:water	28.6	0.325	HPLC analysis resolved: 4-OH-THPA 2.7% TRR 0.031 ppm THPA 5.5% TRR 0.062 ppm 3-/4-OH-S-53482-SA [d] 1.6% TRR 0.015 ppm 4-OH-Flumioxazin 1.6% TRR 0.015 ppm 3-OH-Flumioxazin 1.2% TRR 0.014 ppm OH-Flumioxazin [b] 0.5% TRR 0.005 ppm Flumioxazin 0.4% TRR 0.004 ppm Plus additional unknowns totaling 15.6% TRR (each <5% TRR).
Nonextractable	22.0	0.250	Subjected to protease digestion (36-38 C for 16 hours).
Hydrolysate	18.2	0.207	HPLC analysis resolved: 4-OH-THPA 3.1% TRR 0.035 ppm THPA 4.7% TRR 0.053 ppm 3-/4-OH-Flumioxazin [c] 2.2% TRR 0.025 ppm OH-Flumioxazin [b] 0.5% TRR 0.005 ppm Flumioxazin 0.3% TRR 0.003 ppm Plus additional unknowns totaling 7.4% TRR.
Nonextractable	N/R	N/R	N/A.
Kidney (TRR = 0.887 ppm)			
ACN	50.9	0.451	HPLC analysis resolved: 4-OH-THPA 6.0% TRR 0.053 ppm THPA 5.8% TRR 0.052 ppm 3-/4-OH-S-53482-SA [d] 2.1% TRR 0.018 ppm 4-OH-Flumioxazin 7.4% TRR 0.066 ppm 3-OH-Flumioxazin 6.5% TRR 0.058 ppm OH-Flumioxazin [b] 2.0% TRR 0.018 ppm Flumioxazin 6.4% TRR 0.057 ppm Plus additional unknowns totaling 13.8% TRR (each <5% TRR).

Table 10. Distribution of total radioactive residues in eggs and tissues from laying hens dosed with [^{14}C]flumioxazin at a feeding level of 9.01 ppm in the diet for 14 consecutive days

Fraction	% TRR	ppm	Characterization/Identification [a]
ACN:water	21.5	0.190	HPLC analysis resolved: 4-OH-THPA 4.0% TRR 0.035 ppm THPA 3.6% TRR 0.032 ppm 3-/4-OH-S-53482-SA [d] 2.1% TRR 0.019 ppm 4-OH-Flumioxazin 1.0% TRR 0.009 ppm 3-OH-Flumioxazin 0.3% TRR 0.003 ppm OH-Flumioxazin [b] 1.0% TRR 0.009 ppm Flumioxazin 0.6% TRR 0.005 ppm Plus additional unknowns totaling 8.8% TRR (each <5% TRR).
Nonextractable	24.4	0.216	Subjected to protease digestion (36-38 C for 16 hours).
Hydrolysate	17.9	0.158	HPLC analysis resolved: 4-OH-THPA 2.9% TRR 0.025 ppm THPA 3.8% TRR 0.034 ppm 3-/4-OH-Flumioxazin [c] 2.2% TRR 0.019 ppm OH-Flumioxazin [b] 0.5% TRR 0.004 ppm Flumioxazin 0.4% TRR 0.003 ppm Plus additional unknowns totaling 8.0% TRR.
Nonextractable	N/R	N/R	N/A.
Muscle, breast (TRR = 0.138 ppm)			
ACN	62.1	0.086	HPLC analysis resolved: 4-OH-THPA 4.8% TRR 0.007 ppm THPA 7.5% TRR 0.010 ppm 4-OH-Flumioxazin 12.0% TRR 0.017 ppm 3-OH-Flumioxazin 11.4% TRR 0.016 ppm OH-Flumioxazin [b] 3.2% TRR 0.004 ppm Flumioxazin 10.6% TRR 0.015 ppm Plus unknowns totaling 11.7% TRR (each <5% TRR).
ACN:water	4.1	0.006	HPLC analysis resolved: 4-OH-THPA 1.5% TRR 0.002 ppm THPA 0.8% TRR 0.001 ppm 4-OH-Flumioxazin 0.2% TRR <0.001 ppm 3-OH-Flumioxazin 0.2% TRR <0.001 ppm OH-Flumioxazin [b] 0.2% TRR <0.001 ppm Flumioxazin <0.1% TRR <0.001 ppm Plus unknowns totaling 1.3% TRR.
Nonextractable	32.6	0.045	Subjected to protease digestion (36-38 C for 16 hours).

Table 10. Distribution of total radioactive residues in eggs and tissues from laying hens dosed with [^{14}C]flumioxazin at a feeding level of 9.01 ppm in the diet for 14 consecutive days

Fraction	% TRR	ppm	Characterization/Identification [a]
Hydrolysate	26.9	0.037	HPLC analysis resolved: 4-OH-THPA 2.4% TRR 0.003 ppm THPA 4.6% TRR 0.006 ppm 3-/4-OH-Flumioxazin [c] 2.6% TRR 0.004 ppm OH-Flumioxazin [b] 0.7% TRR 0.001 ppm Flumioxazin 0.6% TRR 0.001 ppm Plus additional unknowns totaling 15.3% TRR.
Nonextractable	N/R	N/R	N/A.
Muscle, thigh (TRR = 0.175 ppm)			
ACN	57.7	0.101	HPLC analysis resolved: 4-OH-THPA 4.6% TRR 0.005 ppm THPA 7.0% TRR 0.012 ppm 4-OH-Flumioxazin 9.9% TRR 0.017 ppm 3-OH-Flumioxazin 7.8% TRR 0.014 ppm OH-Flumioxazin [b] 3.6% TRR 0.006 ppm Flumioxazin 11.3% TRR 0.020 ppm Plus unknowns totaling 12.5% TRR (each <5% TRR).
ACN:water	9.3	0.016	HPLC analysis resolved: 4-OH-THPA 2.7% TRR 0.005 ppm THPA 2.7% TRR 0.005 ppm 4-OH-Flumioxazin 0.6% TRR 0.001 ppm 3-OH-Flumioxazin 0.2% TRR <0.001 ppm OH-Flumioxazin [b] 0.3% TRR 0.001 ppm Flumioxazin 0.1% TRR <0.001 ppm Plus unknowns totaling 2.6% TRR.
Nonextractable	36.6	0.064	Subjected to protease digestion (36-38 C for 16 hours).
Hydrolysate	32.7	0.057	HPLC analysis resolved: 4-OH-THPA 2.8% TRR 0.005 ppm THPA 6.2% TRR 0.011 ppm 3-/4-OH-Flumioxazin [c] 4.2% TRR 0.007 ppm OH-Flumioxazin [b] 1.2% TRR 0.002 ppm Flumioxazin 0.9% TRR 0.002 ppm Plus additional unknowns totaling 17.1% TRR.

Table 10. Distribution of total radioactive residues in eggs and tissues from laying hens dosed with [^{14}C]flumioxazin at a feeding level of 9.01 ppm in the diet for 14 consecutive days

Fraction	% TRR	ppm	Characterization/Identification [a]
Nonextractable	N/R	N/R	N/A.
Fat, abdominal (TRR = 0.226 ppm)			
ACN	79.3	0.179	HPLC analysis resolved: 4-OH-THPA 0.3% TRR 0.001 ppm THPA 1.2% TRR 0.003 ppm 4-OH-Flumioxazin 8.3% TRR 0.019 ppm 3-OH-Flumioxazin 7.5% TRR 0.017 ppm OH-Flumioxazin [c] 3.7% TRR 0.008 ppm Flumioxazin 53.8% TRR 0.121 ppm Plus unknowns totaling 4.0% TRR.
ACN:water	25.3	0.057	HPLC analysis resolved: 4-OH-THPA 3.0% TRR 0.007 ppm THPA 1.4% TRR 0.003 ppm 4-OH-Flumioxazin 4.9% TRR 0.011 ppm 3-OH-Flumioxazin 3.5% TRR 0.008 ppm OH-Flumioxazin [c] 2.0% TRR 0.005 ppm Flumioxazin 2.4% TRR 0.005 ppm Plus unknowns totaling 7.5% TRR (each <5% TRR).
Nonextractable	10.3	0.023	N/A.
Skin with fat (TRR = 0.667 ppm)			
ACN	34.3	0.229	HPLC analysis resolved: 4-OH-THPA 0.6% TRR 0.004 ppm THPA 2.5% TRR 0.017 ppm 4-OH-Flumioxazin 7.3% TRR 0.049 ppm 3-OH-Flumioxazin 4.5% TRR 0.030 ppm OH-Flumioxazin [b] 2.3% TRR 0.015 ppm Flumioxazin 9.9% TRR 0.066 ppm Plus unknowns totaling 6.7% TRR (each <5% TRR).
ACN:water	16.7	0.111	HPLC analysis resolved: 4-OH-THPA 2.5% TRR 0.016 ppm THPA 1.7% TRR 0.011 ppm 4-OH-Flumioxazin 2.9% TRR 0.019 ppm 3-OH-Flumioxazin 1.5% TRR 0.010 ppm OH-Flumioxazin [b] 0.8% TRR 0.005 ppm Flumioxazin 0.9% TRR 0.006 ppm Plus unknowns totaling 6.2% TRR (each <5% TRR).
Nonextractable	42.6	0.284	Subjected to protease digestion (36-38 C for 16 hours).

Table 10. Distribution of total radioactive residues in eggs and tissues from laying hens dosed with {^{14}C]flumioxazin at a feeding level of 9.01 ppm in the diet for 14 consecutive days

Fraction	% TRR	ppm	Characterization/Identification [a]		
Hydrolysate	28.2	0.188	HPLC analysis resolved:		
			4-OH-THPA	1.2% TRR	0.008 ppm
			THPA	6.7% TRR	0.045 ppm
			3-/4-OH-Flumioxazin [c]		
				3.4% TRR	0.023 ppm
			OH-Flumioxazin [b]	0.8% TRR	0.006 ppm
			Flumioxazin	0.7% TRR	0.005 ppm
			Plus additional unknowns totaling 14.9% TRR.		
Nonextractable	N/R	N/R	N/A.		

[a] The %TRR values for characterized/identified metabolites were calculated by the study reviewer. The petitioner had normalized results to 100% recovery (recovery = total extractable + unextractable; actual recoveries were 87.5-126.9%). Results were "un-normalized" by the study reviewer.

[b] The position of hydroxylation was not known.

[c] Mixture of 3-OH-flumioxazin and 4-OH-flumioxazin.

[d] Mixture of 3-OH-S-53482-SA and 4-OH-S-53482-SA.

Table 11. Summary of radioactive residues characterized/identified in eggs and tissues from laying hens dosed with [THP-^{14}C]flumioxazin at a feeding level equivalent to 9.01 ppm in the diet for 14 consecutive days

	Egg yolk, Day 7 (TRR = 0.414 ppm)		Egg yolk, Day 13 (TRR = 0.630 ppm)		Egg white, Day 7 (TRR = 0.036 ppm)		Egg white, Day 13 (TRR = 0.024 ppm)		Liver (TRR = 1.137 ppm)	
Fraction	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]										
Flumioxazin	9.4	0.039	8.5	0.053	0.7	<0.001	1.5	<0.001	6.9	0.079
3-OH-Flumioxazin	3.8	0.016	3.9	0.025	–	–	–	–	6.9	0.079
4-OH-Flumioxazin	5.7	0.024	5.9	0.037	–	–	–	–	10.7	0.121
3-OH/4-OH-Flumioxazin [b]	–	–	1.7	0.011	–	–	–	–	2.2	0.025
THPA	7.2	0.030	10.6	0.067	23.7	0.009	15.4	0.004	14.3	0.163
TPA	–	–	–	–	17.1	0.006	13.7	0.003	–	–
3-OH-THPA	–	–	–	–	17.5	0.006	14.6	0.004	–	–
4-OH-THPA	8.2	0.034	6.6	0.041	27.1	0.010	23.9	0.006	7.5	0.085
3-OH-S-53482-SA	1.1	0.005	1.1	0.007	–	–	–	–	6.0	0.068
4-OH-S-53482-SA	33.6	0.139	47.5	0.299	–	–	–	–		
OH-Flumioxazin [c]	5.2	0.022	5.3	0.033	0.5	<0.001	1.3	<0.001	3.1	0.035
Total identified	**74.2**	**0.307**	**91.0**	**0.573**	**86.6**	**0.031**	**70.4**	**0.017**	**57.5**	**0.654**
Characterized										
Unknowns (ACN, ACN:water) [d]	20.5	0.085	26.2	0.165	11.6	0.004	10.3	0.003	29.5	0.335
Unknowns (protease hydrolysate)	–	–	2.70	0.017	–	–	–	–	7.4	0.084
Total characterized/identified	**94.7**	**0.393**	**119.9**	**0.755**	**98.2**	**0.036**	**80.6**	**0.020**	**94.5**	**1.074**
Nonextractable	9.4	0.039	N/R [f]	N/R	6.5	0.002	6.5	0.003	N/R [f]	N/R

Table 11. Summary of radioactive residues characterized/identified in eggs and tissues from laying hens dosed with [THP-^{14}C]flumioxazin at a feeding level equivalent to 9.01 ppm in the diet for 14 consecutive days

Fraction	Kidney (TRR = 0.887 ppm)		Muscle, breast (TRR = 0.139 ppm)		Muscle, thigh (TRR = 0.175 ppm)		Fat, abdominal (TRR = 0.226 ppm)		Skin with fat (TRR = 0.667 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]										
Flumioxazin	7.4	0.065	11.3	0.016	12.3	0.021	56.3	0.127	11.5	0.077
3-OH-Flumioxazin	6.9	0.061	11.6	0.016	8.0	0.014	11.0	0.025	6.0	0.040
4-OH-Flumioxazin	8.4	0.075	12.1	0.017	10.5	0.018	13.2	0.030	10.2	0.068
3-OH/4-OH-Flumioxazin [b]	2.2	0.019	2.6	0.004	4.2	0.007	–	–	3.4	0.023
THPA	13.2	0.117	13.0	0.018	6.2	0.011	2.6	0.006	10.9	0.073
TPA	–	–	–	–	–	–	–	–	–	–
3-OH-THPA	–	–	–	–	–	–	–	–	–	–
4-OH-THPA	12.8	0.114	8.7	0.012	2.8	0.005	3.3	0.007	4.2	0.028
3-OH-S-53482-SA	4.2	0.037	–	–	–	–	–	–	–	–
4-OH-S-53482-SA			–	–	–	–	–	–	–	–
OH-Flumioxazin [c]	3.5	0.031	4.0	0.006	5.1	0.009	5.8	0.013	3.9	0.026
Total identified	**58.5**	**0.519**	**63.2**	**0.088**	**66.3**	**0.116**	**92.1**	**0.208**	**50.1**	**0.334**
Characterized										
Unknowns (ACN, ACN:water) [d]	22.6	0.200	13.0	0.018	15.1	0.026	11.4	0.026	12.9	0.086
Unknowns (protease hydrolysate)	8.0	0.071	15.3	0.021	17.1	0.030	–	–	14.9	0.099
Total characterized/identified	**89.1**	**0.790**	**91.5**	**0.127**	**98.4**	**0.172**	**103.5**	**0.233**	**50.2**	**0.335**
Nonextractable	N/R [e]	N/R	N/R [e]	N/R	N/R [e]	N/R	10.3	0.023	N/R [e]	N/R

[a] See Attachment 1 for chemical structure of identified metabolites.
[b] Mixture of 3-OH-flumioxazin and 4-OH-flumioxazin.
[c] The position of hydroxylation could not be determined.
[d] The petitioner stated that individual metabolites were <5% TRR (<1% for egg white).
[e] The radioactivity levels in solids following protease digestion were not reported.

Storage stability

The petitioner verified the stability of the parent compound in eggs and tissues by fortifying liver, kidney, muscle, skin, egg white, and egg yolk samples from control hens with [^{14}C]flumioxazin. At least 90% of the fortified residues were found to be unchanged parent after 71-77 days of frozen storage. In addition, the petitioner re-extracted and analyzed samples of egg white, egg yolk, liver, kidney, muscle, fat, and skin with fat after 3-4 months of additional frozen storage. Comparison of the HPLC chromatograms of the extracts indicated that the metabolic profile was stable in these matrices during 3-4 months of storage.

The petitioner provided the following dates (Table 12) of sample collection, extraction, and analysis to allow the Agency to determine whether the submitted storage stability data are sufficient to support the metabolism study (electronic transmission to D. Kenny, 1/9/2001).

Table 12. Metabolism of [^{14}C(3,4,5,6-Tetrahydro)Phthalimide]S-53482 in Laying Hens

Sample	Sampling Date	Date of Initial Extraction	Date of Initial HPLC	Date of Final Extraction	Date of Final HPLC
Egg Yolk, Day 7	11-15-93	1-13-94	1/13-21/94	5-11-94	6-8-94
Egg Yolk, Day 13	11-21-93	1-14-94	1/14-2/3/94	5-12-94	6-7-94
Egg White, Day 7	11-15-93	1-11-94	1-11-94	5-9-94	6-17-94
Egg White, Day 13	11-21-93	1-12-94	1-12-94	5-10-94	NA
Liver	11-22-93	12-14-93	12-15-93	4-28-94	6/6-6/8/94
Kidney	11-22-93	12-16-93	12-17-93	4-29-94	6/6-6/9/94
Breast Muscle	11-22-93	12-20-93	12/22-12/27/93	5-2-94	6/6-6/9/94
Thigh Muscle	11-22-93	11-29-93	1/3-1/4/94	5-3-94	NA
Gizzard	11-22-93	1-18-94	1/19-1/20/94	5-6-94	6/6-6/8/94
Abdominal Fat	11-22-93	1-24-94	1/24-1/25/94	5-4-94	NA
Skin Fat	11-22-93	1-21-94	1/21-1/24/94	5-5-94	6-7-94

NA: Not Analyzed

All analyses were completed in less than 7 months from the sampling dates. The data indicate that significant changes in the metabolic profiles did not occur during the study's analytical phase.

Proposed metabolic pathway in poultry

Based on the results of the metabolism study, the petitioner proposes that flumioxazin is metabolized in poultry by hydroxylation of the cyclohexene ring in the THP moiety, cleavage of the imide linkage, and incorporation of sulfonic acid in the THP moiety.

Conclusions:

The submitted hen metabolism study is acceptable. Following oral administration of [THP-^{14}C]flumixoazin to laying hens for 14 consecutive days at a feeding level of 9.0 ppm (2000x the MTDB for poultry), the TRR were 0.025-0.041 ppm in egg white, 0.009-0.760 ppm in egg yolk, 1.137 ppm in liver, 0.138-0.175 ppm in muscle, 0.226 ppm in fat, and 0.667 ppm in skin with fat. Residues in egg white appeared to remain constant over the dosing period while residues in egg yolks appeared to plateau on Day 11 of the study at 0.638 ppm.

Approximately 50->100% TRR were characterized/identified in eggs and tissues. The parent, flumioxazin, was the major metabolite identified in fat, at 56.3% TRR (0.127 ppm), but was present at lower levels in all other matrices (0.7-12.3% TRR, <0.001-0.079 ppm). 4-OH-S-53482-SA was the major metabolite identified in egg yolk, at 33.6-47.5% TRR (0.139-0.299 ppm) and was also identified in liver (as a mixture with the 3-OH analog; 6.0% TRR, 0.068 ppm). THPA, its anhydride (TPA), and its 3-OH and 4-OH derivatives were the major metabolites identified in egg whites each at 13.7-27.1% TRR (0.003-0.010 ppm); THPA and 4-OH-THPA were also identified in other hen matrices at 2.6-14.3% TRR (0.005-0.163 ppm). Other identified metabolites were hydroxy derivatives of flumioxazin (3.1-13.2% TRR, <0.001-0.121 ppm).

Nature of the Residue in Animals-Summary

The results of the ruminant and poultry metabolism studies with both phenyl and THP labeled flumioxazin show that the major metabolic pathways are hydrolysis of the imide, hydroxylation of the cyclohexene ring, and the equivalent of addition of sulfonic acid to the alkene function. The MARC has determined that the residues of concern in ruminants are the parent, 3-OH-flumioxazin, 4-OH-flumioxazin, and metabolites B, C, and F. In poultry, the residues of concern are parent, 3-OH-flumioxazin, 4-OH-flumioxazin, and 4-OH-S-53482-SA (D272652, R. Loranger, 3/12/2001). Although feeding studies are not required for the proposed uses on peanuts and soybeans, the above analytes should be determined in any such studies which are needed in future petitions.

OPPTS GLN 860.1340: Residue Analytical Methods - Plants

Valent previously submitted descriptions of GC/NPD methods (Valent Methods RM-30A, RM-30B, and RM-30C) for determining residues of flumioxazin *per se* in/on soybean RACs and processed commodities. These methods were reviewed by the Agency in conjunction with the temporary tolerance petition for soybeans (PP#3G4250; DP Barcode D194594, J. Garbus, 9/21/94), and deemed acceptable for determining residues of flumioxazin in/on soybean commodities. The principal method for determining residues in RACs (Method RM-30A) has undergone both a successful independent laboratory validation and a PMV trial (J. Garbus, 1/12/96). Following the Agency's PMV trial, the proposed enforcement method was revised (MRID 43935509, RM-30A-1, 1/8/96) to include clarifications and minor changes suggested by the Analytical Chemistry Branch along with specifications for an alternate GC column and parameters for use as a confirmatory method.

For both Methods RM-30A and RM-30A-1, flumioxazin residues in plant samples are extracted overnight with acetone:water (4:1, v/v) and filtered. The extract is diluted with an aqueous 5% NaCl solution and partitioned with dichloromethane (DCM). Residues in the DCM fraction are dried over sodium sulfate, concentrated to dryness, redissolved in hexane, and partitioned into ACN. Residues are again concentrated to dryness, redissolved in hexane:EtOAc (2:1, v/v), and cleaned up using a Florisil column eluted with hexane:EtOAc (2:1, v/v). Residues in the resulting eluate are concentrated, redissolve in acetone, and analyzed by GC/NPD.

Methods RM-30B and RM-30C are modifications to RM-30A for determining flumioxazin residues in oil and soapstock, respectively. Crude and refined oils are extracted by dissolving in hexane and partitioning with ACN. Residues in the resulting ACN phase are then purified using a Florisil column as described above and analyzed by GC/NPD. For soapstock, residues are acidified with aqueous 1N HCl and extracted with acetone:5% aqueous NaCl:DCM (3:1:2, v/v/v). Residues in the resulting DCM fraction are then cleaned up and analyzed as per Method RM-30A above.

The reported method LOD for flumioxazin in soybean commodities is 0.01 ppm, and the LOQ is reported as 0.02 ppm. However, in the PMV trial, Method RM-30A was successfully validated by the Agency down to an LOQ of 0.01 ppm using soybean forage, hay and seeds, and the LOD was reported to be 0.005 ppm.

In conjunction with the crop field trials and processing studies on peanuts (MRID 44295054, 44295055, and 44013002) and soybeans (MRID 44295056), the petitioner submitted concurrent recovery data using the above GC/NPD methods. Control samples of peanut and soybean commodities were fortified with flumioxazin at 0.02 ppm or 0.1 ppm (nutmeats only). With the exception of a few marginal recoveries (8 out of 138), concurrent recoveries of flumioxazin were acceptable, with overall recoveries

of 63-125% for peanut commodities and 67-130% for soybean commodities (Table 13). Apparent residues of flumioxazin were <LOD (<0.01 ppm) in/on all control samples. Adequate representative sample calculations and chromatograms were submitted. Sample analyses were performed by Valent U.S.A., Dublin, CA.

Table 13. Concurrent method recoveries of flumioxazin from peanut and soybean commodities using GC/NPD methods (Valent Methods RM-30A, -30A-1, -30B, and -30C)

Crop MRID	Matrix	Method	Fortification level (ppm)	# of samples	% Recovery	
					Range [a]	Ave. ± SD
Peanut 44013002	vines	RM-30A	0.02	4	84-95	85 ± 7.4
	hay		0.02	4	83-87	
	nutmeats		0.02	4	73-90	
	hulls		0.02	4	73-90	
	whole nuts	RM-30A	0.02	1	86	NA
	hulls		0.02	1	76	NA
	nutmeat		0.02	1	85	NA
	presscake		0.02	2	84, 91	NA
	soapstock	RM-30C	0.02		91	NA
	oil [b]	RM-30B	0.02	5	82-125 (1)	96 ± 17
44295054	vines	RM-30A	0.02	6	76-113	91 ± 15
	hay	RM-30A	0.02	5	63-78 (3)	71 ± 7.1
	nutmeats		0.02	8	80-99	86 ± 6.1
	hulls		0.02	7	72-93	80 ± 7.9
	presscake		0.02	2	86, 99	NA
	oil [b]	RM-30B	0.02	5	87-120	106 ± 13
	soapstock	RM-30C	0.02	1	72	NA
44295055	Nutmeats	RM-30A-1	0.02, 0.1	4	95-105	101 ± 4
Soybean 44295056	forage	RM-30A	0.02	29	67-120 (2)	92 ± 14
	hay		0.02	19	69-130 (2)	89 ± 17
	seeds		0.02	18	71-112	84 ± 9.2
	seed	RM-30A	0.02	1	75	NA
	hulls		0.02	1	113	NA
	meal		0.02	1	96	NA
	lecithin		0.02	1	80	NA
	oils	RM-30B	0.02	2	80, 85	NA
	soapstock	RM-30C	0.02	2	89, 92	NA

[a] Values in parentheses indicate the number of samples with recoveries outside the 70-120% acceptable range.
[b] Includes crude and refined (bleached and unbleached) oil.

Valent also submitted method descriptions (MRID 44295054) for two GC/MS methods (Valent Methods RM-30M and RM-30P) for determining free and conjugated residues of 1-OH-HPA, the major metabolite in soybeans, in/on soybean and peanut commodities.

For Method RM-30M, free and conjugated residues of 1-OH-HPA are extracted from peanut and soybean commodities (except oil and soapstock) by refluxing in 2.5 N HCl for 3 hours. After cooling, residues are filtered, diluted with water, and cleaned-up using an Extrelut-20® column eluted with EtOAc. Residues are concentrated to dryness, dissolved in acetone, and then methylated by refluxing for 30 minutes in triisopropanolamine containing dimethyl sulfate. Methylated residues (HPADME) are diluted with NaCl saturated water, partitioned into hexane, concentrated, redissolved in hexane:ethyl ether (1:2, v/v), and cleaned up using a Florisil column eluted with hexane:ethyl ether (1:2, v/v). The methylated residues are then concentrated, redissolved in acetone, and analyzed by GC/MS scanning for the 157 m/e ion. Residues are calculated as 1-OH-HPA. The reported LOD is 0.01 ppm and the LOQ is 0.02 ppm.

A similar GC/MS method (RM-30P) is used for determining free and conjugated residues of 1-OH-HPA in oil and soapstock matrices. This method is identical to Method RM-30M except that following the initial acid extraction, residues are partitioned against hexane (discarding the hexane phase) prior to cleanup on the Extrelut-20® column. The reported LOD and LOQ for 1-OH-HPA in these matrices are 0.01 and 0.02 ppm, respectively.

For validation of Methods RM-30M and -30P, control samples of soybean forage, hay, meal, hulls, and oil and peanut soapstock were fortified with 1-OH-HPA at 0.02 ppm and/or 0.1 ppm. With the exception of three low recoveries (63-69%) from soapstock and one high recovery (128%) from hay, method recoveries of 1-OH-HPA were acceptable with average recoveries of 74-118% for the various matrices (Table 12). Apparent residues of 1-OH-HPA were <LOD in/on control samples. Adequate representative sample calculations and chromatograms were submitted. Sample analyses were performed by Valent U.S.A., Dublin, CA.

The petitioner also submitted concurrent method recovery data for Method RM-30M and RM-30P in conjunction with the crop field trials and processing studies on peanuts (MRID 44295054) and soybeans (MRID 44295056). Concurrent recoveries of 1-OH-HPA were 63-119% from peanut nutmeats and processed fractions fortified at 0.02 ppm (Table 14), and recoveries were 67-100% from soybean seeds and processed fractions fortified at 0.02 ppm, with the exception of one high recovery (154%) from soybean lecithin, an unregulated commodity. Apparent residues were <LOD (<0.01 ppm) in/on

all control samples, except for one hull sample (0.01 ppm). Adequate representative sample calculations and chromatograms were submitted. Sample analyses were performed by Valent U.S.A., Dublin, CA.

Table 14. Method recoveries of 1-OH-HPA from peanut and soybean commodities using GC/MS methods (Valent Methods RM-30M and RM-30P)

Crop MRID	Matrix	Method	Fortification level (ppm)	# of samples	% Recovery	
					Range [a]	Ave. ± SD
Method Validation Recoveries						
Soybean 44295056	hulls	RM-30M	0.02	6	71-94	82 ± 8.9
	forage		0.02, 0.1	6	75-118	97 ± 18
	hay		0.1	3	106-128 (1)	117 ± 11
	meal		0.02	3	70-113	98 ± 24
	oil	RM-30P	0.02, 0.1	9	72-96	86 ± 7.2
	soapstock [b]		0.02, 0.1	9	63-87 (3)	74 ± 8.6
Concurrent Method Recoveries						
Peanut 44295054	nutmeats	RM-30M	0.02	1	84	NA
	hulls		0.02	1	74	NA
	presscake		0.02	2	73, 119	NA
	oil [c]	RM-30P	0.02	4	63-108 (1)	87 ± 19
	soapstock		0.02	1	75	NA
Soybean 44295056	seeds	RM-30M	0.02	14	71-100	81 ± 7.8
	seed	RM-30M	0.02	1	71	NA
	hulls		0.02	1	67	NA
	meal		0.02	1	76	NA
	lecithin		0.02	1	154	NA
	oil [c]	RM-30P	0.02	2	87, 100	NA
	soapstock		0.02	1	78	NA

[a] Values in parentheses indicate the number of samples with recoveries outside the 70-120% acceptable range.

[b] Peanut soapstock was used for method validation.

[c] Includes crude and refined (bleached and unbleached) oil.

Radiovalidation of Valent GC/NPD Method RM-30A

In conjunction with the peanut metabolism study, the petitioner also submitted radiovalidation data (MRID 44013001) for the proposed tolerance enforcement method, Valent Method RM-30A, using samples from the peanut metabolism study.

Samples of nutmeats, hulls, and vines from plants treated with [THP-^{14}C]flumioxazin at 1x the maximum proposed rate were extracted and analyzed using the GC/NPD method RM-30A, along with control samples and control samples fortified with flumioxazin at 0.02 ppm. Sample analyses were conducted within 8 months of harvest, and are supported by adequate storage stability data.

Using Method RM-30A, residues of flumioxazin were <0.01 ppm in/on control and ^{14}C-treated samples of nutmeats, hulls, and vines from the metabolism study. Recovery of flumioxazin from a single fortified (0.02 ppm) sample each of nutmeats, hulls, and vines was 78, 74, and 91%, respectively. These results are in agreement with the radio-HPLC method used in the metabolism study which also did not detect (<0.001 ppm) flumioxazin residues in these samples.

As flumioxazin residues were not detected (<0.001 ppm) in the above peanut metabolism study, samples from this metabolism study are of limited use for radiovalidation of the proposed enforcement method.

In conjunction with the requested sugarcane metabolism study, the petitioner should radiovalidate Method RM-30A-1 using mature cane samples containing quantifiable residues of flumioxazin.

<u>*Conclusions*</u>:

The GC/NPD methods, Valent Methods RM-30A, RM-30A-1, RM-30B, and RM-30C are adequate for collecting data on residues of flumioxazin *per se* in/on peanut and soybean commodities. These methods were validated by the petitioner down to an LOQ of 0.02 ppm and have reported LODs of 0.01 ppm.

The proposed enforcement method, Valent Method RM-30A, has undergone a successful ILV trial and has been successfully validated down to an LOQ of 0.01 ppm by the Agency in a PMV trial using soybean seed, forage and hay. A revised copy of the method (RM-30A-1), including changes suggested by ACB, has been submitted. GC/NPD Method RM-30A-1 should be radiovalidated in conjunction with the requested sugarcane metabolism study.

The confirmatory procedure in Method RM-30A-1 involves a change in the GC column, but not the detector. Because of the relative lack of specificity in these methods, HED

recommends that a more specific confirmatory method (e.g., MS detection) be required as a condition of registration. Alternatively, the petitioner could submit an interference study showing whether other pesticides registered on soybeans and peanuts interfere with the analysis of flumioxazin.

The GC/MS methods, Valent Methods RM-30M and RM-30P, are adequate for collecting data on residues of the metabolite 1-OH-HPA in/on peanuts and soybeans and their processed commodities. The LOQ for these methods is 0.02 ppm, and the LOD is reported to be 0.01 ppm.

OPPTS GLN 860.1340: Residue Analytical Methods - Animals

Valent has submitted a description of a GC method using thermionic specific detection (TD; similar to NPD) for determining residues of flumioxazin in animal commodities (MRID Numbers 44295052 and 44295053), along with method validation data and radiovalidation data using samples from the above goat and poultry metabolism studies. Method development and validation was conducted by Ricerca (Painesville, OH).

The method for analysis of residues in animal commodities is similar to Valent Method RM-30A. For the animal method, flumioxazin residues are extracted from homogenized animal matrices with acetone. The extract is filtered and diluted with aqueous 5% NaCl, and residues are partitioned into DCM and concentrated to dryness. Residues are redissolved in hexane, partitioned into ACN, backwashed with hexane, and concentrated to dryness. Residues are then dissolved in hexane:EtOAc (2:1, v/v) and purified using a Florisil column topped with sodium sulfate and eluted sequentially with hexane and hexane:EtOAc (2:1, v/v). For most animal matrices, residues in the final Florisil column eluate are then concentrated to dryness, redissolved in acetone and analyzed by GC/TD. For selected matrices (milk, goat liver, and egg yolks), an additional silica gel column cleanup is required. For these matrices, residues are further purified using a silica gel (3% deactivated) column topped with sodium sulfate and eluted sequentially with DCM and hexane:EtOAc (2:1, v/v). Residues in the final column eluate are then analyzed by GC/TD.

For method validation, homogenized control samples of goat milk, muscle, and liver, and chicken liver, muscle, egg whites and yolks were fortified with flumioxazin at 0.01 and 1.0 ppm. Overall recoveries of flumioxazin from goat commodities were 67-119% with average recoveries of 90 ± 20%, 103 ± 15%, and 93±11% for milk, liver, and muscle, respectively (Table 15). For poultry commodities, overall recoveries of flumioxazin were 62-132% with average recoveries of 84 ± 10%, 92 ± 16%, 85± 22%, and 97 ± 28%, for egg whites, yolks, muscle, and liver, respectively. Apparent residues of flumioxazin were <LOQ (<0.01 ppm) in/on all control samples. Adequate

representative sample calculations and chromatograms were submitted. The validated LOQ for flumioxazin residues in animal commodities is 0.01 ppm.

Using ^{14}C-samples from the goat and poultry metabolism studies above, the petitioner also compared residues of flumioxazin determined by the above GC/TD method to the Radio-HPLC method used in the metabolism studies. Duplicate samples of goat milk, muscle and liver and poultry egg whites, egg yolks (4 samples), muscle (breast and loin), and liver from the metabolism studies were analyzed using the above GC/TD method, along with control and fortified control samples.

Results from these analyses are compared to results from the metabolism studies in Table 16. For the goat matrices, the results of the two methods were consistent. Although the radio-HPLC method detected flumioxazin in goat liver (0.0039 ppm) and muscle (0.0003 ppm), it was present at levels below the LOQ (<0.01 ppm) for the GC/TD method. However, results from the poultry matrices indicate that the GC/TD method does not adequately recover endogenous flumioxazin residues from animal commodities. Using the GC/TD method, flumioxazin was detected only in poultry liver at 0.02 ppm, although residues of flumioxazin reported in the metabolism study were also well above the method LOQ (0.01 ppm) in egg yolks and muscle. The levels found in liver by the GC/TD method were also well below the residue levels reported for liver (0.076 ppm) in the metabolism study. In addition, no samples of poultry fat were analyzed using the GC/TD method, although this matrix had the highest reported levels of flumioxazin (0.127 ppm) in the metabolism study.

Table 15. Recovery of flumioxazin from animal commodities using a GC/thermionic specific detection method

Animal MRID	Matrix	Fortification level (ppm)	# of samples	% Recovery	
				Range [a]	Avg. ± SD
Ruminants 44295052	milk	0.01	3	101-116	90 ± 20
		1.00	3	67-76 (1)	
	liver	0.01	3	96-119	103 ± 15
		1.00	3	80-113	
	muscle (loin)	0.01	1	96	93 ± 11
		1.00	5	81-114	
Poultry 44295054	egg whites	0.01	3	71-91	84 ± 10
		1.00	3	82-93	
	egg yolks	0.01	4	73-123 (1)	92 ± 16
		1.00	3	91-98	
	muscle (breast)	0.01	3	84-124 (1)	85 ± 22
		1.00	3	62-82 (2)	

Table 15. Recovery of flumioxazin from animal commodities using a GC/thermionic specific detection method

Animal MRID	Matrix	Fortification level (ppm)	# of samples	% Recovery	
				Range [a]	Avg. ± SD
	liver	0.01	3	75-132 (1)	97 ± 28
		1.00	3	62-112 (1)	

[a] Values in parentheses indicate the number of samples with recoveries outside the 70-120% acceptable range.

Table 16. Residues of flumioxazin in ^{14}C-samples from goat and poultry metabolism studies determined using GC/thermionic specific detection and Radio-HPLC methods

Animal MRID	Sample	Flumioxazin residues (ppm)	
		GC/TD method	Radio-HPLC method
Ruminants 44295052	milk	<0.01, <0.01 [a]	ND [b]
	liver	<0.01, <0.01	0.0039
	muscle (loin)	<0.01, <0.01	0.0003
Poultry 44295054	egg whites	<0.01, <0.01[c]	ND, ND [d]
	egg yolks	<0.01, <0.01,<0.01, <0.01 [c]	0.048, 0.039 [d]
	muscle (breast)	<0.01, <0.01	0.015
	muscle (thigh)	<0.01, <0.01	0.020
	liver	0.02, 0.02	0.076

[a] Composite milk sample from study Day 4.
[b] Composite milk sample from study Day 2.
[c] Composite egg sample from study Day 12.
[d] Composite egg sample from study Days 13 and 7, respectively.

Conclusions:

The proposed GC/TD method is not adequate for determining flumioxazin residues in animal commodities. Although recoveries of flumioxazin were generally acceptable from control samples of goat and poultry commodities fortified at 0.01 and 1.0 ppm, recoveries from fortified samples of poultry muscle (85 ± 22%) and liver (97 ± 28%) were quite variable. In addition, radiovalidation data using ^{14}C-samples from the poultry metabolism study indicate that the method does not adequately recover endogenous flumioxazin residues from animal commodities.

However, for purposes of this petition, tolerance enforcement and data collection methods for flumioxazin residues in animal commodities are not required, as neither livestock feeding studies nor animal tolerances are required at the present time.

OPPTS GLN 860.1360: Multiresidue Method Testing

Valent submitted data depicting the testing of flumioxazin through FDA Multiresidue Methods (MRM) in Pesticide Analytical Manual, Volume I (MRID Number 44295057). Testing was conducted by Corning Hazleton, Inc. (Madison, WI).

The report references the FDA Multiresidue Protocols designated A, C, and F from PAM, Vol. I, third edition dated 1/94. The current revision of PAM, Vol. 1, dated 10/97 groups the methods in chapters according to Multiclass MRMs (formerly protocols D and E) and Selective MRMs (methods for particular classes of chemicals, e.g., protocol A for N-methylcarbamates). These data are summarized in this report and will be forwarded to the FDA for review.

Protocol A (Section 401: Method for N-Methylcarbamates)

Flumioxazin was tested through Section 401 (Module DL2) using HPLC with a fluorescence detector (excitation - 288 nm, emission - 330 nm). The relative retention time of flumioxazin was 0.21 compared to carbofuran, and 1243 ng of flumioxazin were required for 50% FSD. As standard concentrations of flumioxazin below 10 μg/mL were not detected, no further work was conducted under Protocol A.

Protocol B (Section 402: Method for Acids and Phenols)

Flumioxazin was not tested through Section 402 as it does not possess a carboxylic acid or phenol moiety.

Protocol C (Gas Chromatographic Screening)

Flumioxazin was dissolved in acetone:isooctane (10:90, v/v) and tested using Module DG 5 with nitrogen-phosphorus detection (NPD). Using an isocratic oven temperature of 230°C, the actual retention time of flumioxazin was 17.6 minutes, and 68.1 ng of flumioxazin was required for 50% FSD. The relative retention time of flumioxazin with respect to chlorpyrifos was 17.9.

Protocol F (Sections 304: Method for Fatty Foods)

The recovery of flumioxazin from the Florisil column using either the mixed ether or methylene chloride elution system was zero; therefore, no additional work was performed on this compound under Protocol F.

OPPTS GLN 860.1380: Storage Stability Data

Peanut Commodities.

In conjunction with a magnitude of the residue study on peanuts (1994, MRID 44013002), Valent submitted interim data depicting the frozen (-20 C) storage stability of flumioxazin in peanut RACs for up to approximately 300 days and in peanut processed commodities for up to 30 days.

Control samples of macerated peanut vines, hay, nutmeats, and hulls were weighed into plastic bags and fortified with flumioxazin at 0.10 ppm. Samples of presscake, crude oil, and soapstock were weighed into plastic bottles, fortified with flumioxazin at 0.10 ppm, and placed in freezer storage. At each sampling interval, a control sample, a freshly-fortified sample, and two stored fortified samples were analyzed for each matrix using Valent's Residue Method RM-30A, RM-30B, or RM-30C, as described above. A second freezer storage stability study was conducted, as described above, on peanut soapstock, except sampling occurred at 0, 15, and 31 days, to confirm the results of the first study. Residues in control samples were <0.01 ppm. All extracts were analyzed within two days of extraction. Adequate representative chromatograms and data worksheets were provided.

The data indicate that residues of flumioxazin are stable at -20°C in peanut vines, hay, nutmeats, and hulls for 296 to 300 days, and peanut processed commodities, except soapstock, for up to 31 days. Residues of flumioxazin were found to decline in soapstock during freezer storage, declining by 41% after 15 days and 55% after 31 days. This commodity is not considered a significant food or feed. The results of the storage stability study are presented in Table 17.

In the peanut field trials, the maximum sample storage interval prior to extraction for analysis of flumioxazin was 210 days for nutmeats and hulls, 203 days for vines, and 182 days for hay. Peanut processed fractions were stored frozen for 16-57 days prior to analysis for flumioxazin and 17-39 days prior to analysis for 1-OH-HPA.

Table 17. Stability of flumioxazin residues fortified in peanut RACs and processed commodities at 0.1 ppm and stored at -20 °C

Crop/Matrix	Storage Interval (days)	Fresh Fortification %Recovery	Stored Sample % Recovery	Stored Sample [a] Corrected % Recovery
Peanut vines	0	NA	96, 95, 99	NA
	20	99	97, 97	98, 98
	40	103	100, 105	97, 102
	147	100	110, 111	110, 111
	300	100	92, 100	92, 100
Peanut hay	0	NA	94, 92, 101	NA
	20	95	100, 101	105, 106
	41	84	96, 93	114, 111
	142	112	117, 128	104, 114
	296	73	92, 74	126, 101
Peanut nutmeats	0	NA	87, 89, 85	NA
	20	94	86, 84	91, 89
	40	102	105, 92	103, 90
	147	105	86, 74	82, 70
	300	77	93, 92	121, 119
Peanut hulls	0	NA	105, 89, 88	NA
	20	92	89, 98	97, 107
	41	95	97, 91	102, 96
	142	100	93, 91	93, 91
	296	75	92, 124	123, 165
Presscake	0	NA	108, 107, 111	NA
	30	96	119, 111	124, 124
Crude Oil	0	NA	115, 119, 114	NA
	31	98	133, 123	136, 126
Peanut Soapstock	0	NA	96, 109, 108	NA
	30	93	57, 57	61, 40
Peanut Soapstock (Repeat Study)	0	NA	104, 98	NA
	15	111	64, 67	58, 60
	31	97	44, 44	45, 45

[a] Average % recovery of stored samples corrected for average fresh fortification recoveries.

Soybean Commodities

In conjunction with a magnitude of the residue study on soybeans (1996, MRID 44295056), Valent submitted data from an interim study depicting the frozen storage stability of 1-OH-HPA in soybean seed stored at -20°C for up to 290 days. Studies depicting the freezer storage stability of flumioxazin in/on soybean RACs were

previously reported (PP#3G4250; DP Barcode D194594, J. Garbus, 9/21/94) and indicated that flumioxazin is stable in frozen soybean forage, hay and seeds for at least 12 months.

In the present study, control samples of macerated soybean seeds were weighed into plastic bags and fortified with 1-OH-HPA at 0.10 ppm, and placed in storage at -20°C. At each sampling interval (0, 43, and 290 days), a control sample, a freshly-fortified sample, and two stored fortified samples were analyzed using Valent's Residue Method RM-30M, as described above. Residues of 1-OH-HPA in control samples were <0.01 ppm. All extracts were analyzed within 25 days of extraction. Adequate representative chromatograms and data worksheets were provided. The data indicate that residues of 1-OH-HPA are stable in soybean seeds at -20°C for up to 290 days (Table 18).

In the soybean field trials, samples of forage, hay, and seeds were stored frozen for a maximum of 382, 315, and 280 days prior to analysis for flumioxazin, and seeds were stored for up to 245 days prior to analysis for 1-OH-HPA. In the processing studies, samples were analyzed for flumioxazin and 1-OH-HPA within one month of sampling.

Conclusion:

The available storage stability data adequately support the peanut and soybean field trial and processing studies.

Table 18. Stability of 1-OH-HPA residues fortified in soybean seed at 0.1 ppm each and stored at -20 °C

Crop/Matrix	Storage Interval (days)	Fresh Fortification %Recovery	Stored Sample % Recovery	Stored Sample [a] Corrected % Recovery (Mean)
Soybean seed	0	88	92, 69	83
	43	83	109, 67	106
	290	125	113, 153	106

[a] Average % recovery of stored samples corrected for average fresh fortification recoveries.

OPPTS GLN 860.1500: Crop Field Trials

Peanut

The petitioner submitted three studies depicting flumioxazin residues in/on peanuts from plants treated with flumioxazin at one to five times the proposed maximum seasonal rate (MRID Numbers 44013002, 44295054, and 44295055). The trials were conducted during 1992, 1993, and 1996.

Four trials (1994, MRID 44013002) were conducted during the 1992 growing season in AL, TX, GA, and NC. At each test site, flumioxazin (51% DF) was applied once as a preemergence (2 tests) or pre-plant incorporated (2 tests) application at 0.094-0.097 lb ai/A (1x the proposed maximum rate). A single preemergence application of flumioxazin (51% DF) was also applied to separate plots in GA and NC at 0.47-0.48 lb ai/A (5x) to produce peanuts for processing studies. All applications were made with tractor mounted booms using 20 gallons of spray mixture per acre.

Another 5 trials (1996, MRID 44295054) were conducted at the 1x rate during 1993 in AL, FL, GA, NC, and TX. Flumioxazin (51% WP) was applied as either a single preemergence (2 tests) or pre-plant incorporated (3 tests) application at 0.095-0.099 lb ai/A (1x). A single application of flumioxazin (51% WP) was also applied to separate test plots in GA and TX at 0.48 or 0.49 lb ai/A (5x) to produce peanuts for the processing studies. Applications were made with a tractor mounted spray boom using 20 gallons of spray mixture per acre.

In 1996, another 5 trials (MRID 44295055) were conducted in AL, FL, GA, OK, and TX. In each test, flumioxazin (51% WP) was applied as a single preemergence application at 0.091-0.098 lb ai/A (1x) within four days of planting. Applications were made with a tractor mounted spray boom using 20 gallons of spray mixture per acre.

For the 1992 study, peanuts were harvested at normal crop maturity (110-148 DAT) using a small plot combine. One control and two treated samples of each commodity were collected from each test. Peanut vines were collected immediately after digging, and samples of hay and whole nuts were collected after 6-8 days of field drying. Whole nuts were dried in an oven for 36 to 48 hours and shelled, except those shipped for processing. All samples were kept in freezer storage at -20°C until being shipped by overnight carrier (on dry ice) to Valent's Dublin Laboratory for analysis. RAC samples were stored for 97 to 119 days before extraction and all extracts were analyzed within two days.

In the 1993 trials, one control and two treated samples each of peanut vines, hay, and peanuts were collected from each test at normal crop maturity (97-152 DAT) using small plot combines. Samples of vines were collected immediately after digging, and samples of hay and whole nuts were collected after 3-8 days of drying in the field. Additional samples of vines were also collected from two field sites (NC and GA) at 14, 21, and 28 DAT to examine residue decline. All samples were kept in freezer storage at -20°C until being shipped by overnight carrier (on dry ice) or by freezer truck (ACDS) to the Valent Technical Center for analysis. All samples were extracted for analysis within 210 days of harvest or processing.

In the 1996 study, one control and two treated samples of peanut nutmeats were harvested from each plot at crop maturity (131 to 154 days after application) using small

plot combines. Whole nuts were allowed to dry in the field for 4-19 days before sampling. All samples were kept in freezer storage -20 C until shipped by overnight carrier (on dry ice) or by freezer truck (ACDS) to the Valent Technical Center for analysis. All samples were extracted for analysis within 87 days of harvest.

Residues of flumioxazin in the peanut vines, hay, nutmeats, and hulls were determined by Valent's Residue Method RM-30A and RM-30A-1, discussed above. In conjunction with the 5x application rate used for the processing study, residues of the analyte 1-OH-HPA, in the peanut nutmeats and hulls were determined by Valent's Residue Method RM-30M. Adequate method recoveries were obtained for each analyte. Concurrent recoveries of flumioxazin and 1-OH-HPA were 63-113% from all peanut RACs fortified with flumioxazin at 0.02-0.1 ppm. Apparent residues were <0.01 ppm (<LOQ) in/on all untreated peanut commodity samples. Adequate representative sample calculations and chromatograms were submitted.

Following a preemergence or pre-plant incorporated application of flumioxazin (51% DF or WP) at 1x the maximum proposed rate, residues of flumioxazin were <0.01 ppm in/on 18 samples each of peanut vines and hay from nine tests harvested at normal crop maturity (97-152 DAT), and in/on12 samples of vines collected from two tests at 14-28 DAT. Flumioxazin residues were also <0.01 ppm in/on 26 samples of nutmeats from 13 tests and in/on 18 samples of hulls from nine tests. In four tests conducted at 5x the maximum label rate, residues of flumioxazin were <0.01 ppm in/on 8 samples of nutmeats and 6 samples of hulls; two hull samples from one test at 5x (TX) had flumioxazin residues of 0.04 ppm. Results for analyses of the 1-OH-HPA metabolite are discussed under 860.1520 (Processed Food/Feed).

Geographic representation and the number of the peanut field trials conducted are adequate. The petitioner provided residue data on peanuts from a total of 13 tests conducted at 1x the proposed maximum rate in Region 2 (eight tests), Region 3 (two tests), Region 6 (one test), and Region 8 (two tests).

Conclusions:

The submitted residue data on peanut are adequate. However, as the lowest fortification level in the peanut RACs was 0.02 ppm, HED recommends that the nutmeat tolerance be set at 0.02 ppm. A revised Section F should be submitted.

Soybean

The petitioner submitted data (citation shown below) from 18 field trials depicting residues of flumioxazin and 1-OH-HPA in/on soybean RACs (MRID No. 44295056). The trials were conducted in AR, IA (2), IL(3), IN (2), MO (2), MN, MS (2), NC (2), NE (1), and OH (2), during 1992 and 1993.

In eighteen tests (Table 19), flumioxazin (51% DF or WP) was applied once to soybeans as either a pre-plant incorporated or preemergence application at 0.094-0.098 lb ai/A (1x the maximum proposed rate) within 5 days of planting. Applications were made using ground equipment at 16-29 gallons of water per acre. The spray mixture in six of the tests included 1% crop oil concentrate. In addition, flumioxazin (51% DF or WP) was applied once preemergence at an exaggerated rate (0.47-0.49 lb ai/A; 5x) to soybeans at four test sites (2 in MS and 2 in IL), in order to provide seed for processing.

A single control and duplicate treated samples of each RAC were collected from each 1x test. Soybean forage was sampled at approximately 21, 28, and 40 DAT in each test, and also at 13 or 14 DAT in four tests. Hay samples were cut 80-123 DAT and allowed to air-dry in the field for 1-8 days prior to sampling. Seed was collected at normal crop maturity (112-160 DAT). All samples were shipped by freezer truck (ACDS) or by overnight carrier on dry ice to the Valent Technical center and stored at -20°C.

Prior to extraction for analysis of flumioxazin, frozen sample storage intervals were 16-382 days for forage, 28-315 days for hay, and 18-280 days for seeds. Seeds were also stored frozen for 211-245 days prior to analysis for 1-OH-HPA. The available storage stability data adequately support the storage intervals in the current study.

Residues of flumioxazin in the soybean forage, hay, and seed were determined by Valent's Residue Method RM-30A, discussed above. Residues of the analyte 1-OH-HPA in the soybean seeds were determined by Valent's Residue Method RM-30M. Adequate method recoveries were obtained for both analytes. Concurrent recoveries of flumioxazin from soybean forage, hay, and seed fortified at 0.02 ppm (LOQ) were 67-120%, 67-130%, and 71-112%, respectively. Concurrent recoveries of 1-OH-HPA from soybean seed fortified at 0.02 ppm (LOQ) were 71-100%. Apparent residues were <0.01 ppm (<LOD) in/on all untreated soybean commodity samples. Adequate representative sample calculations and chromatograms were submitted.

Following a pre-plant or preemergence application at 1x, residues of flumioxazin were <0.01 ppm in/on 106 out of 110 samples of soybean forage collected from 13-42 DAT; residues were 0.02 ppm in/on two treated forage samples harvested 28 DAT from one test and 0.02-0.03 ppm in/on two samples harvested 28-DAT from another test. Residues of flumioxazin were also <0.01 ppm in/on all 36 samples of hay and 36 samples of seed from the 1x tests, and were <0.01 ppm in/on all 8 samples of seed from plants treated at 5x. Residues of the metabolite 1-OH-HPA were <0.01 ppm in/on 27 out of 28 samples of seed from 14 tests at 1x and in/on all 8 samples from the 4 tests conducted at 5x. One seed sample from a 1x test had 1-OH-HPA residues of 0.01 ppm, but reanalysis of this sample in duplicate found residues of <0.01 ppm.

Geographic representation and the number of the soybean field trials conducted are adequate. The petitioner provided residue data on soybeans from 18 tests conducted at

1x the proposed maximum rate in Region 2 (two tests), Region 4 (three tests), and Region 5 (thirteen tests).

The data for forage at various time points (i.e., occasional residues above LOQ), plus the results for hay (all <0.01 ppm), indicate that residues do not increase over the growing season.

In addition to the field trial data above, residues of flumioxazin were <0.01 ppm in all samples of forage, hay and seeds from 24 field trials conducted at the 1x rate in 1989 and 1990 and submitted in conjunction with the EUP (PP#G4250, DP Barcode D194594, 9/21/94., J. Garbus).

Conclusions:

The submitted residue data on soybeans are adequate. However, as the lowest fortification level in the soybean RACs was 0.02 ppm, HED recommends that the soybean seed tolerance be set at 0.02 ppm. A revised Section F should be submitted.

Table 19. Information from 1992 and 1993 field trials on the use of flumioxazin (51% DF or WP) in soybeans

Trial Location (trial #)	EPA Region	Application Data			Sample Information [b]		
		Type [a]	Formulation	Rate (lb ai/A)	Commodity	sampling intervals (DAT) [c]	# samples
1992 Field Trials							
Goldsboro, NC (V-1039-A)	2	PPI	51% DF	0.094	forage	22, 29, 41	6
					hay	123 + 8	2
					seeds [d]	154	2 [e]
Seymour, IL (V-1039-B)	5	PPI	51% DF	0.094	forage	21, 28, 40	6
					hay	102 + 6	2
					seeds [d]	130	2
Little Rock, AR (V-1039-C)	4	PPI	51% DF	0.094	forage	21, 28, 40	6
					hay	110 + 4	2
					seeds [d]	146	2
Noblesville, IN (V-1039-D)	5	PRE [f] (no till)	51% DF	0.094	forage	28, 40	4
					hay	97 + 6	2
					seeds [d]	131	2
Waukee, IA (V-1039-F)	5	PRE [f] (no-till)	51% DF	0.094	forage	14, 21, 28, 39	8
					hay	98 + 7	2
					seeds [d]	129	2

Table 19. Information from 1992 and 1993 field trials on the use of flumioxazin (51% DF or WP) in soybeans

Trial Location (trial #)	EPA Region	Application Data			Sample Information [b]		
		Type [a]	Formulation	Rate (lb ai/A)	Commodity	sampling intervals (DAT) [c]	# samples
New Holland, OH (V-1039-G)	5	PRE	51% DF	0.094	forage	22, 29, 42	6
					hay	106 + 5	2
					seeds [d]	132	2
Greenville, MS (V-1039-H)	4	PRE	51% DF	0.094	forage	13, 20, 28, 39	8
					hay	99 + 6	2
					seeds [d]	127	2
				0.47 (5x)	seeds [d]	127	2
Seymour, IL (V-1039-J)	5	PRE	51% DF	0.094	forage	21, 28, 40	6
					hay	98 + 5	2
					seeds [d]	126	2
				0.47 (5x)	seeds [d]	126	2
Leonard, MO, (V-1039-M)	5	PRE [f] (no-till)	51% DF	0.091	forage	21, 28, 40	6 [g]
					hay	91 + 6	2
					seeds [d]	126	2
1993 Field Trials							
Jamesville, NC (V-10719-A)	2	PPI	51% WP	0.096	forage	21, 28, 40	6
					hay	122 + 4	2
					seeds [d]	160	2
Theilman, MN (V-10719-B)	5	PPI	51% WP	0.095	forage	28, 40	4
					hay	101 + 6	2
					seeds [d]	160	2
York, NE (V-10719-C)	5	PPI	51% WP	0.095	forage	21, 28, 40	6
					hay	85 + 4	2
					seeds	126	2
Noblesville, IN (V-10719-D)	5	PRE [f] (no till)	51% WP	0.098	forage	21, 27, 40	6
					hay	108 + 6	2
					seeds	138	2
Leonard, MO (V-10719-E)	5	PRE [f] (no-till)	51% WP	0.095	forage	21, 28, 40	6
					hay	88 + 3	2
					seeds	123	2
Webster City, IA (V-10719-F)	5	PRE [f] (no-till)	51% WP	0.096	forage	28, 41	4
					hay	80 + 7	2
					seeds	112	2
New Holland, OH (V-10719-G)	5	PRE (no-till)	51% DF	0.095	forage	22, 31, 40	6

Table 19. Information from 1992 and 1993 field trials on the use of flumioxazin (51% DF or WP) in soybeans

Trial Location (trial #)	EPA Region	Application Data			Sample Information [b]		
		Type [a]	Formulation	Rate (lb ai/A)	Commodity	sampling intervals (DAT) [c]	# samples
					hay	88 + 1	2
					seeds [d]	127	2
Greenville, MS (V-10719-H)	4	PRE	51% WP	0.097	forage	14, 21, 28, 41	8
					hay	98 + 4	2
					seeds [d]	127	2
				0.49 (5x)	seeds [d]	127	2
Seymour, IL (V-10719-J)	5	PRE	51% WP	0.095	forage	14, 22, 28, 40	8 [h]
					hay	83 + 7	2
					seeds [d]	112	2
				0.48 (5x)	seeds [d, i]	112	2

[a] Pre-plant incorporated (PPI) or a preemergence (PRE) application to conventional or no till fields.
[b] Residues of flumioxazin and 1-OH-HPA (only seeds analyzed) were <0.01 ppm in each sample unless otherwise indicated.
[c] DAT = days after treatment; values for hay indicate the days from application to cutting + the number of days samples were allowed to dry in the field.
[d] Designated seed samples were analyzed for residues of both flumioxazin and 1-OH-HPA.
[e] One seed sample had 1-OH-HPA residues at 0.01 ppm, but duplicate reanalysis found residues at <0.01 ppm.
[f] Spray mixture included a crop oil concentrate at 1%.
[g] Residues of flumioxazin were 0.02 and 0.03 ppm in the two 21-DAT forage samples.
[h] Duplicate analyses found residues of flumioxazin at 0.02 ppm in the two 28-DAT forage samples.
[i] Soybean seeds from the 1993 5x test in Seymour, IL were used in the processing study.

OPPTS GLN 860.1520: Processed Food/Feed

Peanuts

The petitioner submitted data from two studies (MRIDs 44013002 and 44295054) depicting flumioxazin residues in/on peanut processed commodities from plants treated with flumioxazin at 5x the proposed maximum seasonal rate.

Flumioxazin (51% DF or WP) was applied as a single preemergence application at 0.47-0.48 lb ai/A (5x) at test sites in GA and NC in 1992 and at 0.48-0.49 lb ai/A (5x) at tests sites in GA and TX in 1993. All applications were made with tractor mounted booms using 20 gallons of spray mixture per acre.

One control, two treated samples, and a bulk treated (50 lb) sample of whole peanuts were harvested from each 5x test at crop maturity (101-152 DAT) using small plot combines. All plots were allowed to dry in the field for 3-8 days before sampling for peanuts. All samples were placed in frozen storage (-20°C). Bulk samples of frozen whole peanuts from the NC (1992) and GA (1993) tests were shipped by overnight carrier (on dry ice) to Engineering Biosciences Research Center, Texas A&M University, College Park, TX for processing. Whole peanuts were separated into nutmeats and hulls, and nutmeats were processed into presscake, crude oil, refined, bleached and deodorized oil, and soapstock using simulated commercial procedures. Processed fractions were frozen (-20°C) shipped by overnight carrier (on dry ice) to the Valent Technical Center for analysis. Processed samples from the 1992 study were stored at -20°C for up to 16 days prior to extraction for analysis of flumioxazin. Processed samples from the 1993 study were stored at -20°C for 16-22 days (except soapstock, 57 days) prior to extraction for analysis of flumioxazin, and 17-39 days prior to extraction for analysis of 1-OH-HPA. All extracts were analyzed within 7 days of extraction.

Residues of flumioxazin in the peanut nutmeats, hulls, and presscake were determined by Valent's Residue Method RM-30A, discussed above. Residues of flumioxazin in the peanut oils and soapstock were determined by Valent's Residue Methods RM-30B and RM-30C, respectively. Residues of the analyte 1-OH-HPA, in the peanut nutmeats, hulls, and presscake were determined by Valent's Residue Method RM-30M, and in all oil samples by Valent's Residue Method RM-30P. Adequate method recoveries were obtained for each analyte. Concurrent recoveries of flumioxazin and 1-OH-HPA were 76-125% from all peanut commodities fortified with flumioxazin at 0.02-0.1 ppm. Apparent residues were <0.01 ppm (<LOD) in/on all untreated peanut commodity samples. Adequate representative sample calculations and chromatograms were submitted.

In the 1992 processing study, residues of flumioxazin were <0.01 ppm in a single sample each of nutmeats, hulls, presscake, crude and refined oils, and soapstock from peanuts treated at 5x. In the 1993 processing study, residues of flumioxazin and 1-OH-HPA were each <0.01 ppm in a single sample each of nutmeats, presscake, crude and refined oils, and soapstock from peanuts treated at 5x. Hulls from the 1993 test had flumioxazin residues of <0.01 ppm and 1-OH-HPA residues of 0.02 ppm; however, the control sample of hulls also had apparent 1-OH-HPA residues of 0.01 ppm.

Conclusions:

The submitted peanut processing studies are adequate and indicate that tolerances are not required for flumioxazin residues in peanut processed commodities.

Soybeans

The petitioner submitted data (MRID 44295056) from a processing study using soybeans treated at 5x the proposed maximum label rate. As indicated in the field trial data above, flumioxazin (51% DF or WP) was applied as a single preemergence application to soybeans at 0.47-0.49 lb ai/A (5x) in four tests conducted during 1992 and 1993 in MS and IL. Bulk samples for control and treated seed from one of these tests (Seymour, IL, 1993) was shipped frozen by overnight courier to Texas A&M University, Food Protein Research and Development Center, Byran, TX for processing. After a total of 166 days of frozen storage, seed samples were processed using simulated commercial processing procedures into meal, hulls, crude and refined oils, lecithin, deodorized distillates, and soapstock. Immediately after processing, samples were shipped frozen by overnight courier to the analytical laboratory where the samples were stored at -20°C.

As all samples were analyzed within 38 days of processing, no storage stability data were generated for soybean processing commodities. Flumioxazin freezer storage stability studies on peanut processing commodities showed good storage stability for 31 days in all commodities except soapstock.

Residues of flumioxazin in the soybean seed and processed fractions were determined using one of the GC/NPD Methods (RM-30A, RM-30B or RM-30C), discussed above. Residues of the analyte 1-OH-HPA in the soybean seed and processed fractions were determined by GC/NPD Methods RM-30M or RM-30P, discussed above. Adequate method recoveries were obtained for each analyte. Concurrent recoveries of flumioxazin from soybean seed fortified at 0.02 ppm (LOQ) ranged from 67-120%. Concurrent recoveries of 1-OH-HPA from soybean seed and processed commodities fortified at 0.02 ppm (LOQ) ranged from 71-100% from seed, and 67- 100% for processed commodities, except lecithin (154% recovery) and deodorized (50% recovery). Apparent residues of both flumioxazin and 1-OH-HPA were <0.01 ppm in/on all untreated soybean commodity samples. Adequate representative sample calculations and chromatograms were submitted.

Residues of flumioxazin and 1-OH-HPA were each <0.01 ppm in all samples of soybean commodities tested, except one sample of soapstock which had flumioxazin residues of 0.028 ppm. However, reanalysis of the soapstock sample found flumioxazin residues of < 0.01 ppm.

Conclusions:

The submitted soybean processing study is adequate and indicates that tolerances are not required for flumioxazin residues in soybean processed commodities. The residues of flumioxazin and 1-OH-HPA were <0.01 ppm in soybean seed harvested 112 days following a single pre-emergence application of flumioxazin at 0.48 lb ai/A (5x rate), and in all regulated soybean processed commodities.

OPPTS GLN 860.1480: Meat/Milk/Poultry/Eggs

Based upon the proposed tolerances for peanuts and soybeans, the MTDBs for flumioxazin residues in cattle and poultry diets are 0.0035 and 0.0045 ppm, respectively (Table 20).

Ruminant and poultry feeding studies were not submitted with the current petition and none are required. Based on data from the available poultry and goat metabolism studies, which were conducted at >2,000x the MTDB, there is no reasonable expectation that quantifiable residues of flumioxazin will occur in livestock commodities [40 CFR 180.6(a)(3)]. Therefore, livestock feeding studies and tolerances for animal commodities are not required at the present time. If the use of flumioxazin is expanded to include other livestock feed items, the need for feeding studies will be reevaluated.

Table 20. Calculation of theoretical maximum dietary burdens of flumioxazin residues in livestock diets. [a]

Feed Commodity	% Dry Matter [b]	% Diet [b]	Proposed Tolerance (ppm)	Dietary Contribution (ppm) [c]
Beef and Dairy Cattle				
soybean, seed	89	15	0.01	0.0017
peanut, meal	85	15	0.01	0.0018
TOTAL BURDEN				**0.0035**
Poultry				
soybean, seed	NA	20	0.01	0.0020
peanut meal	NA	25	0.01	0.0025
TOTAL BURDEN				**0.0045**
Swine				
soybean, seed	NA	25	0.01	0.0025
peanut, meal	NA	15	0.01	0.0015
TOTAL BURDEN				**0.0040**

[a] As the petitioner has proposed an appropriate feeding restriction for soybean forage and hay, these commodities were not included in the dietary burden calculation.
[b] Table 1 (August 1996).
[c] Contribution = [tolerance / % DM (if cattle)] X % diet).

OPPTS GLN 860.1850: Confined Accumulation in Rotational Crops

In conjunction with the previous EUP request for use of flumioxazin on soybeans (PP#3G4250, DP Barcode D194594, J. Garbus, 9/21/94), the petitioner submitted an acceptable confined rotational crop study in which [phenyl-^{14}C]flumioxazin was applied to soil at 0.096 or 0.19 lb ai/A (1x and 2x rates for soybeans/peanuts; 0.25 and 0.5x rate for sugarcane). At a 30-day PBI, TRRs were <0.01 ppm in all rotational crop commodities from the 1x treatment, except wheat straw (0.013 ppm). For the 2x treatment, TRRs were 0.005-0.029 ppm in rotational crop commodities from the 30-day PBI. Chromatographic analyses of straw and chaff extracts indicated that flumioxazin and its metabolites were each present at <0.01 ppm in straw. There are currently no rotational crop restrictions on the proposed label.

With the current petition, Valent has submitted an additional confined rotational crop study (MRID No. 44295049) depicting the metabolism of [THP-^{14}C]flumioxazin in representative rotational crops. The in-life phase and analytical phases of the study were performed by PTRL West, Inc., Richmond, CA.

The [THP-^{14}C]flumioxazin had a radiochemical purity of >97% and a specific activity of 4.04 GBq/mmol (685,980 dpm/μg). The test substance was dissolved in acetone and applied directly to the surface of a series of test plots. Each plot consisted of a 3 ft × 2.5 ft × 2 ft wooden box lined with plastic and filled with a sandy loam soil (78% sand, 10% silt, and 12% clay; 1.2% organic matter; CEC 11.7 meq/100 g; pH 6.7). [THP-^{14}C]Flumioxazin was applied to separate plots at a rate of 0.094 or 0.187 lb ai/A (1x or 2x the soybean/peanut rates; 0.25x or 0.5x the sugarcane rate). (Note that in the remainder of this discussion the 1x/2x designations will be used for the rates). The test plots were subdivided and planted with lettuce, carrots and wheat at 30, 120, 180, or 365 days after treatment, with the 1x treated plot being planted only at the 30-day PBI. The initial crops of lettuce (1x and 2x plots) and carrots (2x plot) planted at the 30-day PBI failed because of phytotoxicity and were replanted at 60 days post-treatment. The 2x-treated lettuce crop again failed and was replanted successfully at 90 days post-treatment. Throughout the study, the test plots were maintained in a screen house that was enclosed and heated during the winter. The crops received water, fertilizer, and maintenance pesticides in accordance with standard agricultural practices. Adequate information pertaining to the growing conditions was provided.

For each PBI, one third of the wheat crop was harvested at an immature stage for wheat forage. Samples of lettuce, carrots (roots and tops), and wheat (grain, straw and chaff) were harvested at crop maturity. The planting to harvest intervals for each commodity were as follows: 66-112 days after planting (DAP) for lettuce, 114-145 DAP for carrot roots and tops, 58-75 DAP for wheat forage, and 129-133 DAP for wheat grain, straw and chaff. Composited soil samples (0-3"and 3-6" depth) were also collected from each plot prior to treatment, immediately after application, at each planting interval, and at each crop harvest. After collection, samples were stored at the field site in freezers prior to being shipped overnight on dry ice to the analytical laboratory, where samples were stored frozen (-20°C) until analysis.

Total radioactive residues (TRR)

At least triplicate subsamples of each RAC and soil sample were combusted and radioassayed by LSC. The LOD for the radioassay was 0.0001 and 0.0006 ppm for soil and plant samples, respectively. The TRRs in/on plant commodities grown in [THP-^{14}C]flumioxazin treated soil are presented in Table 21. Total radioactive residues were approximately 2-4x as high in rotational crops planted following application of the [THP-^{14}C]-label as they were in crops planted in [phenyl-^{14}C]-treated soil from the earlier confined rotational crop study.

For the 1x application, ^{14}C-residues were <0.01 ppm in lettuce (0.004 ppm) and wheat forage (0.006 ppm) from the 60- and 30-day PBI, respectively, but were >0.01 ppm in carrot roots and tops (0.028 and 0.010 ppm) and wheat grain and straw (0.013 and 0.057 ppm) from the 30-day PBI.

For the 2x application, TRR in lettuce was 0.003-0.012 ppm from the 90- to 365-day PBIs. For carrots, TRR in tops and roots from the 60-day PBI were 0.045 and 0.022 ppm, respectively, and declined to 0.013 and 0.005 ppm by the 180-day PBI. For wheat forage, TRRs were 0.004-0.015 ppm with the highest value occurring at the 180-day PBI. For wheat grain and straw, TRRs were 0.005-0.023 ppm and 0.049-0.131 ppm, respectively, with the highest values occurring at the 120-day PBI.

TRRs in soil (0-3") samples were 0.100 ppm immediately following the 1x application and declined to 0.062-0.067 ppm by crop maturity for the 30-day PBI (159-175 days post-treatment). For the 2x treatment, TRRs in soil (0-3") samples averaged 0.194 ppm immediately following application and declined slowly thereafter to 0.074-0.122 ppm at the final crop harvest for the 365-day PBI (431-494 days post-treatment). ^{14}C-Residues were relatively immobile in the soil, with <0.001 ppm in the 3-6" samples from the 1x treatment at up to 175 days post-treatment and ≤0.009 ppm in the 3-6" samples from the 2x treatment at up to 494 days post-treatment.

Table 21. Total radioactive residues in representative rotational crops planted at various intervals following a soil application of [THP-^{14}C]flumioxazin at 0.096 or 0.18 lb ai/A (1x or 2x maximum seasonal rate for peanuts/soybeans)

Crop	Commodity	Plant-back Interval (days)	Sampling Interval (DAP) [a]	Total Radioactive Residues (ppm) [b]	
				1x rate	2x rate
Lettuce	leaves	60/90 [c]	112/82	0.004	0.003
		120	75	NA	0.006
		180	73	NA	0.012
		365	66	NA	0.004
Carrot	tops	30/60 [c]	145/115	0.028	0.045
		120	133	NA	0.026
		180	114	NA	0.013
		365	129	NA	0.013
	roots	30/60 [c]	145/115	0.010	0.022
		120	133	NA	0.010
		180	114	NA	0.005
		365	129	NA	0.004
Wheat	forage	30	65	0.006	0.008
		120	75	NA	0.011
		180	58	NA	0.015
		365	66	NA	0.004
	straw	30	129	0.057	0.072
		120	133	NA	0.131
		180	128	NA	0.062
		365	129	NA	0.049
	chaff	30	129	0.026	0.033
		120	133	NA	0.043
		180	128	NA	0.027
		365	129	NA	0.016
	grain	30	129	0.013	0.017
		120	133	NA	0.023
		180	128	NA	0.008
		365	129	NA	0.005

[a] DAP = days after planting.

[b] Expressed in [^{14}C]flumioxazin equivalents.

[c] At the 30-day PBI, lettuce planted in 1x and 2x treated soil and carrots planted in 2x treated soil failed due to phytotoxicity and were replanted at 60 days post-treatment; the 2x-treated lettuce crop again failed and was replanted successfully at 90 days post-treatment.

Extraction and hydrolysis of residues

Plant samples with TRRs of ≥0.01 ppm were used for characterization of ^{14}C-residues. Samples were initially extracted with acetone:water (4:1 v/v) and filtered. If ^{14}C-residues remaining in the post-extraction solids (PES) still accounted for >0.01 ppm, the residual solids were then reextracted with acetone:0.1 N HCl. If ^{14}C-residues in the PES fraction were still >0.01 ppm, the sample was then extracted by refluxing with ACN:0.25 N HCl (4:1, v/v) for 4 hours. A final extraction with aqueous 6N HCl at reflux for 4 hours was conducted on PES fractions from carrot tops and wheat straw from the 2x application at a 30-day PBI. Fractionation of ^{14}C-residues in rotational crop samples is presented in Table 22. Solvent fractions containing ≥0.01 ppm of radioactivity were analyzed by HPLC, and selected fractions were also analyzed by TLC.

With the exception of wheat grain, solvent extraction adequately released the radioactivity from crop samples with TRRs of >0.01 ppm. The initial solvent extraction (ACN:water) released 37.6-82.5% of the TRR from crop matrices, excluding wheat grain (5.4-12.6% TRR released), and radioactivity remaining in the final PES fractions was ≤0.01 ppm for all RACs except wheat grain and straw.

For further characterization of ^{14}C-residues in PES fractions from wheat grain and straw, the final PES fractions from 2x-treated, 120-day PBI wheat grain (0.020 ppm) and straw (0.014 ppm) were subjected to a series of extraction/hydrolyses designed to fractionate the PES sample into various natural plant compounds. These PES fractions were sequentially extracted by (i) extraction with 0.05 M Phosphate (P_i) buffer at pH 7; (ii) enzymatic hydrolysis with α-amylase in P_i buffer (pH 7) at 30°C for 20 hours to release starch, (iii) enzymatic hydrolysis with pronase (0.05 M Tris buffer, pH 7.2) at 25°C for 20 hours to release proteins; (iv) extraction with 0.05 M EDTA (pH 4.5) at 70°C for 20 hours to release pectins; (v) extraction with aqueous glacial acetic acid and sodium chlorite at 70°C for 4 hours to release lignins; (vi) extracted with aqueous potassium hydroxide (24%) at 25°C for 24 hours to release hemicellulose; and (vii) hydrolyzed with concentrated sulfuric acid at 25°C for 4 hours to digest cellulose. The resulting extracts/hydrolysates were filtered or centrifuged, and radioassayed.

Results from the extraction/hydrolyses of the wheat grain and straw PES fractions are presented in Table 23. The sequential extractions/hydrolyses adequately released the radioactivity remaining in these PES fractions; ≤0.004 ppm of radioactivity remained in the residual solids. Of the solubilized radioactivity, no single fraction accounted for >0.005 ppm; therefore, these fractions were not further analyzed.

Table 22. Fractionation of ^{14}C-residues in RACs harvested from representative rotational crops grown in a sandy loam soil treated with [THP-^{14}C]flumioxazin at 1x and 2x the soybean/peanut rates

Fraction	1x rate: 30-day Plant-back Interval									
	Carrot tops		Carrot roots		Wheat straw		Wheat grain		Wheat Chaff	
	%TRR [a]	ppm [b]	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TTR	ppm
Acetone:H_2O	**61.6** [c]	**0.017**	78.9	0.008	**52.3**	**0.030**	7.2	0.001	**47.5**	**0.012**
Acetone:0.1N HCl	7.0	0.002	5.5	0.001	16.3	0.009	NA	--	14.3	0.004
ACN:0.25 HCl (4 hr reflux)	NA [d]	--	NA	--	12.0	0.007	NA	--	NA	--
PES	19.5	0.006	12.6	0.001	13.7	0.008	75.1	0.009	40.0	0.010
	2x rate: 30-day Plant-back Interval									
Fraction	Carrot tops		Carrot roots		Wheat straw		Wheat grain		Wheat Chaff	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TTR	ppm
Acetone:H_2O	**49.0**	**0.022**	**57.2**	**0.013**	**46.9**	**0.034**	5.4	0.001	**37.6**	**0.012**
Acetone:0.1N HCl	8.3	0.004	4.2	0.001	**15.1**	**0.011**	NA	--	14.7	0.005
ACN:0.25 HCl (4 hr reflux)	17.2 [e]	0.003	NA	--	22.0 [f]	0.009	NA	--	14.1	0.005
PES	5.2	0.002	9.0	0.002	3.9	0.003	77.2	0.013	22.5	0.007
	2x rate: 120-day Plant-back Interval									
Fraction	Carrot tops		Carrot roots		Wheat forage		Wheat straw		Wheat grain	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TTR	ppm
Acetone:H_2O	**63.2**	**0.016**	66.9	0.007	68.7	0.008	**61.4**	**0.080**	12.6	0.003
Acetone:0.1N HCl	7.7	0.002	5.9	0.001	5.4	0.001	17.8	0.023	NA	--
ACN:0.25 HCl (4 hr reflux)	NA	--	NA	--	NA	--	9.0	0.012	NA	--
PES	27.3	0.007	22.9	0.002	19.5	0.002	10.5 [g]	0.014	87.4 [g]	0.020
	2x rate: 180-day Plant-back Interval									
Fraction	Lettuce		Carrot tops		Wheat forage		Wheat straw		Wheat chaff	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Acetone:H_2O	47.5	0.006	58.8	0.008	57.4	0.009	**43.1**	**0.027**	**39.1**	**0.011**
Acetone:0.1N HCl	8.9	0.001	NA	--	NA	--	**21.1**	**0.013**	18.3	0.005
ACN:0.25 HCl (4 hr reflux)	NA	–	NA	--	NA	--	9.2	0.006	11.7	0.003
PES	34.3	0.004	52.2	0.007	29.3	0.004	12.6	0.008	22.3	0.006
	2x rate: 365-day Plant-back Interval									
Fraction	Carrot tops		Wheat straw		Wheat chaff		NA		NA	
	%TRR	ppm	%TRR	ppm	%TRR	ppm				
Acetone:H_2O	47.3	0.006	**82.5**	**0.040**	**68.8**	**0.011**				
PES	35.0	0.005	33.4	0.016	29.9	0.005				

[a] %TRR values are not corrected for recovery.
[b] Expressed in [^{14}C]flumioxazin equivalents.
[c] **Bolded** fractions were analyzed by HPLC.

[d] NA = Not applicable.
[e] Fraction includes radioactivity released by aqueous 6N HCL reflux for 4 hours (11.4% TRR, 0.005 ppm).
[f] Fraction includes radioactivity released by aqueous 6N HCL reflux for 4 hours (10% TRR, 0.007 ppm).
[g] PES fractions from 120-day PBI wheat grain and straw were subjected for further extraction (see Table 21).

Table 23. Fractionation of PES fractions from wheat grain and straw of 2x treated plants from the 120-day PBI

Fraction	120-Day PBI			
	Wheat grain (0.023 ppm) [a]		Wheat straw (0.131 ppm)	
	%TRR [b]	ppm [c]	%TRR	ppm
Solvent extracted [d]	12.6	0.003	88.2	0.116
PES	87.4	0.020	10.5	0.014
Phosphate buffer	7.4	0.002	1.8	0.002
Starch fraction	11.9	0.003	0.6	0.001
Protein fraction	9.0	0.002	ND [e]	--
Pectin fraction	3.3	0.001	0.4	0.001
Lignin fraction	4.9	0.001	3.9	0.005
Hemicellulose fraction	20.6	0.005	2.4	0.003
Cellulose fraction	14.6	0.003	4.0	0.005
Residual solids	12.3	0.003	2.7	0.004

[a] Values in parentheses are the TRR for each sample.
[b] Values are not normalized for the percent recovery.
[c] Expressed in [^{14}C]flumioxazin equivalents.
[d] See Table 20.
[e] ND= not determined.

Characterization/identification of residues

Radioactive residues in acetone:water and acetone:HCl fractions containing >0.01 ppm of radioactivity (see Table 20) were analyzed and quantified by reverse-phase HPLC using a C_{18} column eluted using a gradient of ACN to water (containing 0.01% trifluoroacetic acid). Unlabeled reference compounds were co-chromatographed with treated samples and were detected using a UV detector (254 nm). ^{14}C-Residues were detected and quantified using an in-line radioactivity monitor or by LSC of collected fractions. Selected solvent fractions were also analyzed by TLC on silica gel plates using various solvent systems. For TLC analyses, reference compounds were visualized

under a UV light (254 nm) and ^{14}C-residues were detected using a radioisotopic imaging system. Quantitation of ^{14}C-residues in acetone:water fractions from selected rotational crops grown in soil treated at 1x or 2x with [THP-^{14}C]flumioxazin is summarized in Table 24.

For the 1x-treated crop samples (30-day PBI) with radioactivity >0.01 ppm in the acetone:water extracts, HPLC analyses indicated that the majority of extractable ^{14}C-residues were comprised of unknown polar components (28-29% TRR; 0.007-0.015 ppm). Low levels of flumioxazin were detected (1.1-10.7% TRR; ≤0.003 ppm) in carrot tops and wheat chaff and straw, along with trace amounts (≤3.3% TRR, <0.001 ppm) of the metabolite 482-HA. Trace amounts (<0.001 ppm) of IMOXA and 482-CA were also tentatively detected in wheat straw and carrot tops, respectively.

For rotational crops grown in 2x-treated soil, only acetone:water fractions from carrot tops (30- and 120-day PBIs) and roots (30-day PBI) and wheat chaff and straw (all PBIs) had sufficient radioactivity (>0.01 ppm) for analysis. As with the 1x-treated samples, the majority of extractable ^{14}C-residues were comprised of unknown polar components (~10-30% TRR; 0.002-0.015 ppm). Flumioxazin was identified in these sample extracts at 0.4-26.9% of the TRR, but amounted to ≤0.009 ppm in all samples except wheat straw from the 120-day PBI (25.2% TRR, 0.033 ppm). Trace amounts of the metabolites 482-HA (≤3.7% TRR, ≤0.002 ppm), IMOXA (≤1.0% TRR, <0.001 ppm), and 482-CA (≤2% TRR, <0.001) were tentatively detected in carrot and wheat samples; however, the presence of these metabolites was not confirmed. Other metabolites tentatively identified in wheat straw from the 120- or 180-day PBIs included: SAT-I-OH-482 (6% TRR, 0.008 ppm), 1-OH-HPA (5.8% TRR, 0.004 ppm), THPA (6.7% TRR, 0.004 ppm), and TPA (6.6% TRR, 0.0004 ppm).

No ^{14}C-residues were identified in the acetone:HCl fractions from wheat straw (2x rate, 30- and 12-day PBIs). HPLC analyses showed a single polar region of radioactivity eluting with the column void, and TLC analysis of the same fraction showed radioactivity remaining at the origin.

Table 24. Summary of ^{14}C-residues detected by HPLC in the initial acetone/water extracts											
Crop/matrix	TRR (ppm) [a]	Residues (ppm) in acetone/water extract [b]									
		flumioxazin		482-HA		IMOXA		482-CA		Polar ^{14}C-residues [c]	
		% TRR [d]	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
1x rate, 30-day PBI											
Carrot tops	0.028	10.7	0.003	3.3	<0.001	ND	--	1.3	<0.001	28.6	0.008
Wheat chaff	0.026	1.1	<0.001	0.7	<0.001	ND	--	ND	--	26.9	0.007
Wheat straw	0.057	3.5	0.002	1.3	<0.001	0.9	<0.001	ND	--	26.3	0.015
2x rate, 30-day PBI											
Carrot tops	0.045	4.4	0.002	3.2	0.001	0.2	<0.001	0.6	<0.001	28.9	0.013
Carrot roots	0.022	22.7	0.005	0.7	<0.001	0.4	<0.001	ND	--	31.8	0.007
Wheat chaff	0.033	0.4	<0.001	1.3	<0.001	ND	--	ND	--	21.2	0.007
Wheat straw	0.072	4.2	0.003	1.6	<0.001	0.8	<0.001	0.8	<0.001	16.7	0.012
2x rate, 120-day PBI											
Carrot tops	0.026	26.9	0.007	1.0	<0.001	1.0	<0.001	0.5	<0.001	23.1	0.006
Wheat chaff	0.043	5.3	0.002	3.7	0.002	0.3	<0.001	1.3	<0.001	34.9	0.015
Wheat straw [e]	0.131	25.2	0.033	1.3	<0.001	ND	--	2.0	<0.001	9.2	0.012
2x rate, 180-day PBI											
Wheat chaff	0.027	8.3	0.002	2.1	<0.001	0.8	<0.001	ND	--	20.1	0.005
Wheat straw [f]	0.062	15.3	0.009	ND	--	ND	--	ND	--	2.8	0.002
2x rate, 365-day PBI											
Wheat chaff	0.016	7.4	0.001	ND	--	ND	--	ND	--	28.3	0.005
Wheat straw	0.049	13.3	0.007	ND	--	ND	--	ND	--	10.8	0.005

[a] All ^{14}C-residues are expressed in [^{14}C]flumioxazin equivalents.
[b] The presence of flumioxazin in carrot tops and wheat straw and chaff was confirmed by TLC analysis; all other residues were detected only by HPLC.
[c] Polar ^{14}C-residues were comprised of radioactivity eluting with or near the column void volume.
[d] %TRR values are not corrected for %recovery and were calculated by the reviewer.
[e] The metabolite SAT-1-OH-482 was also tentatively detected in wheat straw at 6% of the TRR (0.008 ppm).
[f] Other metabolites tentatively detected include: 1-OH-HPA (5.8% TRR, 0.004ppm), THPA (6.7% TRR, 0.004 ppm), and TPA (6.6% TRR, 0.0004 ppm).

Storage Stability

To verify the frozen storage stability of ^{14}C-residues during the study, the analytical laboratory compared HPLC analyses of extracts from carrot tops and roots and wheat chaff and straw obtained at the beginning and end of the analytical phase of the study.

Samples of carrot tops and roots (2x rate, 30-day PBI) were initially extracted within 2 months of harvest, and samples of wheat chaff and straw (2x rate, 30-day PBI) were initially extracted within 3 months of harvest. Sample extracts were analyzed by HPLC within 1 month of extraction. After an additional ~9 months of frozen storage, the original samples were reextracted and the original extracts and new extracts were analyzed by HPLC. The HPLC chromatograms indicated that the metabolite profile was stable during the 9 months of storage. No additional storage stability data are required to support this confined rotational crop study.

Conclusions:

The submitted confined rotational crop study is adequate for the proposed uses on peanuts and soybeans. Although TRR values for all RACs were slightly higher in the present study, results from the present study are consistent with results from the previous confined rotational crop study using [phenyl-^{14}C]flumioxazin (PP#3G4250, DP Barcode D194594, 9/21/94, J. Garbus).

Following a 1x application of [THP-^{14}C]flumioxazin to a sandy loam soil, TRRs were 0.004-0.057 ppm in RACs from representative rotational crops planted 30 days post-treatment (60 days for lettuce, because of phytotoxicity). Extraction and analysis of plant samples with TRRs >0.01 ppm indicated that the majority of extractable ^{14}C-residues were comprised of unknown polar components each present at ≤0.015 ppm. Low levels of flumioxazin (≤0.003 ppm) were detected in carrot tops and wheat chaff and straw, along with trace amounts (<0.001 ppm) of the metabolites 482-HA, IMOXA, and 482-CA.

Following a 2x application, TRRs were 0.003-0.072 ppm in RACs from representative rotational crops planted 30 days post-treatment (90 days for lettuce and 60 days for carrots, due to phytotoxicity). Although the highest TRR values were obtained at the 120-day PBI (0.131 ppm in wheat straw), ^{14}C-residues generally declined at longer PBIs. As with the 1x-treated plant samples, the majority of extractable ^{14}C-residues were comprised of unknown polar components. Flumioxazin was detected at ≤0.009 in sample extracts from carrot tops and roots, and wheat chaff and straw, with the exception of wheat straw (0.033 ppm) from the 120-day PBI. Other metabolites tentatively identified included: 482-HA (≤0.002 ppm), IMOXA (<0.001 ppm), 482-CA (<0.001) in carrot and wheat samples and SAT-1-OH-482 (0.008 ppm), 1-OH-HPA (0.004 ppm), THPA (0.004 ppm), and TPA (0.0004 ppm) in wheat straw from later PBIs. The MARC has determined that the residue of concern in rotational crops is the parent compound (D272652, R. Loranger, 3/12/2001)

As TRR levels in rotational crops grown in soil treated at 1x were >0.01 ppm at the 30-day PBI, the 51% WP and DF product labels should be amended to specify a 30-day restriction on the planting of rotational crops other than peanuts or soybeans.

With respect to the proposed use on sugarcane, the highest rate used in the confined study was only 0.5x the maximum proposed rate. Taking into account that 0.03 ppm parent flumioxazin was found at the confined study's higher rate, limited rotational crop field studies reflecting the 0.383 lb ai/A rate will be needed to support the use on sugarcane. The petitioner may wish to consult with HED as to which crops to include in these studies.

cc: Douglas Dotson, RAB2 Reading File, Petition File Numbers 7F4841 and 0F6171

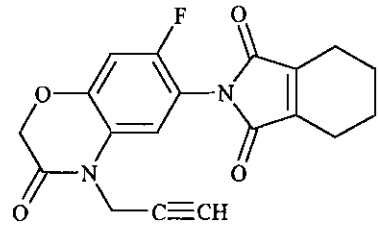
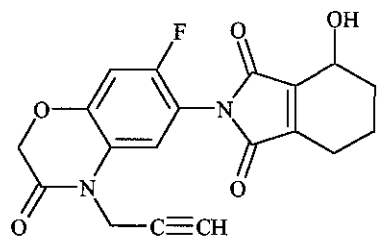
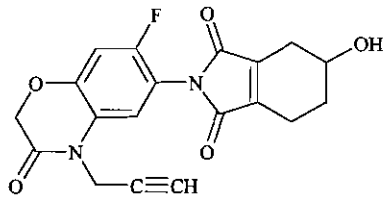
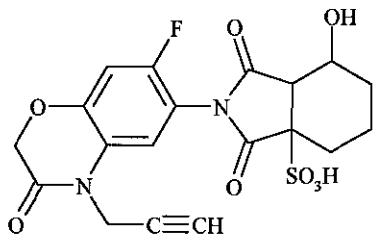
ATTACHMENTS 1 and 2

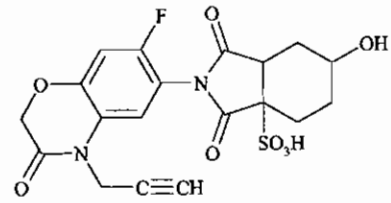
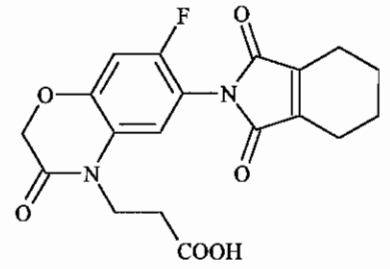
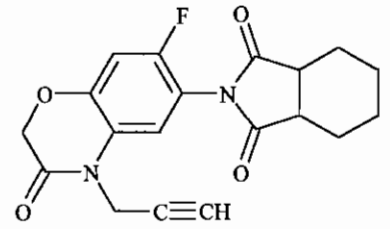
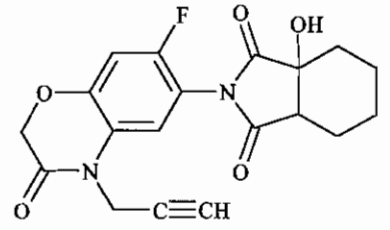
PERMANENT TOLERANCE PETITION
(PP#7F4841) FOR USE OF FLUMIOXAZIN
ON PEANUTS AND SOYBEANS

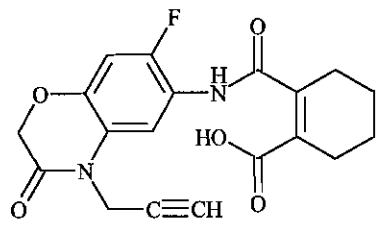
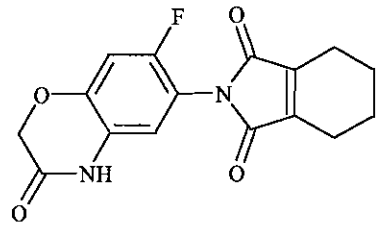
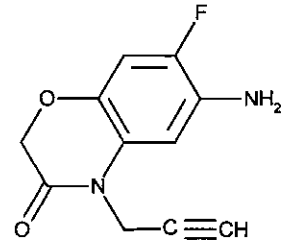
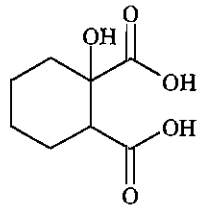
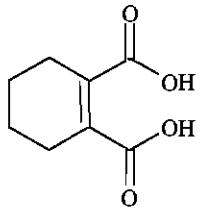
(DP Barcode D259493)

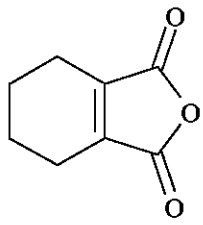
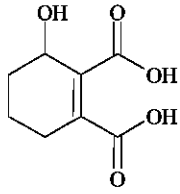
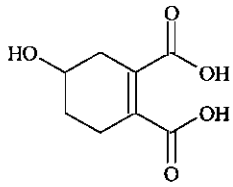
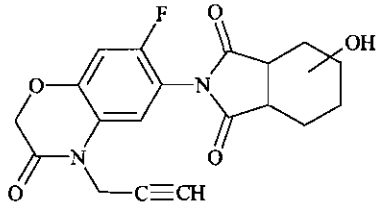
Attachment 1

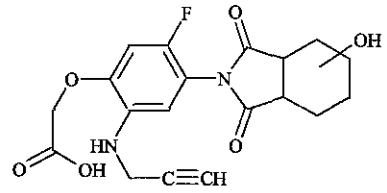
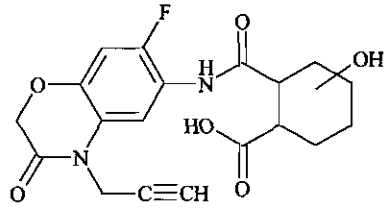
Table 1. Chemical names and structures of flumioxazin and its metabolites in plants and animals.

Common name Code(s)/chemical name	Chemical Structure	Matrices
Flumioxazin V-53482; S-53482 2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propynyl)-2*H*-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3(2H)-dione		Rotational crops (carrots and wheat) Goat liver and muscle Hen eggs, liver, muscle, fat, and skin with fat
3-OH-Flumioxazin 3-OH-S-53482 7-fluoro-6-(3-hydroxy-3,4,5,6-tetrahydrophthalimido)-4-(2-propynyl)-2H-1,4-benzoxazin-3(4H)-one		Goat liver and kidney Hen egg yolk, liver, muscle, fat, and skin with fat
4-OH-Flumioxazin 4-OH-S-53482 7-fluoro-6-(4-hydroxy-3,4,5,6-tetrahydrophthalimido)-4-(2-propynyl)-2H-1,4-benzoxazin-3(4H)-one		Goat milk, liver, kidney, and muscle Hen egg yolk, liver, muscle, fat, and skin with fat
3-OH-S-53482-SA 7-fluoro-6-(1-sulfo-3-hydroxy-1,2-cyclohexanedicarboximido)-4-(2-propynyl)-2H-1,4-benzoxazin-3(4H)-one		Hen egg yolk and liver

Common name Code(s)/chemical name	Chemical Structure	Matrices
4-OH-S-53482-SA 7-fluoro-6-(1-sulfo-4-hydroxy-1,2-cyclohexanedicarboximido)-4-(2-propynyl)-2H-1,4-benzoxazin-3(4H)-one		Hen egg yolk and liver
482-CA 2-[7-fluoro-3-oxo-6-(3,4,5,6-tetrahydrophthalimido)-2H-1,4-benzoxazin-4-yl] propionic acid		Rotational crops (carrots and wheat)
SAT-482 6-(cis-1,2-cyclohexane-dicarboximido)-7-fluoro-4-(2-propynyl)-2H-1,4-benzoxazin-3(4H)-one		Goat liver and kidney
SAT-1-OH-482		Rotational crops (wheat straw)

Common name Code(s)/chemical name	Chemical Structure	Matrices
482-HA N-[7-fluoro-3-oxo-4-(2-propynyl)-2H-1,4-benzoxazin-6-yl]-3,4,5,6-tetrahydrophthalamic acid		Rotational crops (carrots and wheat)
IMOXA 2-[7-fluoro-3,4-dihydro-3-oxo-2*H*-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3(2H)-dione		Rotational crops (carrots and wheat)
APF 6-amino-7-fluoro-4-(2-propenyl)-2H-1,4-benzoxazin-3(4H)-one		Soybean forage and hay
1-OH-HPA 1-hydroxy-*trans*-1,2-cyclohexanedicarboxylic acid		Rotational crops (wheat straw)
THPA 3,4,5,6-tetrahydrophthalic acid		Rotational crops (wheat straw) Goat liver and kidney Hen eggs, liver, muscle, fat, and skin with fat

Common name Code(s)/chemical name	Chemical Structure	Matrices
TPA 3,4,5,6-tetrahydrophthalic anhydride		Rotational crops (wheat straw) Hen egg white
3-OH-THPA 3-hydroxy-1-cyclohexene-1,2-dicarboxylic acid		Hen egg white
4-OH-THPA 4-hydroxy-1-cyclohexene-1,2-dicarboxylic acid		Goat milk, liver, kidney, and muscle Hen eggs, liver, muscle, fat, and skin with fat
Metabolite B; **Metabolite F**		Goat milk, liver, and kidney

Common name Code(s)/chemical name	Chemical Structure	Matrices
Metabolite C	 or 	Goat milk, kidney, and muscle

Attachment 2

INTERNATIONAL RESIDUE LIMIT STATUS

Chemical Name:	Common Name: **Flumioxazin**	X Proposed tolerance □ Reevaluated tolerance □ Other	Date:

Codex Status (Maximum Residue Limits)	U. S. Tolerances
X No Codex proposal step 6 or above □ No Codex proposal step 6 or above for the crops requested	Petition Number: PP#7F4841 DP Barcode: D259493 Other Identifier:
Residue definition (step 8/CXL): N/A	Reviewer/Branch: J. Early, Dynamac
	Residue definition: Flumioxazin

Crop (s)	MRL (mg/kg)	Crop(s)	Tolerance (ppm)

Limits for Canada	Limits for Mexico
X No Limits □ No Limits for the crops requested	X No Limits □ No Limits for the crops requested
Residue definition: N/A	Residue definition: N/A

Crop(s)	MRL (mg/kg)	Crop(s)	MRL (mg/kg)

Notes/Special Instructions:
S. Funk, 06/07/2000

Rev. 1998

13544

028299

Chemical:	**Flumioxazin**
PC Code:	**129034**
HED File Code	**11000 Chemistry Reviews**
Memo Date:	**03/12/2001**
File ID:	**DPD259493**
Accession Number:	**412-02-0010**

HED Records Reference Center
01/23/2002