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

SEP 28 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: PP#3G4250: New Chemical EUP: V-53482, Flumioxazin on Soybeans. Evaluation of Analytical Methods and of Residue Data. MRID Nos. 428840-11 thru -14; 428840-16 thru -19. DP Barcodes D194587 and D194594. CBTS Nos. 12469 and 12470

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and

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Valent U.S.A. Corporation, Walnut Creek, CA, has petitioned for temporary tolerances for the herbicide V-53482, also known as S-53482, (flumioxazin) [7-fluoro-6-[(3,4,5,6-tetrahydro)phthalimido)]-4-(2-propynyl)-1,4-benzoxazin-3(2H)-one] in or on soybean seed and soybean forage at 0.01 ppm.

**Conclusions and Recommendations**

1. The product chemistry data for Product Chemistry Series 61-1, 2, and 3, for Product Chemistry Series 62-1, 2, and 3, and for Series 63 for the technical grade active ingredient were submitted and reviewed as part of a request for a non-food use registration of flumioxazin. (See MRID's 42684901 - 42684904.) RD concluded (A. Smith, memo of April 16, 1993) that the data met the product chemistry requirements of 40CFR 158.150-158.190 and that no additional information was needed. The product chemistry data requirements for flumioxazin have been met.

2. We conclude that the proposed label is suitable for the proposed use in an EUP. However, formulated flumioxazin is packaged in water-soluble packets with application rates given in terms of acres per packet rather than lbs or

ounces per acre. This may lead to confusion especially when the suggested seasonal maximum application is given as 3 ounces per acre. CBTS would suggest the label state the application rates in ounces per acre together with a table giving the number of packets per acre needed to achieve that rate.

3. The protocol for the proposed EUP mixes metric and English units together in giving rates. We would suggest that all units be given in the English system with which the majority of the agricultural community is familiar. This is not an objection to the proposed EUP program. Our comments regarding the expression of rates and units also used should be considered in proposing the label for permanent registration.

4. The proposed tolerance expression specifies rates in metric units. Although it is not necessary to specify rates in a Section F tolerance proposal, the petitioners wording in this instance again shows the confounding of English and metric units.

5a. The plant metabolism studies are adequate for delineating a degradative pathway for flumioxazin.

5b. For the purposes of this EUP only we will consider the parent as the residue of concern for plants. However it is evident that the major components of the residue in plants are 3,4,5,6-tetrahydro-phthalic acid (THPA); and 1-hydroxy-trans-1,2-cyclohexanedicarboxylic acid (1-OH-HPA).

5c. We consider the animal metabolism studies as adequate in delineating the degradative pathways of flumioxazin in ruminants and poultry. However, if additional uses are requested for flumioxazin resulting in higher residues on feed items, additional studies of ruminant metabolism may be needed.

5d. About 35% of the recovered radioactivity in animal tissues and milk was identified, the predominant metabolite being 482-HA, a metabolite not found in the plant metabolism study. Anywhere from 5 to 24 discrete peaks or areas of radioactivity were detected that are characterized as unknown. However, the profile of metabolites characterized in the ruminant study does not resemble that found with soybean plants.

5e. In poultry tissue and eggs, identification of known metabolites accounted for from 10 to 55% of the total tissue radioactivity. The profile of metabolites in the residues of poultry tissues and eggs is different from that found in soybeans and in ruminant tissues. In this instance the major component of the residue is parent flumioxazin.

5f. For the purposes of this EUP only we will consider the parent as the residue of concern for ruminants and poultry. However it is evident that the major component of the residue in ruminants is the metabolite 482-HA while in poultry tissues the parent is the predominant component of the residue.

5g. For the purposes of this EUP only we will consider the parent as the residue of concern for plants, ruminants, and poultry. As noted in

Conclusion 8, tolerances for residues in animal commodities are not needed at this time.

5h. The components of the residue of this low application herbicide that need to be regulated in conjunction with a permanent tolerance petition needs clarification. CBTS in conjunction with HED's Metabolism Committee will determine which components of the residue need to be regulated and how the tolerance expression should be worded. We expect to use our experience with an analogous low application herbicide, flumiclorac-pentyl, for guidance and precedent.

6a. The analytical methodology described in this presentation and used to generate residue data appears to be suitable for the required enforcement method.

6b Documentation is provided to show that the method has been independently validated as required by PR 88-5.

6c. For the purposes of this EUP petition we will accept the demonstration of the storage stability of flumioxazin residues in soybean matrices for 12 months.

6d. The method for the parent must be validated by the Agency's analytical chemistry section. Concomitant with the establishment of temporary tolerances for this EUP, we will forward the method for Agency validation to expedite the establishment of permanent tolerances.

6e. No data is presented for the behavior of flumioxazin in multiresidue testing protocols. Such data will be needed for the establishment of permanent tolerances for flumioxazin.

6f. No confirmatory methodology was submitted with this petition. A confirmatory method including validation and representative chromatograms should be submitted with the permanent tolerance request.

7a. Field trial results with soybeans and results from processing studies with fractions derived from treated rac's support the proposed temporary tolerances for this EUP. However, although trials were conducted with soybean hay, no tolerance has been proposed for this commodity. As no residues were reported in any rac or processed commodity, the requested tolerances of 0.01 ppm are adequate and represent the limit of quantitation of the proposed analytical method.

7b. Tolerances are proposed for soybean seed and forage but not for hay. Soybean hay is an agricultural of commodity of concern as a component of animal diets. As a temporary tolerance is not proposed for this commodity, the petitioner should propose a tolerance of 0.01 ppm.

8. It is unlikely that sufficient residues will be present in/on feed stuffs derived from flumioxazin treated commodities of this petition to result in detectable secondary residues in animal tissues, milk, and eggs.

This is a Section 180.6 (a)3 situation with respect to secondary residues in animal tissues, milk, and eggs. Tolerances for these commodities are not needed.

9. The planting of a rotational crop after a normal harvest interval for treated soybeans will not result in any detectable residues in the rotated crop. Thus rotational crop restrictions for flumioxazin are not needed.

Recommendation

We recommend for the proposed EUP and its associated temporary tolerances provided that a tolerance of 0.01 ppm is proposed for soybean hay. The other deficiencies cited above in Conclusions 2, 3, 4, 5h, 6e, and 6f should be addressed in the petition for the permanent tolerance request.

**Product Chemistry**

The present submission includes a table summarizing the physical-chemical characteristics of the technical flumioxazin and of two end use products. Also included is a CSF for one of the formulated end-use products.

Comment:

According to the petitioner, the product chemistry data for Product Chemistry Series 61-1, 2, and 3, for Product Chemistry Series 61-1, 2, and 3, and for Series 63 were submitted for the technical grade active ingredient as part of a request for a non-food use registration of flumioxazin. (See MRID's 42684901 - 42684904.) The data was reviewed by Alfred Smith, (memo of April 16, 1993) who concluded that the product met the product chemistry requirements of 40CFR 158.150-158.190 and that no additional information was needed. A copy of this review is attached.

Conclusion:

The product chemistry data requirements for flumioxazin have been met.

**Residue Chemistry**

**Directions for Use**

General

Flumioxazin is intended to be used as a selective herbicide for the pre- or post-emergence control of susceptible broadleaf weeds and grasses in soybeans at low rates. The end use product contains 51% active material formulated as a wettable powder or granule in water-soluble packets. Each packet contains 6 ounces of formulated material.

For pre-emergence use, flumioxazin can be used alone or in tank-mixes with Lasso (alachlor), Dual (metalochlor), or Prowl (pendimethalin). Flumioxazin can be applied sequentially as a pre-emergent use following trifuralin, Sonalan (ethalfluralin), or Prowl (pendimethalin). For post-emergence use prior to the emergence of the crop, flumioxazin can be used alone or in tank-mixes with Lasso, Dual, Prowl, Roundup (glyphosate), Gramoxone Extra (paraquat), Select (clethodim), Bronco (alachlor plus glyphosate), Pursuit (imazethapyr), Pursuit Plus (imazethapyr plus pendimethalin), Scepter (imazaquin), Squadron (imazaquin plus pendimethalin), or Weedone LV4 (2,4-D). A crop oil concentrate should be added to the post-emergent tank-mixes, except those with Roundup or Gramoxone Extra. All of the co-tank-mix pesticides have established tolerances on soybeans.

Soybeans

The label directions for the amount to be applied are unique in that they specify the number of acres that can be treated with one 6-ounce packet of the formulated herbicide. The rates of application depend upon the weed species to be controlled, the type of soil, the organic matter and clay

content of the soil, the cultivation method, and the stage at which the herbicide is applied. Depending upon the specifics of these conditions, 4, 3, 2.4, or 2 acres can be treated per 6-ounce packet. These rates are equivalent to 0.76, 1.02, 1.28, or 1.53 ounces ai/A. A maximum of 3 ounces (1.53 ounces ai) formulated material per acre per season is permitted.

Application volumes are 10 to 30 gallons of water per acre for conventional tillage, a minimum of 20 gallons for burndown in reduced or no-tillage applications, or up to 30 gallons if dense vegetation or crop residues are present.

There are no restriction on rotational crops other than those imposed by the other components of tank-mixes. There are restrictions upon application to water, whether directly to irrigation systems, surface water, intertidal areas, or areas of potential runoff or from equipment wash water.

Comment

Application rates are generally given as an amount (ounces, grams, etc) per acre. Couching the application rates in terms of acres per packet rather than ounces per acre may lead to confusion especially when the suggested seasonal maximum application is given as 3 ounces per acre. CBTS would suggest the label state the application rates in ounces per acre together with a table giving the number of packets per acre needed to achieve that rate.

We conclude that the proposed label is suitable for the proposed use in an EUP. However, for a permanent tolerance we suggest the petitioner revise the label to give application rates in terms of ounces per acre with a supplementary table indicating the number of packets to use to achieve these rates.

**Proposed Experimental Use Program**

Soybeans

Valent proposes to conduct 57 trials at 17 sites in all major soybean growing states over a two year period. Of the 1,260 acres to be treated, most sites (38) will be devoted to reduced tillage/no-tillage treatments; 16 sites will be devoted to conventional tillage ; and 3 sites to trials of worker exposure. The total maximum application rate will be 3 oz formulated (43.4 grams a.i.) per acre for a total of 89,938 grams (198.1 lbs) of active ingredient over the two years including the trials of worker exposure. If the worker exposure trials are excluded the petitioner calculates that 155 lbs of active ingredient will be required

Comment:

The description of the proposed EUP mixes metric and English units together in giving rates. We would suggest that all units be given in the English system with which the majority of the agricultural community is familiar.

The proposed EUP protocol also demonstrates the difficulty in achieving the suggested rates by treating a number of acres per packet. For example, if the proposed 15 acre site in MD is to be treated at a maximum of 3 ounces formulated per acre this requires 45 ounces or 7.5 packets. Giving application rates in terms of acres per packet does not allow for situations such as this that requires fractional packets.

Conclusion

We have no objections to the proposed EUP program. Our comments regarding the expression of rates and units used should be considered in proposing the label for permanent registration.

Proposed Tolerances

Valent U.S.A. Corporation, Walnut Creek, CA, has petitioned for temporary tolerances for the active ingredient flumioxazin (V-53482) [7-fluoro-6-[(3,4,5,6-tetrahydro)phthalimido)]-4-(2-propynyl)-1,4-benzoxazin-3(2H)-one] in or on soybean seed and soybean forage at 0.01 ppm when applied at a maximum of 43.4 grams per acre per season.

Comment

Tolerances are proposed for soybean seed and forage but not for hay. As soybean hay is also a regulated RAC, the same tolerance of 0.01 ppm should be proposed for this commodity. It is not necessary to specify rates in a Section F tolerance proposal. The petitioner should submit a revised Section F proposing tolerances of 0.01 ppm for soybean seed, forage, and hay.

**Nature of the Residue in Plants**

**Soybeans:**

V-53482, labeled uniformly with $^{14}C$ in its phenyl ring (Sp. Ac.=179 mCi/mmol; radiochemical purity = 98.2%) or at positions 1 and 2 of the tetrahydrophthalimide ring (Sp. Ac.=105 mCi/mmol; radiochemical purity = 98.7%), was applied to the soil of pots containing soybean seeds 3 days after planting. Application rates were equivalent to 40 gms/acre (low rate, approximately equal to proposed label maximum seasonal rate of 43.4 grams/acre) and to 80 grams/acre (2x maximal label rate). Plants were grown under greenhouse conditions to half-maturity (53 days) and sampled for forage and forage hay or to full maturity (138 days) and sampled for seed, pods, and straw.

Aliquots of the sampled material were combusted and total radioactivity of the sample determined by liquid scintillation counting. Additional samples were extracted with solvents and aliquots of extracts subjected to TLC and HPLC. Immature plants (forage and forage hay) and mature soybeans were repeatedly extracted with acetone/water (4/1), and partitioned against hexane and ethyl acetate. The post-extraction-residues were subjected to enzymatic and chemical hydrolytic procedures to release potential bound residues. Aliquots of the extracts and of post-extraction-residues were

combusted for the determination of total radioactivity and subjected to chromatographic procedures for the determination of metabolites. Metabolites of V-53482 were determined by comparing mobilities and retention times of the metabolites with those of synthesized potential metabolites.

Mature pods and straw were not further examined as these commodities according to the petitioner are not defined as RAC's.

Results

Total Radioactivity of $^{14}C$ Residues of V-53482 as V-53482 Equivalents in Soybean Plant Samples after the Application of 40 grams/Acre (1X).

| | Phenyl-$^{14}C$ (ppm) | | %* | THP-1,2-$^{14}C$ (ppm) | | %* |
|---|---|---|---|---|---|---|
| PHI | 53 Days | 138 Days | | 53 Days | 138 Days | |
| Forage | 0.055 | ----- | 0.6 | 0.069 | ----- | 0.7 |
| Forage Hay | 0.155 | ----- | | 0.257 | ------ | |
| Seeds | ----- | 0.033 | 0.1 | ----- | 0.245 | 0.3 |
| Pods | ----- | 0.060 | 0.1 | ----- | 0.326 | 0.8 |
| Straw | ----- | 0.152 | 0.6 | ----- | 0.207 | 0.6 |

* Percentage of applied radioactivity

Total Radioactivity of $^{14}C$ Residues of V-53482 as V-53482 Equivalents in Soybean Plant Samples after the Application of 80 grams/Acre (2X).

| | Phenyl-$^{14}C$ (ppm) | | %* | THP-1,2-$^{14}C$ (ppm) | | %* |
|---|---|---|---|---|---|---|
| PHI | 53 Days | 138 Days | | 53 Days | 138 Days | |
| Forage | 0.108 | ----- | 0.7 | 0.196 | ----- | |
| Forage Hay | 0.348 | ----- | | 0.617 | ------ | |
| Seeds | ----- | 0.055 | 0.1 | ----- | 0.177 | 0.3 |
| Pods | ----- | 0.118 | 0.1 | ----- | 0.551 | 0.8 |
| Straw | ----- | 0.176 | 0.3 | ----- | 0.254 | 0.6 |

* Percentage of applied radioactivity

$^{14}C$ Distribution in Soybean Forage

| Label and Rate | Phe.- 1X | | Phe.- 2X | | THP - 1X | | THP - 2X | |
|---|---|---|---|---|---|---|---|---|
| | ppm | % | ppm | % | ppm | % | ppm | % |
| Extractable | 0.039 | 69.2 | 0.081 | 70.5 | 0.044 | 61.2 | 0.124 | 70.8 |
| Non-Extractable | 0.018 | 30.8 | 0.033 | 29.5 | 0.029 | 38.8 | 0.051 | 29.2 |

$^{14}C$ Distribution in Soybean Hay

| Label and Rate | Phe.- 1X | | Phe.- 2X | | THP - 1X | | THP - 2X | |
|---|---|---|---|---|---|---|---|---|
| | ppm | % | ppm | % | ppm | % | ppm | % |
| Extractable | 0.017 | 67.7 | 0.230 | 68.9 | 0.161 | 60.1 | 0.354 | 61.3 |
| Non-Extractable | 0.059 | 35.3 | 0.104 | 31.1 | 0.106 | 39.9 | 0.224 | 38.7 |

$^{14}C$ Distribution in Soybean Seeds

| Label and Rate | Phe.- 1X | | Phe.- 2X | | THP - 1X | | THP - 2X | |
|---|---|---|---|---|---|---|---|---|
| | ppm | % | ppm | % | ppm | % | ppm | % |
| Extractable | 0.012 | 35.9 | 0.022 | 45.0 | 0.145 | 66.5 | 0.108 | 66.3 |
| Non-Extractable | 0.023 | 64.1 | 0.029 | 55.0 | 0.072 | 33.5 | 0.051 | 33.7 |

Identification of Metabolites

Chromatographic analyses of the extracts revealed the presence of the parent and 4 major metabolites identified as: N[-7-fluoro-3-oxo-4-(2-propynyl)-2H -1,4-benzoxazin-6-yl] -3,4,5,6-tetrahydrophthalamic acid (482-HA); 6-Amino-7-fluoro-4-(2-propynyl)-1,4-benzoxazin-3(4H)-one (APF); 3,4,5,6-tetrahydrophthalic acid (THPA); and 1-hydroxy-trans-1,2-cyclohexanedicarboxylic acid (1-OH-HPA). (See attached figures for structures)

Beside the metabolites identified in the acetone/water extracts, additional components were detected at low levels (0.002 to 0.006 ppm). Two to 5 unidentified metabolites were seen in the hexane phase of the partition and 11 to 32 peaks in the ethyl acetate phase.

After the unextractable residues were subjected to enzymatic, acid, or base hydrolysis and reextracted, 2- 10 minor, unidentified peaks were seen. The only identified metabolites released by the hydrolytic procedures were THPA and 1-OH-HPA.

$^{14}C$ residues of V-53482 and its Metabolites as V-53482 Equivalents in Soybean Plant Samples Treated at 40 grams/Acre (1X).

| | Phenyl-$^{14}C$ (ppm) | %TRR | THP-1,2-$^{14}C$ (ppm) | %TRR |
|---|---|---|---|---|
| Forage | | | | |
| V-53482 | 0.004 | 6.1 | <LOQ of 0.001 | |
| 482-HA | <LOQ of 0.001 | | N.D. | |
| APF | <LOQ of 0.001. | | ----- | |
| THPA* | ---- | | 0.002 | 2.6 |
| 1-OH-HPA* | ---- | | 0.011 | 15.3 |
| Hay | | | | |
| V-53482 | 0.007 | 4.4 | 0.006 | |
| 482-HA | N.D. | | N.D. | |
| APF | <LOQ of 0.003. | | ----- | |
| THPA* | ---- | | 0.013 | 4.8 |
| (As bound THPA) | | | (<LOQ of 0.005) | |
| 1-OH-HPA* | ---- | | 0.085 | 31.5 |
| (As bound 1-OH-HPA) | | | (0.042) | (15.6) |
| Seeds | | | | |
| V-53482 | N.D. | | <LOQ of 0.004 | |
| 482-HA | N.D. | | N.D. | |
| APF | N.D. | | ----- | |
| THPA* | ---- | | 0.013 | 6.0 |
| (As bound THPA) | | | (<LOQ of 0.007) | |
| 1-OH-HPA* | ---- | | 0.092 | 42.2 |
| (As bound 1-OH-HPA) | | | (0.022) | (10.2) |

$^{14}C$ residues of V-53482 and its Metabolites as V-53482 Equivalents in Soybean Plant Samples Treated at 80 grams/Acre (2X).

| | Phenyl-$^{14}C$ (ppm) | %TRR | THP-1,2-$^{14}C$ (ppm) | %TRR |
|---|---|---|---|---|
| Forage | | | | |
| V-53482 | 0.006 | 5.5 | 0.008 | 4.4 |
| 482-HA | 0.001 | 0.7 | N.D. | |
| APF | <LOQ of 0.001. | | ----- | |
| THPA* | ---- | | 0.007 | 4.6 |
| 1-OH-HPA* | ---- | | 0.045 | 25.2 |
| (As bound 1-OH-HPA) | | | (0.017) | (9.4) |
| Hay | | | | |
| V-53482 | 0.017 | 5.2 | 0.030 | 5.1 |
| 482-HA | N.D. | | N.D. | |
| APF | <LOQ of 0.003. | | ----- | |
| THPA* | ---- | | 0.049 | 8.6 |
| (As bound THPA) | | | (0.018 | 3.1 |

$^{14}C$ residues of V-53482 and its Metabolites as V-53482 Equivalents in Soybean Plant Samples Treated at 80 grams/Acre (2X). (continued)

| | Phenyl-$^{14}C$ (ppm) | %TRR | THP-1,2-$^{14}C$ (ppm) | %TRR |
|---|---|---|---|---|
| Hay (continued) | | | | |
| 1-OH-HPA* | ---- | | 0.150 | 25.8 |
| (As bound 1-OH-HPA) | | | (0.082) | (14.1) |
| Seeds | | | | |
| V-53482 | N.D. | | <LOQ of 0.003 | |
| 482-HA | N.D. | | N.D. | |
| APF | N.D. | | ----- | |
| THPA* | ---- | | 0.006 | 4.0 |
| (As bound THPA) | | | (<LOQ of 0.003) | |
| 1-OH-HPA* | ---- | | 0.063 | 37.9 |
| (As bound 1-OH-HPA) | | | (0.008) | (5.0) |

* Free plus bound residue

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From these results the petitioner concludes that at the proposed label rate (40 grams/A) residues of parent and metabolites are low (0.01 ppm or less) with the exception of 1-OH-HPA. To account for the metabolic profile, the petitioner has proposed a degradative pathway in which the parent is cleaved to form 482-HA, followed by further hydrolysis to APF and THPA. Hydration of THPA then leads to 1-OH-HPA. (See attached figures)

Comment

The plant metabolism studies are adequate for delineating a degradative pathway for flumioxazin.

The petitioner did not examine soybean hay in their metabolic studies as it did not consider soybean hay a rac. The petitioner is wrong in not considering soybean hay as a RAC. Soybean hay is an agricultural of commodity of concern as it is a component of animal diets. (See Table II of the Residue Chemistry Guidelines) As a temporary tolerance is not proposed for this commodity, the petitioner should propose a tolerance of 0.01 ppm (see the Magnitude of the Residue in Plants section below) or the label should be revised to carry a restriction against the feeding of soybean hay.

For the purposes of this EUP only we will consider the parent as the residue of concern for plants. However, it is evident that the major components of the residue are the metabolites THPA and 1-OH-HPA and that the parent is present as a relatively low percentage of the total residue.

What components of the residue of this low application herbicide need to be regulated is a matter that needs clarification and will be considered in conjunction with the permanent tolerance petition. CBTS in conjunction with HED's Metabolism Committee will determine which components of the residue

need to be regulated and how the tolerance expression should be worded. We expect to use our experience with an analogous low application, low risk herbicide, flumiclorac-pentyl, for guidance and precedent.

**Nature of the Residue in Animals:**

Lactating Ruminants.

Two lactating dairy goats were fed the equivalent of 11.8 ppm of flumioxazin uniformly labeled in the phenyl ring (Sp. Ac.=179 mCi/mol; radiochemical purity = 98.2%) for 5 consecutive days. A untreated dairy goat served as control. Urine, feces, and milk were collected during the dosing period. The animals were sacrificed 6 hrs. after the last dose. The milk, feces and selected tissues were analyzed for retained radioactivity. The results were as follows:

Percentage of administered radioactive dose in tissues, milk, and excreta of animals fed $^{14}C$ flumioxazin.

| | Goat 1 | | Goat 2 | |
|---|---|---|---|---|
| Matrix | % | ppm | % | ppm |
| Rear Leg Muscle | 0.01 | 0.014 | 0.01 | 0.013 |
| Front Leg Muscle | 0.01 | 0.014 | 0.01 | 0.012 |
| Omental Fat | <0.01 | 0.006 | <0.01 | 0.005 |
| Perirenal Fat | <0.01 | 0.006 | <0.01 | 0.004 |
| Kidney | 0.02 | 0.182 | 0.01 | 0.110 |
| Blood | <0.01 | 0.019 | <0.01 | 0.025 |
| GI Tract | 0.81 | 0.296 | 0.01 | 0.246 |
| GI Tract Contents | 14.21 | 2.207 | 14.34 | 2.256 |
| Liver | 0.19 | 0.209 | 0.12 | 0.165 |
| Milk | 0.05 | 0.029 | 0.17 | 0.034 |
| Urine | 15.59 | | 15.72 | |
| Feces | 50.27 | | 50.16 | |
| Total | 80.16 | | 81.17 | |

Tissues and milk were subjected to solvent extraction and protease digestion. Aliquots of fractions of the separation procedures were subjected to HPLC and TLC chromatography with determination of radioactivity. Retention times and $R_f$'s of radioactivity were compared with those of postulated metabolites. See attached figure for identification and structures of metabolites cited as abbreviations.

Identification and Quantitation of Residues of Flumioxazin in Tissues and Milk of Animals Fed $^{14}C$ Flumioxazin.

| | Matrix | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Liver | | Kidney | | Muscle | | Milk | |
| | % | ppm | % | ppm | % | ppm | % | ppm |
| Metabolite | | | | | | | | |
| 3-OH & 4-OH SA | 1.8 | 0.004 | ND | <0.001 | ND | <0.001 | 6.5 | 0.002 |
| 482-HA | 9.8 | 0.020 | 8.7 | 0.016 | 4.2 | <0.001 | 14.4 | 0.004 |
| APF | 3.8 | 0.008 | 5.8 | 0.011 | 3.5 | <0.001 | 0.2 | <0.001 |
| 4-OH Flumioxazin | 6.5 | 0.014 | 13.7 | 0.025 | 1.6 | <0.001 | 1.5 | <0.001 |
| 3-OH Flumioxazin | 4.2 | 0.009 | 6.2 | 0.011 | 1.2 | <0.001 | 1.8 | <0.001 |
| Flumioxazin | 4.7 | 0.010 | 0.2 | <0.001 | 1.2 | <0.001 | ND | <0.001 |
| Total | 30.8 | | 34.6 | | 11.7 | | 24.4 | |

From these results, the petitioner concludes that the main metabolic transformations in goats involve the tetrahydrophthalimide moiety: 1) the cleavage of the imide linkage, 2) hydroxylation of the cyclohexene ring, and 3) incorporation of a sulfonic group. (See attached figure)

Comment

At best only about 35% of the recovered radioactivity in a tissue was identified, the predominant metabolite being 482-HA, a metabolite not found in the plant metabolism study. Anywhere from 5 to 24 discrete peaks or areas of radioactivity were detected that are characterized as unknown.

However, the profile of metabolites characterized in the ruminant study does not resemble that found with soybean plants. This may be due to the fact that flumioxazin labeled in the tetrahydrophthalimide moiety was not employed as in plant studies or that the animal metabolism is different than that of plants.

If additional uses are requested for flumioxazin resulting in higher residues on feed items, additional studies of ruminant metabolism may be needed.

Laying Hens.

Ten laying hens were fed daily the equivalent of 9.9 ppm flumioxazin uniformly labeled in the phenyl ring (Sp. Ac.=179 mCi/mol; radiochemical purity = 98.2%) for 14 days. Four untreated hens served as controls. Eggs and excreta were collected daily during the dosing period. The animals were sacrificed 4 hrs. after the last dose. The eggs, excreta, and selected tissues were analyzed for retained radioactivity. The results were as follows:

Quantitation of $^{14}C$ Residues in Tissues, Eggs, and Excreta of Hens Fed $^{14}C$ Flumioxazin

| Sample | ppm* | %** |
|---|---|---|
| Thigh Muscle | 0.050 | 0.03 |
| Breast Muscle | 0.040 | 0.04 |
| Fat | 0.074 | 0.02 |
| Liver | 0.237 | 0.08 |
| Kidney | 0.272 | 0.02 |
| Heart | 0.161 | <0.01 |
| Gizzard | 0.104 | 0.02 |
| Intestinal Contents | 0.716 | 1.17 |
| Intestine | 0.620 | 0.26 |
| Skin | 0.143 | 0.04 |
| Ovary | 0.250 | 0.23 |
| Excreta | ----- | 92.61 |
| Egg Yolk | 0.437*** | 0.35 |
| Egg White | 0.018*** | <0.01 |
| Total | | 94.90 |

* Values for pooled tissues of 10 hens
** Percentage of total administered radioactivity
*** Maximum found during 14 days

Tissues and eggs were subjected to solvent extraction and protease digestion. Aliquots of fractions of the separation procedures were subjected to HPLC and TLC chromatography with determination of radioactivity. Retention times and $R_f$'s of radioactivity were compared with those of postulated metabolites. See attached figure for identification and structures of metabolites cited as abbreviations.

Identification and Quantitation of Residues of Flumioxazin in Tissues and Eggs of Hens Fed $^{14}C$ Flumioxazin.

| | Matrix | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Liver | | Kidney | | Egg White | | Egg Yolk | |
| Metabolite | % | ppm | % | ppm | % | ppm | % | ppm |
| 3-OH SA | 0.7 | 0.002 | 2.7 | 0.008 | ND | ND | 0.2 | ND |
| 4-OH SA | ND | ND | ND | ND | ND | ND | ND | ND |
| 482-HA | 1.2 | 0.003 | 0.1 | ND | 20.0 | 0.004 | 0.1 | 0.002 |
| APF | 3.1 | 0.007 | 4.8 | 0.013 | 23.2 | 0.004 | 0.6 | 0.015 |
| 4-OH | 3.9 | 0.009 | 7.2 | 0.020 | ND | ND | 1.1 | 0.004 |
| 3-OH | 2.6 | 0.006 | 3.1 | 0.008 | ND | ND | 0.5 | 0.002 |
| Flumioxazin | 9.1 | 0.022 | 6.9 | 0.019 | ND | ND | 3.8 | 0.016 |
| Total | 20.6 | | 24.8 | | 43.2 | | 9.8 | |

Identification and Quantitation of Residues of Flumioxazin in Tissues and Eggs of Hens Fed $^{14}C$ Flumioxazin.

| | Matrix | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Fat | | Skin | | Thigh Muscle | | Breast Muscle | | Gizzard | |
| | % | ppm | % | ppm | % | ppm | % | ppm | % | ppm |
| Metabolite | | | | | | | | | | |
| 3-OH & 4-OH SA | 1.2 | ND | 0.3 | ND | 4.0 | 0.002 | 1.1 | ND | 3.5 | 0.003 |
| 482-HA | ND | ND | 6.9 | 0.010 | 5.5 | 0.003 | 1.2 | ND | 1.8 | 0.002 |
| APF | ND | ND | 1.1 | 0.001 | 7.7 | 0.004 | 10.4 | 0.004 | 8.8 | 0.009 |
| 4-OH | 3.7 | 0.003 | 1.6 | 0.002 | 6.8 | 0.003 | 7.7 | 0.003 | 5.8 | 0.006 |
| 3-OH | 2.6 | ND | 2.6 | 0.004 | 5.6 | 0.003 | 6.7 | 0.003 | 7.0 | 0.007 |
| Flumioxazin | 48.8 | 0.046 | 24.7 | 0.035 | 9.9 | 0.005 | 13.9 | 0.006 | 25.9 | 0.027 |
| Total | 54.7 | | 37.2 | | 39.5 | | 41.0 | | 52.8 | |

Comment

In poultry tissue and eggs, identification of known metabolites accounted for from 10 to 55% of the total tissue radioactivity. The profile of metabolites in the residues of poultry tissues and eggs is different from that found in soybeans and in ruminant tissues. In this instance the major component of the residue is parent flumioxazin.

Conclusion

We consider the animal metabolism studies as adequate in delineating the degradative pathways of flumioxazin in ruminants and poultry. In ruminants about 35% of the recovered radioactivity in a tissue was identified, the predominant metabolite being 482-HA, a metabolite not found in the plant metabolism study. The profile of metabolites in the residues of poultry tissues and eggs was different from that found in soybeans and in ruminant tissues, the major component of the residue being the parent flumioxazin.

For the purposes of this EUP, we will consider the residue of concern for animal metabolism to be the parent, flumioxazin. However, the consideration regarding the residue of concern discussed above for plant metabolism apply to the animal studies also. It is evident that the major component of the residue in ruminant tissues is the metabolite 482-HA and the parent in poultry tissues.

What components of the residue of this low application herbicide if any, need to be regulated for animal tissues is a matter that needs clarification and will be considered in conjunction with the permanent tolerance petition. CBTS in conjunction with HED's Metabolism Committee will determine which components of the residue need to be regulated and how the tolerance expression should be worded. We expect to use our experience with an analogous low application herbicide, flumiclorac pentyl ester, for guidance and precedent.

**Analytical Methodology:**

**Proposed Enforcement Method:**

Soybean RAC's and Processed Fractions

The methods for the determination of residues of flumioxazin in soybean crops and in processing fractions are essentially similar but involve different procedures for the extraction of the different matrices. After extraction and cleanup quantitative determination is by gas chromatography with a nitrogen specific detector.

Soybean Plant Parts

Method RM-30A

Finely ground samples are shaken with acetone /water 1:1, allowed to stand overnight and filtered. The residue is reextracted with acetone/water and the filtrates combined. The filtrate is twice partitioned against dichloromethane, the dichloromethane layer is taken to dryness and the residue dissolved in hexane. The hexane phase is twice partitioned against acetonitrile with the acetonitrile phase taken to dryness. The residue from this step is taken up in ethyl acetate and applied to a florisil column. Elution is with hexane/ethyl acetate 2/1. The eluate is taken to dryness, and the residue dissolved in acetone. Aliquots of the acetone phase are injected into a GC column coated with 50% phenyl/methyl silicone. The retention time for flumioxazin under the GC parameters employed is 7.7 minutes. The limit of detection is given as 0.01 ppm using a nitrogen specific detector.

Refined and Crude Soybean Oil

Method RM-30B

The sample is dissolved in hexane. The hexane phase is extracted twice with acetonitrile and the acetonitrile phase is taken to dryness. The residue is dissolved in ethyl acetate and the procedure follows that described above from this step.

Soybean Soapstock

Method RM-30C

The sample is acidified with 1N HCl and extracted with acetone/dichloromethane/NaCl solution. After partitioning the sample twice with aqueous NaCl and dichloromethane, combining and concentrating the dichloromethane phases to dryness, the procedure as described above for soybean RAC's is followed.

Quantitation and Recoveries

The limit of detection for the three procedures is given as 0.01 ppm. Fortification of the various matrices with residues of 0.01 to 0.10 in forage, hay, sand seed; 0.02 in hulls; 0.02 and 0.10 in oil; and 0.02 amd0.10 in soapstock gave recoveries ranging from 80 to 116%.

Confirmation and Validation of Analytical Procedure

An independent validation of the methods for residues of flumioxazin in/on soybeans is presented with this submission. The study was conducted by Hazelton Wisconsin, Inc following Method RM-30A without consultation with Valent according to PR 88-5. Soybean seeds were fortified with either 0.01 or 0.05 ppm of flumioxazin and carried thru the analytical procedure. Recoveries ranged from 96.2 to 111%.

Demonstration of Equivalence of Analytical Methods

Samples of radioactive treated plant material used in the plant metabolism studies were analyzed by the proposed enforcement method. A rough equivalence was demonstrated in that the residue levels in the metabolism studies as shown by radiometry were below the limits of detection of the analytical method and no residue were detected by the analytical method.

Storage Stability Studies

The stability of residues of flumioxazin was determined in fortified seeds, forage, and hay a 12 month period. Samples of these matrices were macerated, fortified with 0.1 ppm of flumioxazin, initially analyzed for flumioxazin, stored in a freezer, and reanalyzed at intervals.

| Storage Time (months) | % Recovery | | |
|---|---|---|---|
| | Matrix | | |
| | Forage | Hay | Seed |
| 0 | 102 | 79 | 86 |
| 1 | 88 | 92 | 100 |
| 3 | 78 | 83 | 104 |
| 6 | 85 | 78 | 91 |
| 8 | 96 | 67 | 100 |
| 10 | -- | 92 | --- |
| 12 | 112 | 89 | 105 |

Comment

For the purposes of this EUP petition we will accept the demonstration of the storage stability of flumioxazin residues in soybean matrices for 12 months.

Behavior in Multiresidue Testing Protocols

No data is presented for the behavior of flumioxazin in multiresidue testing protocols. Such data will be needed for the establishment of permanent tolerances for flumioxazin.

Confirmatory methodology

No confirmatory methodology was submitted with this petition. A confirmatory method including validation and representative chromatograms should be submitted with the permanent tolerance request.

Summary of Analytical Methodology

The analytical methodology described in this presentation and used to generate residue data appears to be suitable for the required enforcement method. Documentation is provided to show that the method has been independently validated as required by PR 88-5. Additionally, the method must be validated by the Agency's analytical chemistry section. Concomitant with the establishment of temporary tolerances for this EUP, we will forward the method for Agency validation to expedite the establishment of permanent tolerances.
No confirmatory methodology was submitted with this petition. A confirmatory method including validation and representative chromatograms should be submitted with the permanent tolerance request.

**Magnitude of the Residue in Plants**

Residue Trials

Soybeans

Twenty-four residue trials were conducted in 1989 and 1990 in 11 states representing 78% of soybean producing areas. The formulated pesticide was applied at rates of 0.09 to 0.47 lbs/A ( 1.44 to 7.52 ounces active). According to the petitioner these are equivalent to 1X to 5X the suggested label rates. Applications were made as pre-emergent, pre-plant incorporated, or no till applications.

In 1989, trials were conducted at sites in Arkansas, Missouri, Minnesota, Indiana, Iowa, Ohio, Nebraska and Louisiana. Additional trials were conducted in these states plus Illinois, Mississippi, and Tennessee in 1990.

Most trials were conducted with the 50 WPG formulation. Forage samples were harvested 40 to 67 days after 1 application of 0.09 lbs active/acre (In 1 instance 0.28 lbs ai/A was applied). Nineteen forage samples were analyzed. In all samples, no detectable residues of flumioxazin per se (>or = to 0.01 ppm) were found. Samples for hay were harvested 40 to 111 days after 1 application of 0.09 lbs active/acre (In 1 instance 0.28 lbs ai/A was applied). Twenty-one hay samples were analyzed. In all samples, no detectable residues (<0.01 ppm) were found. Soybean seed samples were harvested 111 to 149 days after 1 application of 0.09 lbs active/acre (In 2

instances 0.28 lbs ai/A or 0.47 lbs ai/A was applied). Twenty-one seed samples were analyzed. In all samples, no detectable residues (<0.01 ppm) were found.

Three additional trials were conducted with flumioxazin 10 FL a flowable formulation at 0.09 lbs ai/A with 1 pre-emergent application. No detectable residues of flumioxazin were found in forage, hay or seed samples.

Processing Studies (Soybeans)

Processing studies show that seeds from plants treated with 0.47 lbs ai/A (5x the proposed rate according to the petitioner) when processed into hulls, meal, crude oil, refined oil, and soapstock demonstrated no detectable residues of flumioxazin. It should be noted that the seeds for processing contained no detectable residues.

**Summary of Plant Residue Trials**

Field trial results with soybeans and from processing studies with fractions derived from treated rac's support the proposed temporary tolerances for this EUP. However, although trials were conducted with soybean hay, no tolerance has been proposed for this commodity. As no residues were detected in any rac or processed commodity, the tolerances of 0.01 ppm represents the limit of detection of the proposed analytical method. The petitioner should submit a revised Section F requesting a tolerance for soybean hay at 0.01 ppm.

**Meat, Milk, Poultry, and Eggs**

The proposing of secondary residue tolerances is not needed at this time as it is unlikely that sufficient residues will be present in/on feed stuffs derived from flumioxazin treated commodities of this petition to result in detectable secondary residues in animal tissues, milk, and eggs. This is a Section 180.6 (a)3 situation with respect to secondary residues in animal tissues, milk, and eggs. Tolerances for these commodities are not needed.

**Confined Rotational Crop Study**

Radioactive flumioxazin was applied to fallow soil at a rate of 42.5 grams ai per acre (1.5 ounces active = 1X rate) or at 85 grams per acre. In the 1X study crops were planted after 30 days; in the 2X plots, crops were planted after 1, 4, 6, or 12 months. Plants were harvested, sampled, and combusted for the determination of total recovered radioactivity. For some samples, i.e., wheat chaff and straw, HPLC analyses of extracts were conducted.

Residues in Rotational Crops

| Crop | Fallow period of 30 days | |
|---|---|---|
| | Rate | |
| | 1x | 2X |
| | (ppm as flumioxazin) | |
| Wheat Forage | 0.002 | 0.006 |
| Wheat Grain | 0.006 | 0.011 |
| Wheat Chaff | 0.005 | 0.011 |
| Wheat Straw | 0.013 | 0.029 |
| Lettuce | 0.002 | 0.005 |
| Carrot Foliage | 0.002 | 0.010 |
| Carrot Root | 0.001 | 0.005 |

Residue levels greater than 0.01 ppm were seen in wheat straw and chaff in the 2x treatments at the 4 and 6 month intervals. Chromatographic analyses of wheat straw and chaff extracts demonstrated the presence of several known metabolites of flumioxazin, each at less than 0.01 ppm.

Comment

It appears that there is only a very low uptake of flumioxazin or its metabolites in crops planted 1 month after the application of a 1X rate. It appears that the planting of a rotational crop after a normal harvest interval for soybeans would not result in any detectable residues. Thus, no rotational crop restrictions for flumioxazin are needed.

cc: PP3G4250, R.F., Circ., Reviewer
RDI:PE:7/26/94:MF:9/21/94:9/21/94
H7509c:JG:jg:CM2-805a:(703) 305-5405:9/21/94