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

JUL 30 1991

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: PP# 8F3603 - Pyridate (Tough®) on Cabbage, Corn, and Peanuts.
Evaluation of the May 7, 1991 Amendment.
(MRID No. 418739-01 and -02) (CB Nos. 8120, 8121, and 8122) (HED Project No. 1-1512).

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and

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THRU: Robert S. Quick, Section Head
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Agrolinz, Inc. has submitted this amendment consisting of a revised Section D (revised ruminant and poultry ^{14}C-pyridate metabolism/feeding studies) in response to concerns noted on livestock metabolism and feeding studies for future pyridate tolerances requests involving livestock feed items. There are no livestock metabolism deficiencies remaining in PP# 8F3603. The pyridate livestock feeding and metabolism concerns were outlined and summarized in our reviews of December 14, 1989, and February 20, 1990, by E.T. Haeberer. The livestock feeding and metabolism concerns noted in the February 20, 1990, review are listed and repeated in the body of this review, followed by the petitioner's response, then CBTS comments. Our conclusions and recommendation follow.

CONCLUSIONS

1. CBTS Conclusion on **Nature of the Residue - Livestock**

a. Ruminants

The petitioner has identified, using ^{14}C-pyridate in a ruminant metabolism study, no residues of pyridate but residues of its CL-9673 metabolite, and the N- and O-glucuronides of CL-9673 using HPLC-UV and co-chromatographing all detectable components with authentic standards. The petitioner needs to repeat the determinative step to confirm these identities using a different polarity (and with a different gradient) LC column and use a different HPLC detector such as MS. Concerns expressed in deficiency 1 have not been resolved and will remain outstanding in any future pyridate tolerance request involving ruminant feed items.

b. Poultry

The petitioner has identified, using ^{14}C-pyridate in a poultry metabolism study, residues of pyridate, its CL-9673 metabolite, and the N- and O-glucuronides of CL-9673 using HPLC-UV, and co-chromatographing all detectable components with authentic standards. The petitioner needs to repeat the determinative step to confirm these identities using a different polarity (and with a different gradient) LC column, and to use a different HPLC detector such as MS. Concerns expressed in deficiency 2 have not been resolved and will remain outstanding in any future pyridate tolerance request involving poultry feed items.

2. CBTS Conclusion on **Residue Analytical Method**

a. The petitioner agrees to provide a pyridate and its metabolites meat, milk, poultry, and egg method. Until the method has been developed, validated, and accepted by EPA (including passing a PMV), the concern noted in deficiency 4 remains unresolved for future tolerance request involving livestock feed items.

b. Prior to starting any PMV for a pyridate meat and milk method, the petitioner needs to provide independent laboratory validation (ILV) data for pyridate, its CL-9673 metabolite, and both the O- and N-glucuronides of CL-9673 in milk, eggs, liver (poultry and ruminants), two types of fat and muscle tissues in both ruminants and poultry. Recovery data should be generated at the proposed tolerance, limit of detection, highest level detected, and at an intermediate point between the limit of detection and proposed tolerance.

3. CBTS Conclusion on **Magnitude of the Residue - Meat/Milk/ Poultry/Eggs**

a. CBTS concludes the petitioner has tentatively quantitated all HPLC peaks detected in the lactating cow feeding study. The remaining unidentified ^{14}C-material in ruminant tissues of interest need not be further identified. If the petitioner fully resolved deficiency 1 on nature of the residue, then deficiency 5 on magnitude of the residue in ruminants is adequately resolved.

b. CBTS concludes that the petitioner has tentatively quantitated all the HPLC peaks detected in the poultry feeding study. The remaining unidentified ^{14}C-material in poultry tissues of interest need not be further identified. If the petitioner fully resolves deficiency 2 on nature of the residue, then deficiency 6 on magnitude of the residue in poultry is adequately resolved.

4. CBTS Conclusion on **Proposed Tolerances**

a. From feeding exaggerated doses of pyridate to cattle, it is not possible to establish with certainty whether finite residues will be incurred in meat and milk, but there is a reasonable expectation of finite residues [40 CFR 180.6(a)(2)]. Secondary pyridate and its metabolite tolerances are required for meat and milk in any future pyridate petition involving ruminant feed items bearing higher residues.

b. Likewise, from feeding exaggerated pyridate doses to poultry for 28 days, CBTS observes that it is not possible to establish with certainty whether finite residues will be incurred in poultry and eggs, but there is a reasonable expectation of finite residues [40 CFR 180.6(a)(2)]. Secondary pyridate and its metabolite tolerances are required for eggs and poultry in any future pyridate petition involving poultry feed items bearing higher residues.

RECOMMENDATION

There are no outstanding deficiencies for this petition. The Product Manager should relay our concerns noted above in conclusions 1 thru 4 to the registrant so they can be resolved prior to the submission of any future pyridate petition.

DETAILED CONSIDERATIONS

NATURE OF THE RESIDUE - LIVESTOCK
RESIDUE ANALYTICAL METHODS
MAGNITUDE OF THE RESIDUE - MEAT/MILK/POULTRY/EGGS

Deficiencies (from our February 20, 1990 review)

1. In the DEB review of December 14, 1989, we determined that the nature of the residue in lactating ruminants was adequately defined and that the residue of concern was pyridate, its metabolite CL 9673 and its conjugates. The data presented in this submission raised some concerns regarding the nature of the residue in lactating ruminants. The questions raised in Conclusion 5, below, will have to be resolved.

2. As stated in the December 14, 1989 review, the nature of the residue in poultry is not adequately defined. If the petitioner proposes a new use for pyridate which could result in higher residues in animal feed items, a poultry metabolism study will be needed to characterize the nature of the residue.

3. The petitioner has based the 1, 3, and 10X feeding levels in both feeding studies on maximum residues of 1 ppm pyridate in/on alfalfa. The Agency currently does not have a submission for the proposed use of pyridate on alfalfa; therefore, we can draw no conclusions concerning the adequacy of the feeding levels.

4. ^{14}C-Pyridate was used in both feeding studies in order to facilitate qualitative and quantitative determination of pyridate residues. If the petitioner anticipates future uses of pyridate which will lead to residues higher than the 0.03 ppm level proposed for cabbage, corn, and peanuts, and a greater potential for secondary residues in livestock commodity tolerances, a "cold" analytical method will be needed for the analysis of residues in meat, milk, poultry, and eggs suitable for enforcement purposes.

5. The feeding study on lactating cattle is inadequate. The residues which have been identified must be quantitated. In addition, data are needed on the nature of the unidentified peaks, i.e., whether these peaks represent single compounds, and the percent of the total residue which is unidentified.

6. The feeding study on laying hens is inadequate. The residues which have been identified must be quantitated. In addition, data are needed on the nature of the unidentified peaks, i.e., whether these peaks represent single compounds, and the percent of

the total residue which is unidentified. The nature of the residue in poultry remains inadequately defined.

Petitioner's Response (See MRID #418739-01 and -02)

The petitioner's responses to concerns noted above for nature and magnitude of the residue in ruminants and a residue analytical method for pyridate in meat and milk are presented in a study title "Response to EPA Comments on Feeding Study in the Lactating Cow: Quantitation of Metabolites of [^{14}C]-Pyridate Present in Selected Tissues; Addendum 2" by A.M. Johnson et al., dated December 4, 1989 and coded Livestock Research International Report No. 6119.

The petitioner's response to concerns noted above for nature and magnitude of the residues in poultry and a residue analytical method in poultry and eggs are presented in a study titled "Response to EPA Comments on Feeding Study in the Laying Hen: Quantitation of Metabolites of [^{14}C]-Pyridate Present in Selected Tissues; Addendum 1" by A.M. Johnson, et al. dated October 26, 1990 and coded Livestock Research International Report No. 6113.

CBTS Comments

In response to the concern for adequacy of identifying all of the ^{14}C-pyridate radio equivalents in ruminants, the petitioner points out all samples were analyzed by HPLC-UV and all detectable radio components co-chromatographed with authentic reference standards of pyridate and its metabolites. CBTS considers this is a good first or primary identification. However, for positive identification the petitioner is advised to repeat the determinative step to confirm the identity using a different polarity (and with a different gradient) LC column and a different HPLC detector such as MS. We observe there are numerous unidentified analytical responses (UARs) eluting in the vicinity of pyridate metabolites and in the radio detection chromatographic fraction cuts. The petition has not adequately addressed our concerns noted in deficiency 1. They remain unresolved for any future pyridate tolerance request involving ruminant feed items.

The petitioner accepts the need for a cold pyridate meat/milk, poultry and egg method. Until the method has been developed, validated, and accepted by EPA, the concern noted in deficiency 4 remains unresolved for any future tolerance request involving livestock feed items.

CBTS points out that any pyridate and its metabolites meat and milk method will need to complete a petition method validation (PMV) in EPA laboratories. The method needs to be suitable to gather residue data and enforce any proposed pyridate secondary tolerances. Before a PMV can be started on a new method, the petitioner is requested to provide independent laboratory validation (ILV) data to show the method works in another laboratory beside the method developer's. CBTS offers

these suggestions for ILV data on a pyridate and its metabolites in meat and milk method. ILV data need to be presented for pyridate, its CL-9673 metabolite, and for both the N- and O-glucuronide CL-9673 in milk, eggs, liver (both ruminant and poultry), and two muscle and fat tissues for ruminants and poultry. The levels for ILV data are the proposed tolerance, limit (or just above) of detection, the highest residue reported, and an intermediate level between the limit of detection and the proposed tolerance. The limit of quantification is such a point. The petitioner is referred to PR Notice 88-5 for additional guidance on ILV or second laboratory validation data.

The petitioner has quantitated all of the identified metabolites in ruminant muscle, kidney, fat, at the 10 ppm feeding level. No pyridate was detected in any tissue to the 30 dpm or 0.01 ppm level. The CL-9673 metabolite was 100 percent of the 0.02 ppm residue in muscle, 0.2 ppm in liver, and 0.18 ppm in plasma. CL-9673 was found in kidney at 1.81 ppm (96% of the residue) and the O-glucuronide of CL 9673 was at 0.013 (1% for the residue). The remaining 0.08 ppm ^{14}C-pyridate equivalents need not be identified further. While the metabolic profile in urine and bile is not germane to our understanding of the nature of the residue, we note that 12.2 ppm (60% of the residue) in urine and 0.26 ppm (30% of the residue) in bile was the O-glucuronide of CL-9673. While the ^{14}C-pyridate equivalents in the tissues from 1 ppm and 3.3 ppm feeding were not individually identified and quantitated, CBTS feels it is not necessary. The percentage of metabolites identified in the 10 ppm feeding are expected to be nearly the same at 3.3 ppm and 1 ppm feeding.

The petitioner has presented an adequate number of copies of HPLC chromatograms using UV and radio detectors. We note many UARs from the 254 um UV detector with only a single radio labeled band from control and 10 ppm feeding for muscle, kidney, liver, fat, and plasma samples. Two radio labeled peaks were detected in bile and urine samples.

CBTS concludes the petitioner has tentatively quantitated all HPLC peaks detected in the lactating cow metabolism and feeding study. The remaining unidentified ^{14}C-material in the ruminant tissues of interest need not be identified further. If the petitioner confirms the identity of the residues by a different polarity LC column (using a different gradient) and a different detector such as MS; and no other metabolites are identified, then the nature of the pyridate residue in ruminants is adequately understood. Likewise, the magnitude of the pyridate residue in ruminants' feed up to 10 ppm is adequately understood. If the petitioner satisfactorily resolves deficiency 1, then deficiency 5 will be resolved.

For deficiency 3, CBTS again declines to judge the adequacy of these ^{14}C-pyridate metabolism and feeding studies. We note that from feeding exaggerated doses to cattle that it is not possible to establish with certainty whether finite residues will be incurred in meat and milk, but there is a reasonable

expectation of finite residues. Thus, from feeding pyridate treated feed commodities bearing residues at higher levels than the tolerances proposed in PP# 8F 3603 we categorize the use as 180.6(a)(2). Secondary pyridate and its metabolites tolerances are required for meat and milk in any future petition involving ruminant feed items which bear higher residues than those proposed at 0.03 ppm in or on cabbage, corn, and peanuts.

Likewise, in feeding exaggerated doses of ^{14}C-pyridate to poultry for 28 days, we observe that it is not possible to establish with certainty whether finite residues will be incurred in poultry and eggs, but there is a reasonable expectation of finite residues. Thus, from feeding pyridate treated feed commodities bearing residues at levels higher than the tolerances proposed in PP# 8F 3603 to poultry, we categorize the use as 180.6(a)(2). Secondary pyridate and its metabolites tolerances are required for poultry and eggs in any future petition involving poultry feed items which bear higher residues than those proposed at 0.03 ppm in/on cabbage, corn, peanuts.

In response to the concern for adequacy of identifying all of the ^{14}C-pyridate radio equivalents in poultry, the petitioner points out all samples were analyzed by HPLC-UV and all detectable radio components co-chromatographed with authentic reference standards. As with ruminants CBTS considers this is a good first, or primary identification. However, for positive identification the petitioner is advised to repeat the determination step to confirm identities using a different polarity (and with a different gradient) LC column, and a different HPLC detector such as MS. Again, we observe there are numerous UARs eluting in the vicinity of pyridate metabolites and in the radio detection chromatographic fraction cuts. The petitioner has not adequately addressed our concerns noted in deficiency 2. They remain unresolved for any future pyridate tolerance request involving poultry feed items.

The petitioner has adequately quantitated all of the identified metabolites in poultry liver, kidney, breast and leg muscle, and in egg yolks. Pyridate was detected in poultry kidney only at 0.032 ppm (14% of the residue). The major metabolite detected in poultry was the CL-9673 metabolite. In eggs 0.015 ppm (80%) was CL 9673. The other 0.004 ppm was not identified. CBTS concludes it is not necessary to identify the remaining 20 percent or 0.004 ppm. Neither the N- nor the O-glucuronide of CL 9673 was detected in egg yolk, poultry, kidney, liver, breast, or leg muscle. CL-9673 metabolite was 86 percent of the residue or 0.196 ppm in kidney, 0.085 ppm or 94 percent in live and 100 percent of the residue in breast muscle at 0.009 ppm and in leg muscle at 0.014 ppm. While the pyridate metabolic profile in poultry excreta is not germane to our understanding of the nature of the residue, we note the 0.234 ppm (2% of residue) was CL 9673-N-glucuronide and 0.469 ppm (4% of the residue) was CL 9673-O-glucuronide. The ^{14}C-pyridate equivalents in poultry tissues from 1.3 ppm and 4 ppm feeding were not individually

identified or components quantitated. CBTS feels it is not necessary as the percentage of the metabolites detected in the 13 ppm feeding, as discussed above, are not expected to change significantly when lesser amounts of pyridate were fed.

The petitioner has presented an adequate number of copies of HPLC chromatograms using UV detection and radio detection. CBTS observes there are many UARs in the 254 um UV chromatograms. Also the radio detection was more diverse in poultry as opposed to a single large band detected in ruminant tissues. Chromatograms were presented for control and 13 ppm feeding samples for standards, egg whites and yolks, excreta, fat, kidney, liver, breast and leg muscle, and plasma.

CBTS concludes the petitioner has tentatively quantitated all of the HPLC peaks detected in the poultry metabolism and feeding study. The remaining unidentified ^{14}C-material in poultry tissues of interest is quite low and need not be identified further. If the petitioner confirms the identity of the residues by using a different polarity (with a different gradient) LC column and a HPLC different detector, such as MS; and no other metabolites are identified, then the nature of the pyridate residue in poultry is adequately understood. Likewise, the magnitude of the pyridate residue in poultry feed up to 13 ppm is adequately understood. If the petitioner resolves deficiency 2, then deficiency 6 will be resolved.

cc:R.F.,Circu(7),Reviewer(FDG),R.D.Schmitt,Ph.D.,Chief,
PP#8F3603,PID/FOD(Furlow).

H7509C:CBTS:Reviewer(FDG):CM#2:Rm814B:557-0826.
63938:I:WP5.0:C.Disk:KEVRIC:07/12/91:CL:wo:aw:ed:fdg:7/18/91.

RDI:SecHd:R.S.Quick:7/25/91:BrSrSci:R.A.Loranger:7/25/91.