MEMORANDUM

Subject: **PP# 3F04169/3H05655 - Imidacloprid (Confidor®) on Apples, Cottonseed, Potatoes, Meat, Milk, Poultry, and Eggs.**
Review of Residue Data and Analytical Method.
(MRID #s 425561-04 thru -42, 427678-01 thru -03)[CBTS #s 11281, 11282, 11093, 11437, 11438, 11551, 11969, 11970, 11971, 12039, 12040, 12041, 12045, and 12105] {DP Barcodes D185148, D185159, D186042, D188338, D189036, D191806, D191805, D191812, D192248-D192251, and D192476}

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9/15/1993

and

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INTRODUCTION

Miles Inc., Agriculture Division proposes tolerances for residues of the insecticide imidacloprid, trade named Confidor® (1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine) and its metabolites in or on the following raw agricultural commodities: apples at 1 ppm, cottonseed at 6 ppm, cotton forage at 60 ppm, potato tubers at 0.4 ppm, milk at 0.05 ppm, meat, fat, and meat by-products of cattle, goats, hogs, horses, and sheep at 0.2 ppm, eggs at 0.02 ppm, and the meat, fat, and meat by-products of poultry at 0.02 ppm. Food additive tolerances are proposed for dried potatoes at 1.5 ppm and potato chips at 0.7 ppm. Feed additive tolerances are proposed at 9 ppm on cottonseed meal, wet apple pomace at 2 ppm, and dry apple pomace at 7 ppm. In a letter from Miles dated May 6, 1993, and signed by J.S. Thornton the petitioner submitted a revised Section F proposing new higher total imidacloprid tolerances for cottonseed, cotton forage, and cottonseed meal.

EXECUTIVE SUMMARY OF RESIDUE CHEMISTRY DEFICIENCIES

- Additional validation data for formulation enforcement method

- Revised Label/Direction for Use

- Additional identification of residues in cottonseeds

- Residue analytical method
- Additional storage stability data

- Additional field trial residue data for cottonseeds

- Revise tolerances
  cottonseed, cotton forage, and cottonseed meal
  apples and apple pomace
  potatoes, potato chips, and potato waste (wet or dry)
  meat and milk

- Additional data for bovine and poultry feeding studies

- Need field rotational crop studies

CONCLUSIONS

1. CBTS Conclusions on **Product Chemistry/ Chemical Identity**

a. Analysis of the various batches of technical imidacloprid did not reveal any volatile N-nitroso amines to limits of detection of 0.05 ppm to 0.2 ppm. However, the nitrosoimino analog of imidacloprid was detected in all batches analyzed ranging from more than 10 ppm to less than 40 ppm. These data were presented to the HED Metabolism Committee on June 22, 1993. The HED Metabolism Committee concluded these levels were not of toxicological concern, thus there is no need to change the manufacturing process to remove it, nor is it necessary to list it on a CSF.

b. CBTS reiterates that validation data, although alluded to in the description of the analytical method provided for the preliminary analysis, has not been presented and needs to be presented. These validation data should be presented as part of the analytical method used to verify the certified limits for the active ingredient. The petitioner is reminded that the analytical method will be validated as the formulation enforcement method.

2. CBTS Conclusions on **Directions for Use**

a. The petitioner submitted a revised set of directions for use of Confidor® 2 Flowable in an amendment received on June 16, 1993. The significant change for foliar use of Confidor® 2 Flowable on cotton is to add "A spray adjuvant may be used to improve coverage." The petitioner needs to more narrowly define on a revised Confidor® 2 Flowable label which spray adjuvants are acceptable. The present revised label is too general in its instruction for use of adjuvants in foliar applications to cotton since field trial data show the use of an adjuvant in the crop field trials

essentially doubled the total imidacloprid residues on cottonseed and cotton forage.

b. The petitioner has proposed adequate directions for use of imidacloprid on apples and potatoes.

3. CBTS Conclusions on the **Nature of the Residue - Plants**

a. The nature of the imidacloprid residue in apples is adequately understood. Imidacloprid is metabolized by three pathways as follows:

1) hydroxylation of the dihydroimidazole ring of imida-cloprid to form 4-hydroxy, 5-hydroxy, and dihydroxy imida-cloprid followed by the loss of water to form the olefin imidacloprid,

2) reduction and loss of the nitro group on the dihydro-imidazole ring to form the nitrosimine imidacloprid, then the guanidine imidacloprid, and finally the urea imidacloprid, and

3) bridge cleavage of the C-N bond to form the 6-chloro-picolyl alcohol (6-CPA) which rapidly forms the glucoside and 6-chloronicotinic acid (6-CNA) and dihydroimidazole.

The residues of concern in apples are imidacloprid and its metabolites containing the 6-chloropyridinyl moiety.

b. The nature of the imidacloprid residue in cotton forage is partially understood. The imidacloprid residues from cotton leaves consist of 10% unidentified metabolites, 27% unextracted radiolabeled residues, and 25% reported as diffuse and remaining at the origin. The balance of the residues reported are identified and all are below 0.01 ppm. Since CBTS does not consider cotton forage to be a significant livestock feed item and that tolerances will not be necessary the petitioner does not need to provide additional identification of radiolabeled residue in cotton leaves, either from cotton leaves not analyzed or from reanalysis of extracts from the treated leaves.

c. The nature of the imidacloprid residue in cottonseed is not adequately understood when 56% of the residue is unidentified metabolites and is compared to the proposed 6 ppm tolerance for cottonseed. The petitioner is reminded that he is expected to identify at least 90% of the radioactive residue. The petitioner needs to provide additional identification of radiolabeled residue in cottonseeds, either from reserve ^{14}C-imidacloprid cottonseeds not analyzed, or from reanalysis of extracts from cottonseeds from the soil drench application part of the metabolism study. CBTS feels that the unidentified metabolite which is 1.62 ppm imidacloprid equivalents should be easily identified. The petitioner also has the option of repeating the ^{14}C-imidacloprid cotton metabolism study using an exaggerated application rate that approximates the proposed in-furrow, banded at planting application plus 4 foliar applications to generate sufficient radiolabeled residue for identification of metabolites.

d. The nature of the imidacloprid residue in potatoes is adequately understood. It follows the same three metabolic pathways found in apples. The residues of concern in potatoes are imidacloprid and its metabolites containing the 6-chloropyridinyl

moiety.

e. CBTS concludes that the nature of the imidacloprid residue in tomatoes is adequately understood. Imidacloprid is metabolized by the same 3 metabolic pathways identified for apples and potatoes. The residues of concern in tomatoes are imidacloprid and its metabolites that contain the 6-chloropyridinyl moiety.

f. Tentatively, CBTS concludes that the nature of the imida-cloprid residue in eggplants is adequately understood. Imida-cloprid is metabolized by the same 3 metabolic pathways identified for apples and potatoes. The residues of concern in eggplants are imidacloprid and its metabolites that contain the 6-chloropyridinyl moiety.

g. CBTS concludes that the nature of the imidacloprid residue in corn grain, fodder, and forage is adequately understood. Imidacloprid is metabolized by the same 3 metabolic pathways identified for apples and potatoes. The residues of concern in corn are imidacloprid and its metabolites that contain the 6-chloropyridinyl moiety.

4. CBTS Conclusions on the **Nature of the Residue - Livestock**

a. The nature of the imidacloprid residue in ruminants is adequately understood. Imidacloprid is metabolized by three pathways as follows:

1) hydroxylation of the dihydroimidazole ring of imida-cloprid to form 4-hydroxy, 5-hydroxy, plus the glucuronide conjugates of each monohydroxy metabolite, and dihydroxy imidacloprid followed by the loss of water to form the olefin imidacloprid,

2) reduction and loss of the nitro group on the dihydro-imidazole ring to form the aminoguanidine imidacloprid, then the guanidine imidacloprid, and finally the urea imidacloprid, and

3) opening of the dihydroimidazole ring with loss of the ethyl group and subsequent oxidation. The first step is forming the nitroguanidine imidacloprid, next the ring open guanidine which can also form from both the guanidine imidacloprid and the dihydroxy guanidine imidacloprid. This metabolite can form picolylic urea and picolylic amine which is oxidized to 6-chloronicotinic acid (6-CNA) and then conjugates with glycine.

b. The residues of concern in ruminants are imidacloprid and its metabolites containing the 6-chloropyridinyl moiety.

c. The nature of the imidacloprid residue in poultry is adequately understood. Imidacloprid is metabolized by three pathways as follows:

1) hydroxylation of the dihydroimidazole ring of imida-cloprid to form 4-hydroxy, 5-hydroxy, and dihydroxy imida-cloprid followed by the loss of water to form the olefin imidacloprid,

2) reduction and loss of the nitro group on the dihydro-imidazole ring to form the dihydroxyguanidine imidacloprid, and

3) opening of the dihydroimidazole ring with loss of the ethyl group and subsequent oxidation. The first step is forming the nitroguanidine imidacloprid, next the ring open guanidine which can also form from both the guanidine imidacloprid and the dihydroxy guanidine imidacloprid. This metabolite can form picolylic urea and picolylic amine which is oxidized to 6-chloronicotinic acid (6-CNA).

d. The residues of concern in poultry are imidacloprid and its metabolites containing the 6-chloropyridinyl moiety.

5. CBTS Conclusions on **Confined Rotational Crops**

a. The nature of the identified residue in rotational crops is nearly identical to that identified in the primary crops. Imidacloprid is metabolized by the same three metabolic pathways as described for apples and potatoes. The petitioner identified around 45% of the residue in the different rotational plant matrices. When the same matrices were analyzed by the common moiety method for 6-CNA, then 91-96% of the residue was recovered. This characterizes the additional components of the residues as containing the 6-chlorpyridinyl moiety. The petitioner has adequately characterized and identified the nature of the imidacloprid residue in rotational crops.

b. All 3 rotational crops in the confined study had imidacloprid residues when planted 1, 4, and 9 months after imidacloprid soil application. The total imidacloprid residues were all greater then 0.01 ppm from a 1X application. CBTS concludes there is potential for inadvertent imidacloprid residues to occur in non-target crops planted in rotation. Limited field rotation crop studies are necessary for a representative crop at 2 sites per crop for the following 3 crop groups: root and tuber vegetables, leafy vegetables, and cereal grains. At least a total of 6 field trials are necessary all at the 1X application rate.

c. Based on the data presented from the confined imidacloprid accumulation studies CBTS anticipates that the petitioner will need to propose rotational imidacloprid tolerances. A final decision on the need for tolerances and more extensive field trials will be based on the results of the limited field trials.

6. CBTS Conclusion on **Residue Analytical Methods**

The imidacloprid residue analytical methods and their supporting validation data plus the pre-review by the Analytical Chemistry Laboratory for the proposed Tolerance Method Validations were the subject of a separate memorandum by F.D. Griffith, Jr., on June 18, 1993. All discussions on the various methods and deficiencies in the write-up and supporting validation data noted in the June 18, 1993, review are incorporated herein by reference. Since the petitioner has not responded these deficiencies remain outstanding and continue unresolved.

7. CBTS Conclusions on **Storage Stability**

a. The petitioner has presented data to show that residues of imidacloprid and its major metabolites are stable in frozen storage for at least 6 to 9 months in lemons, lettuce, and corn. CBTS considers these storage stability data are acceptable and are supplementary for storage stability of total imidacloprid residues in apples, potatoes, and cottonseed.

b. While the frozen storage stability data show no decline in total imidacloprid residues in potatoes, apples and apple processed commodities, cottonseed processed commodities, and in wheat processed commodities at 3 months, CBTS defers judgement on this data to support the magnitude of the residue crop field trial residue data in this petition until the petitioner has completed the study and submitted the final report. In the interim the petitioner is encouraged to submit an additional interim report that includes the storage stability data for 6, 12, and possibly 18 months.

c. In another frozen storage stability study the results show that total imidacloprid residues in a 1 ppm spiking mixture of imidacloprid plus the guanidine, monohydroxy, and olefin metabolites spiked into sugar beet tops and roots, barley forage, straw, and grain, and sunflower seeds are stable for at least 24 months of frozen storage. These storage stability data are supplementary as there are no tolerance proposals for these commodities in this petition.

d. Analysis of samples from the ^{14}C-imidacloprid plant metabolism studies for corn, cotton, apples, and potatoes showed there is no loss of imidacloprid and its major metabolites (olefin, guanidine, 5-hydroxy, and 6-CNA) during a period of 2 years frozen storage.

8. CBTS Conclusions on the **Magnitude of the Residue - Crop Field Trials**

a. The petitioner's cotton crop field trial residue data do not support the proposed 6 ppm tolerance on cottonseed. None of the imidacloprid on cotton crop field trial residue data have been generated at the proposed use of an in-furrow at planting application (granular or spray) and up to 6 foliar applications at a rate of 0.05 lb a.i. per application with a spray adjuvant for a maximum of 0.5 lb a.i. imidacloprid per acre per season. The petitioner has the options of either generating all new cotton field trial residue data at the proposed use, or proposing a new set of directions which accurately reflect the use pattern for generating the magnitude of the residue data, namely adding a use for treating cotton seed, and having only 2 foliar applications at a rate of 0.24 lb a.i., 7 day repeat application interval and with a 14 day PHI plus the use of the spray adjuvant Silwet L-77. For this use the petitioner needs to present additional crop field residue data from the Texas/New Mexico/ Oklahoma region to improve geographical representation.

b. If the petitioner wishes to keep the current set of directions for use on the proposed label, then he needs to present at least 12 new geographically representative cotton field trials showing the residues on/in cottonseed at the maximum 0.5 lb a.i. proposed use; eg, an in-furrow application (preferably with the granular formulation as higher residues are reported from the use of the granular formulation) followed by up to 6 foliar applications at 0.05 lb per application all with the

spray adjuvant Silwet L-77, 7 day repeat application interval, 14 day PHI with no imidacloprid seed treatments. showing the residues on/in cottonseed.

c. While cotton forage is listed currently in Table 2 as a rac and cattle feed item CBTS does not feel this is a significant feed item at this time. Information we have received indicates cotton forage is not used as a feed item and if it is, then cotton forage has only a very limited use. CBTS will not require any additional crop field trial residue data for imidacloprid on cotton forage. We suggest the petitioner submit a revised label prohibiting the possible use of cotton forage as a feed and also propose a revised section F deleting the proposed total imidacloprid tolerance on cotton forage.

d. The petitioner has presented an adequate amount of geographically representative and varietal apple crop field trial data to show that total imidacloprid residues are not expected to exceed the proposed 1.0 ppm tolerance on apples when Confi-dor® is used as directed.

e. Since the Agency sets tolerances no higher then necessary, the petitioner needs to submit a revised Section F proposing a lower total imidacloprid tolerance for apples at 0.5 ppm. The maximum residue was 0.74 ppm only on 1 sample from a 1.62X exaggerated use, thus when this is extrapolated to a 1X use the expected residue is under 0.5 ppm. This is supported by a majority of the field trial residue data as well as the average residues being at the 0.1-0.2 ppm level.

f. The petitioner has presented an adequate amount of geographically representative and varietal potato crop field trial data to show that total imidacloprid residues are not expected to exceed the proposed 0.4 ppm tolerance on potatoes when Confidor® is used as directed.

g. Since the Agency sets tolerances no higher then necessary, the petitioner needs to submit a revised Section F proposing a lower total imidacloprid tolerance for potatoes at 0.2 ppm. The maximum residues was 0.28 ppm from a 1.67X exaggerated use, thus when this is extrapolated to a 1X use the expected residue is under 0.2 ppm. This is supported by a majority of the field trial residue data as well as the average residues being at the 0.05 ppm level.

9. CBTS Conclusions on the **Magnitude of the Residue - Processed Food/ Feed**

a. The petitioner has conducted a cotton processing study using cottonseed bearing detectable total imidacloprid residues following an exaggerated 7.58X total imidacloprid application. Cottonseeds were processed into cottonseed hulls, meal, crude oil, refined oil, and soapstock. Residues were not detected in crude oil, refined oil, or soapstock, and concentrated at 1.5X only in cottonseed meal. While an imidacloprid FAT is required for cottonseed meal, judgement is deferred on the proposed FAT as there are insufficient crop field trial data available from the proposed imidacloprid use to determine the proper imidacloprid tolerance on cottonseed, and thus the FAT for cottonseed meal.

b. The petitioner has conducted an apple processing study using apples bearing

detectable total imidacloprid residues following an exaggerated 3.14X total imidacloprid application. Apples were processed into apple juice, and wet and dry apple pomace. Residues were detected in apple juice, but did not concentrate, thus no FAT is required. Residues concentrated at 1.6X in wet apple pomace and 6.3X in dry apple pomace. While an imidacloprid FAT is required for wet and dry apple pomace, CBTS prefers the petitioner propose one total imidacloprid tolerance for apple pomace (wet and dried) using a 6X concentration factor, thus avoiding a proliferation of tolerances. The petitioner needs to propose in a revised Section F a total imidacloprid tolerance for apple pomace (wet and dried) at 3 ppm.

c. The petitioner has conducted a potato processing study using potatoes bearing detectable total imidacloprid residues following an exaggerated 7.56X total imidacloprid application. Potatoes were processed into potato chips, wet and dry potato peels, and potato granules. Residues were detected in washed potato tubers, potato granules, and wet potato peels, but did not concentrate; thus no FAT is required. Residues concentrated at 1.3X in potato chips and 2.9X in dry potato peels. While an imidacloprid FAT is required for dry potato peels and potato chips, the Agency sets tolerances no higher then necessary. We prefer the petitioner propose one total imidacloprid tolerance for processed potato waste not dry potato peels, to avoid a proliferation of tolerances and use a 3X concentration factor for potato wastes. The petitioner needs to propose in a revised Section F a total imidacloprid tolerance for processed potato waste at 0.6 ppm and for potato chips at 0.25 ppm.

10. CBTS Conclusions on **Magnitude of the Residue - Meat/Milk/ Poultry/Eggs**

a. Based on the results of the imidacloprid bovine feeding study CBTS concludes that finite residues will actually occur in milk and meat from feeding of imidacloprid treated racs or their processed feed commodities when Confidor® is used as directed. Since this situation falls under 40 CFR 180.6(a)(1) secondary imidacloprid tolerances are required in meat and milk. However, judgement is deferred on the study supporting the proposed 0.05 ppm tolerance in milk and 0.2 ppm in meat, fat, and meat by-products until the petitioner has supplied additional cottonseed crop field trial residue data and the following information to allow CBTS to complete its review of the bovine feeding study.

b. CBTS reiterates that since the feeding study was conducted at Bayer's Research Center in Monheim, Germany the petitioner needs to more completely define normal dairy housing practices so we can compare these to dairy housing practices in the USA. The type of hay needs to be defined as well as what is a high energy dairy concentrate. The petitioner needs to provide a sample label for the concentrate (in English), and the label should list the amount of protein, fat, fiber, and major ingredients that are in the feed. And finally we were unable to locate any data showing the feeds were free from other potentially interfering heavy metals, aflatoxins, and other pesticides. The petitioner needs to provide these data.

c. Based on the results of the imidacloprid poultry feeding study CBTS concludes that finite residues will actually occur in eggs and meat from feeding of imidacloprid treated racs or their processed feed commodities when Confidor® is used as directed.

Since this situation falls under 40 CFR 180.6(a)(1) secondary imidacloprid tolerances are required in eggs and poultry. However, judgement is deferred on the study supporting the proposed 0.02 ppm tolerance in eggs and in poultry meat, fat, and meat by-products until the petitioner has supplied the following information to allow CBTS to complete its review of the poultry feeding study and additional crop field trial data for cottonseed.

d. CBTS reiterates that the petitioner needs to provide a sample label for the poultry feed (in English), and the label should list the amount of protein, fat, fiber, and major ingredients that are in the feed. And finally we were unable to locate any data showing the feeds were free from other potentially interfering heavy metals, aflatoxins, and other pesticides. The petitioner needs to provide these data.

e. The petitioner needs to further identify the poultry breed used in the feeding study so that we may ascertain whether this is a commercially accepted breed.

11. CBTS Conclusion on **Harmonization of Tolerances**

Since there are no Canadian, Mexican, or Codex MRLs/tolerances compatibility is not a problem at this time.

RECOMMENDATION

CBTS cannot recommend for the requested tolerances for residues of imidacloprid and its metabolites containing the 6-chloropyridinyl moiety in apples at 1 ppm, in cottonseed at 6 ppm and in cotton forage at 60 ppm, in potatoes at 0.4 ppm, in milk at 0.05 ppm, in meat, fat, and meat by-products of cattle, goats, hogs, horses, and sheep, in eggs, meat, fat, meat by-products of poultry at 0.02 ppm for the reasons cited above in our Executive Summary and further described in Conclusions 1b; 2a; 3c; 5b and c; 6; 7B; 8a, b, c, e, and g; 9a, b, c; and 10a, b, c, d, and e.

For further consideration of this petition the petitioner should be advised to resolve the deficiencies described in our executive Summary and further detailed in our conclusions above.

DETAILED CONSIDERATIONS

BACKGROUND

This is a first time, food use, permanent tolerance request for imidacloprid (PP# 3F4169). The imidacloprid product chemistry data for the technical material has been previously submitted and reviewed at part of the registration for imidacloprid use on turf and ornamentals (see memorandum dated December 21, 1992, by K.B. Leifer).

The petition passed the new chemical screen and was placed into review on December 24, 1992 (see memorandum from L. Culleen to P. Fenner-Crisp). The due date for

the imidacloprid residue chemistry review is September 30, 1993, per discussion between R. Schmitt (HED) and S. Irene (RD). The review is not in "expedite" status.

No imidacloprid tolerances either temporary or permanent have been established. No special local need registrations for permanent food tolerance have been submitted as of April 1, 1993. CBTS has recommended for Emergency Exemptions (Section 18) for use of imida-cloprid on cotton, cabbage, broccoli and cauliflower, and head and leaf lettuce in Arizona (see memoranda 93AZ0003, 93AZ005, and 93AZ007 by F.D. Griffith, Jr., all dated June 1993). There is a co-pending imidacloprid petition, PP#3F4231, proposing total imidacloprid tolerances on the rac's for the fruiting vegetables crop group at 1 ppm, on the Brassica vegetables crop group at 3.5 ppm, leaf and head lettuce at 3.5 ppm, grapes at 1 ppm, milk at 0.1 ppm, and meat, fat, and meat byproducts of cattle, goats, hogs, horses, and sheep at 0.3 ppm. Also in PP# 3F4231 food additive tolerances are proposed for tomato puree at 2 ppm, and grape juice and raisins at 1.5 ppm. Feed additive tolerances are proposed for wet tomato pomace at 2 ppm, dry tomato pomace at 6 ppm, wet grape pomace at 2.5 ppm, dry grape pomace at 5 ppm, and raisin waste at 15 ppm.

A summary of all plant and animal (rat, ruminant, and poultry) metabolism data were presented to the HED Metabolism Committee on June 22, 1993. The Committee concluded (see memorandum by F.D. Griffith, Jr., dated June 24, 1993) that:

1. no additional plant or animal metabolism studies are needed at this time,

2. levels of the nitrosimino compound in the technical material were not of tox concern, thus there is no need to change the manufacturing process to remove it, nor is it necessary to list it on a revised CSF.

3. residues of the guanidine and nitrosimino imidacloprid metabolites at the levels in the different metabolism studies reported are not toxicologically significant,

4. other imidacloprid metabolites at the levels reported are of no special toxicological concern and that no additional separate regulation of residues is warranted, or are separate additional metabolism or toxicological studies warranted at this time, and

5. there are no scientific objections to the tolerance expression being for imidacloprid and its metabolites containing the 6-chloropyridinyl moiety.

PRODUCT CHEMISTRY/CHEMICAL IDENTITY

No new imidacloprid product chemistry data were submitted with this petition. The product chemistry data were reviewed with the registration for use of imidacloprid on turf and ornamentals and are summarized below.

The petitioner has submitted Confidential Statements of Formula (CSF) for Confidor® 2 Flowable (EPA File Symbol 3125-URI) and for Confidor® 2.5% Granular (EPA File Symbol 3125-URT). The CSFs are dated October 20, 1992, and signed by J.S. Thornton. Review of the status of the inert ingredients for clearance for use on food commodities is now in the purview of the Registration Division.

Product Identity and Composition: GRN 61

The petitioner has named his new insecticide imidacloprid. It is also known as BAY NTN 33893. The trade names are Confidor®, Merit, and Gaucho®. The petitioner has adequately described the composition and structure of imidacloprid. The petitioner has adequately identified the beginning materials and described the batch step wise manufacturing process for the technical material. The petitioner has presented a discussion of the formation of impurities both those known to be present and those theoretically present in technical imidacloprid. Data for this requirement is satisfied. No further product chemistry data are required for this topic.

Analysis and Certification of Product Ingredients: GRN 62

The petitioner analyzed five batches of the technical grade active ingredient (TGAI) material to determine the percentage of active ingredient and to identify impurities at and above 0.1% Technical imidacloprid was found to contain 94% active ingredient. Analysis of the various batches of TGAI did not reveal any volatile N-nitroso amines to the limits of detection of 0.05 ppm to 0.2 ppm. However, the nitrosimino analog of imidacloprid was detected in all batches analyzed ranging from more than 10 ppm to less than 40 ppm. Following the Branch policy these data were presented to the HED Metabolism Committee on June 22, 1993. The committee concluded these levels of the nitrosimino compound were not of tox concern, thus there is no need to change the manufacturing process to remove it, nor is it necessary to list it on a revised CSF.

The analytical method used to determine the percentage of active ingredient and the impurities at and above 0.1% is a reverse phase gradient elution HPLC using a UV detector and quantitation by peak areas.

CBTS reiterates that validation data, although alluded to in the description of the analytical method provided for the preliminary analysis, has not been presented and needs to be presented. These validation data should be presented as part of the analytical method used to verify the certified limits for the active ingredient. The petitioner is reminded that the analytical method will be validated as the formulation enforcement method.

Physical and Chemical Characteristics: GRN 63

The petitioner has presented adequate data defining characteristics such as color, physical state, m.p., density, solubility, v.p., pH, etc. For the TGAI the data are satisfactory for GRN 63-2 through 63-13. CBTS notes that the petitioner has also provided data for GRN 63-14 through GRN 63-21 for the TGAI.

DIRECTIONS FOR USE/LABELING

Imidacloprid is proposed for use as an insecticide to control aphids, leafhoppers, leafminers in apples; **whiteflies**, aphids, thrips and plantbugs on cotton; and aphids, leafhoppers, flea beetles, and Colorado potato beetles on potatoes. The petitioner proposes use of 2 formulations. Imidacloprid is formulated as Confidor® 2 Flowable (EPA File Symbol

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3125-URI), a systemic insecticide for use on apples, cotton, and potatoes. Confidor® 2 Flowable contains 21.4% imidacloprid active ingredient (a.i.), or 2 lbs imidacloprid a.i. per gallon. Imidacloprid is also formulated as Confidor® 2.5 Granular (EPA File Symbol 3125-URT), a systemic insecticide for use on cotton and potatoes. Confidor® 2.5 Granular contains 2.5% imidacloprid a.i.

For the application of the 2.5% granular formulation to cotton the petitioner proposes applying 13 ozs (0.325 ounces or 9.2 grams a.i.) per 1000 row feet at planting as a narrow band in-furrow at or below the seed line. Based on row spacing from 30 inches to 42 inches this is an application of 10.1 to 14.2 lbs of formulation or 0.25 lb to 0.35 lb a.i. imidacloprid per acre. For cotton the maximum imidacloprid application is 0.5 lb per acre per growing season.

For application of the 2.5% granular formulation to potatoes the petitioner proposes applying 9 to 13 ounces (0.325 ozs or 9.2 grams a.i.) per 1000 row feet as a narrow band in-furrow ensuring contact with the seed potatoes. Based on row spacing of 30 to 42 inches this is an application of 9.8 lbs to 11.8 lbs of formulation or 0.18 lb to 0.30 lb a.i. imidacloprid per acre. For potatoes the maximum imida-cloprid application rate is 0.3 lbs per acre per growing season. Thus, a grower may not apply 13 ounces of Confidor® 2.5% Granular per 1000 row feet of potatoes with a row spacing of 30 inches as the amount applied will exceed the maximum use allowed.

The petitioner proposes applying Confidor® 2 Flowable to apples as a dilute, or concentrated foliar spray at a rate of 1.6 ounces per 100 gallons or 6.4 ounces (0.104 lb a.i.) per acre per application. The petitioner suggests thorough uniform coverage for optimum control with no applications during blooming, or when bees are present. The repeat application interval is 10 days and the pre-harvest interval (PHI) is 7 days. The maximum number of applications to apples is 5 per growing season, or 32 fluid ounces of Confidor® (0.52 lbs) per acre per growing season.

For cotton the petitioner proposes a soil application at planting of Confidor® 2 Flowable at a rate of 1.3 fluid ounces per 1000 row feet as a narrow band in-furrow spray at or below the seed line for rows 36 to 42 inches apart. Following these instructions the grower will apply between 0.25 to 0.3 lb a.i. imidacloprid to cotton seed. On cottonseed at planting the maximum application rate is 0.3 lb imidacloprid a.i. The petitioner is also proposing foliar application to cotton at a rate of 3 fl. ounces per acre per application (0.05 lb a.i. imidacloprid). The foliar application to cotton may be by air or ground application. The repeat application interval is 7 days and the PHI is 14 days for cotton. Growers may elect to use all foliar applications (a maximum of 10) provided the maximum imidaclo-prid application does not exceed 0.5 lbs per acre in a growing season, regardless of whether it is all foliar, or split between foliar and soil applications.

The petitioner is proposing a soil application use of Confidor® 2 Flowable on potatoes at a rate of 0.9 to 1.3 fl. ounces per 1000 row feet in a narrow band in-furrow at planting as a direct spray to the potato seed. Following these directions for use a grower will apply Confidor® to potatoes at a rate of 11.2 to 18.9 fl ounces of formulation or 0.18 to 0.3 lb a.i. imidacloprid per acre per application provided the row spacings are 36 to 42 inches. A foliar application of Confidor® 2 Flowable to potatoes is also proposed at a rate of 3 fl. ounces (0.05 lb a.i. imidacloprid) per acre per application. For Confidor® foliar applications there is

a repeat application interval of 7 days and a PHI of 7 days. The maximum number of Confidor® foliar applications to potatoes is 4 applications (0.2 lb a.i. imidacloprid) per acre per growing season. Growers may elect to use only soil imidacloprid application, or they may split the applications between foliar and soil applications as long as the total imidacloprid applied to potatoes does not exceed 0.3 lb a.i. per acre per season.

The petitioner submitted a revised set of directions for use of Confidor® 2 Flowable in an amendment received on June 16, 1993. The date on the revised label is June 2, 1993. The significant change for foliar use of Confidor® 2 Flowable on cotton is to add "A spray adjuvant may be used to improve coverage." The petitioner needs to more narrowly define on a revised Confidor® 2 Flowable label which spray adjuvants are acceptable. The present revised label is too general in its instruction for use of an adjuvant in foliar applications to cotton.

The petitioner has proposed adequate directions for use of imidacloprid on apples and potatoes.

NATURE OF THE RESIDUE - PLANTS

Apples (MRID# 425561-08 and -13)

The petitioner presented the results of an imidacloprid in apples metabolism study in a document titled "Metabolism of [^{14}C] NTN 33893 in Apples" by K. Vogeler, et al., dated February 27, 1992, and coded laboratory project ID M 173 0295-7 and Miles report no. 103216. The petitioner also presented his rationale for choosing the ^{14}C label in the methylene bridge between the rings in a document titled "Rationale for NTN 33893 Radiolabeling Position Used in Plant Metabolism Studies" by E.U. Kaussmann, et al., dated October 22, 1992, and coded Miles report number 103902.

The petitioner used pyridinyl-^{14}C-methyl imidacloprid that had a specific activity of 92.3 uCi/mg with 99.7% radiochemical purity. The radiolabeled imidacloprid was formulated as a 25 WP for use on apples.

The petitioner conducted a two part metabolism and translocation study using 5 Golden Delicious trees. The study was conducted at Bayer's metabolism research center in Monheim, Germany. 80 apples on 4 trees were treated for the metabolism study and two apples on another tree were treated for the translocation study. The apples received 3 applications of imidacloprid approximately 1 month apart. Application was by an Eppendorf syringe to insure uniform application for the metabolism study. Each of the 80 apples received a total of 0.299 mg imidacloprid.

The translocation part of the study received the ^{14}C-imidacloprid on the same day as the metabolism study apples. The apples were covered in plastic to prevent direct contamination and later to prevent condensation of volatile radioactive compounds, then the nearest 5 leaves were treated with ^{14}C-imidacloprid 3 times approximately one month apart with each leaf receiving a total of 0.181 mg a.i. ^{14}C-imidacloprid.

After the last ^{14}C-imidacloprid application, apples in the metabolism study were harvested at 0 and 14 days PHI. After harvest the apples were washed in 3 X 500 mls

CH_3OH, then separated into peal and pulp. The apple leaves, pulp, and the peal were macerated separately with liquid nitrogen, then extracted with methanol/water (1:1) one time and two times with methanol. The extracts were combined and partitioned against hexane. Since the hexane contained no radioactivity it was discarded. The methanol was evaporated off from the water, then the water remainder was cleaned-up on a XAD-4 resin column. The column was first eluted with water, then methanol. The water eluant contained no radioactivity and was discarded. The methanol was solvent exchanged with water, then partitioned with ethyl acetate.

Determination of the parent ^{14}C-imidacloprid and its radiolabeled metabolites was by thin layer chromatography (TLC) using Merck Kisselgel 60 F_{254} plates and 4 different mobile phases in one and two dimensions. Confirmation of low level residues was by use of authentic standards. HPLC was with a Varian 5000 equipped with a RP8, 25 cm X 4 mm (i.d.) column, gradient flow at 1 ml per minute connected to Isco Foxy fraction collector and Raytest "Ramona D" radioactive flow-through detector. The ^1H-NMR spectra were recorded on a Bruker AC 300 spectrometer. Samples were dissolved in CD_3OD. The mass spectra were electron impact using a Finnigan 8230 at 70 eV with a source temperature at 200°C. For total radioactivity measurements solid samples were combusted on a Harvey OX 300 oxidizer. The $^{14}CO_2$ was trapped in a scintillation cocktail of Carbosorb plus Permafluor V and counted on a Philips PW 4700. The petitioner has provided an adequate amount of supporting spectra, chromatograms, and copies of tlc plates to support his conclusions on the nature of the imidacloprid residue in apples.

The ^{14}C-imidacloprid equivalents in apples at 0 day PHI were 1.76 ppm and at 14 days PHI were 1.45 ppm. Approximately 80% of the applied residue was recovered and about 70% of the recovered residue was in the peel.

The petitioner determined the residues in the wash solution. peel, and pulp, then summed the results. The same metabolites were identified in each component of the apples only differing in the amount detected. At 0 day PHI the total ^{14}C-imidacloprid was 1.76 ppm, of which 1.36 ppm (77%) was imidacloprid, <u>per se</u> (1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine). Other residues identified were the 5-hydroxy imidacloprid or WAK 4103 (1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-5-hydroxy-N-nitro-1H-imidazol-2-amine) at 0.038 ppm (2.2%), the dihydroxy imidacloprid or WAK 3772 (1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihyrdodihydroxy-N-nitro-1H-imidazol-2-amine) at 0.014 ppm (0.9%), the olefin imidacloprid or NTN 35884 (1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-1H-imidazol-2-amine) at 0.077 ppm (4.3%), the guanidine imidacloprid or NTN 33823 (1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-1H-imidazol-2-amine) at 0.045 ppm (2.6%), the urea imidacloprid or DIJ 9817 (1-[(6-chloro-3-pyridinyl)methyl]imidazolidine-2-one) at 0.024 ppm (1.3%), the nitrosimine imidacloprid or WAK 3839 (1-[(6-chloro-3-pyridinyl)-methyl]-N-nitroso-2-imino-imidazolidine)at 0.011 ppm (0.6%), and the glucoside of 6-chloropicolyl alcohol (6-CPA) or RBN 1114 at 0.021 ppm (1.3%). The petitioner has identified 1.59 ppm (90.1%) out of 1.76 ppm. 0.13 ppm was extracted but not identified. The petitioner claims there are 26 unknowns, though reviewing the copies of the tlc chromatograms we can not confirm any of the unknowns. Only 0.037 ppm (2.1%) of the residue was unextractable.

The residue profile on apples treated with ^{14}C-imidacloprid at 14 days PHI was quite similar to the 0 day residue profile. The total ^{14}C-imidacloprid residue was 1.44 ppm, of which 0.99 ppm (69%) was imidacloprid, per se. The qualitative identification of imidacloprid metabolite residues for 14 days PHI was the same as at 0 day PHI. Identified residues were the 5-hydroxy imidacloprid or WAK 4103 at 0.039 ppm (2.7%), the dihydroxy imidacloprid or WAK 3772 at 0.016 ppm (1.1%), the olefin imidacloprid or NTN 35884 at 0.082 ppm (5.7%), the guanidine imidacloprid or NTN 33823 at 0.038 ppm (2.4%), the urea imidacloprid or DIJ 9817 at 0.024 ppm (1.7%), the nitrosimine imidacloprid or WAK 3839 at 0.01 ppm (0.7%)., and the glucoside of the 6-chloropicolyl alcohol (6-CPA) or RBN 1114 at 0.031 ppm (2.2%). The petitioner has identified 1.24 ppm (85.5%) out of 1.44 ppm. An additional 0.16 ppm was extracted, but was not identified. The petitioner points out the same problems existed for identifying these 14 day PHI residues as existed in identifying the 0 day PHI residues. Only 0.044 ppm (3%) of the residue was unextracted.

The nature of the imidacloprid residue in apples is adequately understood. Imidacloprid is metabolized by three pathways as follows:

1) hydroxylation of the dihydroimidazole ring of imidacloprid to form 4-hydroxy, 5-hydroxy, and dihydroxy imidacloprid followed by the loss of water to form the olefin imidacloprid,

2) reduction and loss of the nitro group on the dihydroimidazole ring to from the nitrosimine imidacloprid, then the guanidine imida-cloprid, and finally the urea imidacloprid, and

3) bridge cleavage of the C-N bond to form the 6-chloropicolyl alcohol (6-CPA) which rapidly form the glucoside and 6-chloronico-tinic acid (6-CNA) and dihydroimidazole.

The residues of concern in apples are imidacloprid and its metabolites containing the 6-chloropyridinyl moiety.

All of the identified metabolites contain the 6-chloropyridinyl moiety and all metabolites, except 6-CNA and 6-CPA, contain both rings of imidacloprid. CBTS agrees that the proposed residue analytical method will recover the metabolites of concern in that 85 to 90% of the residue is identified and that the proposed common moiety method recovers around 83% of the total residue. The question on whether or not the petitioner needs to do a double labeled metabolism study with a ^{14}C- label in the dihydroimidazole can be resolved by a logical review of the existing data. Only 1-2% (0.02-0.03 ppm) of the residues is identified as 6-CPA/6-CNA, thus it follows that only 1-2% (0.02-0.03 ppm) of the residue contains the metabolites from the dihydroimidazole ring. Based on the October 1989 overview of the residue chemistry guidelines the petitioner has adequately characterized the total dihydroimidazole ring metabolite(s) residue between 0.01 and 0.05 ppm. There is insufficient residue present to make further identifications of metabolites containing only the dihydro-imidazole ring.

The results of the translocation part of the experiment indicate that over half of the radioactivity applied to apple leaves is lost due to volatilization and that very little;, ie, <0.1% of the residue translocated to the fruit.

Cottonseed (MRID# 425561-05)

The petitioner presented the results of an imidacloprid in cottonseed metabolism study in a document titled "Metabolism of NTN 33893 in Cotton After Seed Treatment" by K. Vogeler and A. Brauner dated August 4, 1992, and coded laboratory project ID M 173 0311-6 and Miles report number 103818.

The petitioner used pyridinyl-^{14}C-methyl imidacloprid that had specific activities of 149-150.2 uCi/mg with 99.93% radiochemical purity. The radiolabeled imidacloprid was formulated as a 70 WS for use as a seed treatment for cottonseed and as SL 200 for use as a soil drench.

Cottonseeds, Coker 310 variety, were treated with the ^{14}C-imida-cloprid at a rate of 0.46 mg a.i. imidacloprid per seed or 460 grams a.i. imidacloprid per 100 kg cottonseed. Cottonseed were planted one to a 1 liter pot filled with a loamy silt soil and grown in a greenhouse in Monheim, Germany. Samples were harvested at maturity 210 days later. The samples were divided into seeds, lint, and gin trash. Leaves that fell off during the cotton growth were also collected for analysis.

In a separate experiment the petitioner treated 2 cotton plants as a soil drench with an exaggerated application of ^{14}C-imidacloprid to obtain sufficient radiolabeled material for identification of metabolites. The cotton plants received a total of 30 mg a.i. imidacloprid application between 11 and 145 days after planting for a PHI of 85 days. Samples were harvested at maturity and separated into lint, seed, and gin trash. Leaves that fell off during growth were also collected for analysis.

CBTS would have preferred a cottonseed metabolism study that approximated the proposed in-furrow banded at planting application. However, the results from the ^{14}C-imidacloprid treated cotton seed and soil drench application reasonably approximate the proposed soil application use.

All of the cotton plant material was processed and prepared in the same manner. Samples were macerated in liquid N_2. The cotton seed samples were initially soxhlet extracted in hexane for 5 hours. The cottonseed meal or remainder, lint, gin trash were extracted with methanol/water(1:1) followed by 2 times methanol extractions. The cotton plant samples were then further sequentially extracted with a 36 hours methanol reflux, with a methanol/6 N HCL (1:1) 6 hour reflux; and with a methanol/2N NaOH (3:2) 6 hour reflux for cotton seeds only. The samples were cleaned-up on a XAD-4, 200-400 um, resin column, 50 cm X 30 mm (i.d.). ^{14}C-imidacloprid and its metabolites were eluted off the column with methanol.

Determination of the residues was primarily by TLC using silica gel on glass plates, or on pre-coated aluminum plates (Kisselgel 60 F_{254} from Merck) and several mobile phases either one or two dimension development. The radioactive residues were determined using a Linear Analyzer or exposure to X-ray film. The mass spectra were generated on a Hewlett Packard mass selective detector coupled to HP 5880A gas chromatograph fitted for a 12 meter DB-1 capillary column, temperature programmed. The GC injection were splitless at an injection port temperature of 280°C. The radioactivity in liquid samples was determined by liquid scintillation counting (LSC) using a Philips PW 4700. Solid samples were initially combusted in Harvey OX 300 oxidizer. The $^{14}CO_2$ was trapped in a scintillation cocktail of

Carbosorb plus Permafluor then LSC. The petitioner has provided an adequate amount of supporting spectra, chromatograms, and copies of tlc plates to support his conclusions on the nature of the imidaclo-prid residue in cottonseeds.

Radioanalysis of the cotton plant parts from the ^{14}C-imidacloprid treated seed showed imidacloprid equivalents of 0.0049 ppm in the seed, 0.0019 ppm in the lint, and 0.005 ppm in the gin rash. The low residue levels with a corresponding low level of radioactivity prevented extensive identification of residues. The only compound identified in cottonseed in this part of the metabolism study is 6-CNA at 0.0012 ppm (23% of the residue). There was sufficient residue in the cotton leaves to have part of the residue identified. 0.11 ppm imidacloprid equivalents were detected in the cotton leaves. Of this 0.003 ppm (2.9%) was the parent compound, 0.002 ppm (2.2%) was 6-CNA, 0.011 ppm (9.8%) was guanidine imidacloprid or BEG 5322, 0.012 ppm (11.3%) was a conjugate of 6-CPA, 0.007 ppm (6.3%) was the glucoside of 6-CNA or RBN 1114, 0.002 ppm (1.4%) was 6-CPA or DIJ 9805, 0.002 ppm (1.4%) was the nitrosimine imidacloprid or WAK 3839, and 0.002 ppm (1.5%) was the olefin imidacloprid or NTN 35884. The petitioner has identified 0.041 ppm (37.3%) of the 0.11 ppm in cotton leaves. An additional 10% of the residue is 4 unidentified metabolites with about 25% of the residue reported as diffuse and remaining at the origin. 26.8% of the radioactive residue could not be extracted from cotton leaves.

The nature of the imidacloprid residue in cotton forage is not completely understood. The imidacloprid residues from cotton leaves consist of 10% unidentified metabolites, 27% unextracted radiolabeled residues, and 25% reported as diffuse and remaining at the origin. The balance of the residues reported are identified and all are below 0.01 ppm. Since CBTS does not consider cotton forage to be a significant livestock feed item and that tolerances will not be necessary, the petitioner does not need to provide additional identification of radiolabeled residue in cotton leaves, either from cotton leaves not analyzed or from reanalysis of extracts from the treated leaves.

The results of analysis from the exaggerated application for the soil drench part of the metabolism study did not reveal much additional information. The total imidacloprid equivalents detected in cottonseed were 9.35 ppm. The soxhlet extraction with hexane recovered only 0.05 ppm (0.6%) imidacloprid equivalents which were not further identified. The methanol/water extraction recovered 1.86 ppm (19.9%) of the residue from cottonseed. 0.08 ppm (0.8%) of the residue was identified as the parent compound. The only other compound identified in this extract was 6-CNA at 0.69 ppm (7.4%). 11.7% or 1.09 ppm of the residue in this extract contained unidentified or diffuse components. 4.16 ppm (44.5%) of the residue was recovered by the methanol reflux. 6-CNA was detected at 3.13 ppm (33.5%) and the methyl ester of 6-CNA was 0.24 ppm (2.6%) of the residue. 5 unidentified metabolites totaling 3.03 ppm (32%) were recovered from the methanol/6N HCL reflux. The petitioner has identified only 44% of the 9.35 ppm residue in cottonseed.

When the fact that 56% of the residue is unidentified metabolites is translated to the proposed 6 ppm tolerance for cottonseed, CBTS concludes that the nature of the imidacloprid residue in cottonseed is not adequately understood. The petitioner is reminded that he is expected to identify at least 90% of the radioactive residue. The petitioner needs to provide additional identification of radiolabeled residue in cottonseeds, either from reserve ^{14}C-imidacloprid cottonseeds not analyzed or from reanalysis of extracts from cottonseeds from the soil drench application part of the metabolism study. CBTS feels that the metabolite

that is 1.62 ppm imidacloprid equivalents should be easily identified. The petitioner also has the option of repeating the ^{14}C-imidacloprid cotton metabolism study using an exaggerated application rate that approximates the proposed in-furrow, banded at planting application plus 4 foliar applications to generate sufficient radiolabeled residue for identification of metabolites.

Potatoes (MRID# 425561-06 and -07)

The petitioner presented the results of an imidacloprid in potatoes metabolism study following a foliar application in a document titled "Study on the Metabolism of NTN 33893 After Spray Application to Potatoes" by G. Drager, et. al., dated May 8, 1992, and coded laboratory project number M 173 0 162-0 and Miles report number 103211. The petitioner also presented the results of an imidacloprid in potato metabolism study following a granular application in a document titled "Investigation of the Metabolism of NTN 33893 in Potatoes Following Granular Application" by K. Vogeler, et al., dated July 19, 1991, and coded laboratory project number M 173 0297-9 and Miles report number 103218.

For the granular application the petitioner used pyridinyl-^{14}C-methyl imidacloprid that had a specific activity of 25.5 uCi/mg with 99.7% radiochemical purity. The radiolabeled imidacloprid was formulated as a 5% granular for use on potatoes.

Potatoes, Clivia variety, were treated at planting in furrow with ^{14}C-imidacloprid at a rate of 1 gram per meter (0.05 gram active ingredient). The application rate in the metabolism study is 1.52 grams a.i. (0.165X) per 1000 foot row vs. the proposed use of 9.2 grams a.i. per 1000 foot row. The 6 potatoes were pre-germinated and planted 2 to a row in the 3 row box and grown to maturity. The study was conducted at Bayer's metabolism research center in Monheim, Germany. The mature tubers and withered vines were harvested 129 days after ^{14}C-imidacloprid application. The tubers were separated from the vines, washed, cut into small pieces, and stored frozen at -20°C until analysis.

Both the potato tubers and vines were extracted by successive blendings with methanol/water (2:1 for tuber and 1:1 for vines), methanol, and dichloromethane using an Ultra-Turrax. The extracts were combined and concentrated, then the aqueous remainder was partitioned first with hexane, then with ethyl acetate. The water phase had a XAD-4 resin (20 grams) column (2 cm i.d.) clean-up before TLC analysis of metabolites. The parent imidacloprid and its metabolites were eluted off the column in methanol. The eluant from the XAD-4 column for the tubers was further cleaned-up by using Sep Pac RP 18 cartridges. The ethyl acetate extract went directly to the TLC determination step.

The determination of the parent ^{14}C-imidacloprid and its metabolites residues was primarily by TLC using commercial plates from Merck, precoated silica gel 60 F_{254} on aluminum and four different mobile phases with one or two dimension development. The radioactive residues were determined using a Linear Analyzer, or exposure to X-ray film. Confirmation of low level residues was by co-chromatography of authentic standards and visualization in UV light at 254 mn. HPLC was with a Varian, model 5000, equipped with a RP 8, 25 cm X 4 mm (id) column, gradient flow at 1 ml/min connected to a Gilson fraction collector, model 202 and a Raytest Ramona D radioactivity flow through detector. The ^{1}H-NMR spectra were recorded on a Bruker AC 300. Samples were dissolved in CD_3OD.

The ^{14}C-imidacloprid equivalents in the liquid extracts was determined by liquid scintillation counting (LSC) using a Philips PW 4700 or a LKB Rackbeta 1219. Solid samples were combusted in a Harvey OX 300 oxidizer. The $^{14}CO_2$ was trapped in a scintillation cocktail of Carbosorb plus Permafluor, then LSC. The petitioner used reverse isotope dilution of 6-CNA for structural determination of the 6-CNA in the tuber extracts.

The petitioner has presented an adequate amount of spectra, chromatograms, and copies of TLC plates to support his conclusions on the nature of the imidacloprid residue in potatoes from a granular in-furrow application.

The total ^{14}C-imidacloprid equivalents in the potato vines were 5.76 ppm and in the potato tubers were 0.091 ppm. These residues were the sum of all of the radioresidues determined in the extracts plus the solid remainder. In the potato tubers 0.044 ppm (48.3%) of the 0.091 ppm was identified as the parent imidacloprid. 5-hydroxy imidacloprid or WAK 4103 was 0.007 ppm (8%) and the olefin imidaclo-prid or NTN 335884 was 0.003 ppm (3.1%) of the residue. 6-CNA was 0.009 ppm (9.4%) and the guanidine imidacloprid was 0.01 ppm (11.3%) of the residue. In potato tubers the petitioner identified 0.073 ppm (80.1%) of the 0.091 ppm. An additional 0.012 ppm (13.1%) was the sum of 5 unidentified metabolites and only 0.006 ppm (6.4%) of the residue was unextracted.

The residues detected in the potato vines confirms the metabolic pathway found in potato tubers. In potato vines 1.53 ppm (26.7%) of the 5.76 ppm was the parent imidacloprid. 5-hydroxy imidacloprid or WAK 4103 was 0.26 ppm (4.6%) and the olefin imidacloprid or NTN 35884 was 0.19 ppm (3.3%) of the residue. 6-CNA and the guanidine imida-cloprid were each 0.48 ppm (8.3%) of the residue. Dihydroxy imida-cloprid was 0.02 ppm (0.3%) and the glucoside of 6-CPA or RBN 1114 was 0.08 ppm (1.4%) of the residue. Nitrosimino imidacloprid was 0.015 ppm (2.6%) of the residue. The petitioner has identified 3.19 ppm (55.4%) out of 5.76 ppm. 14 unidentified metabolites with a sum of 0.93 ppm were reported and 1.52 ppm (26.4%) was non-extractable. Starting with a larger total radioresidue in potato vines, and identifying more metabolites helps for tentative identification of metabolites in potato tubers. CBTS feels that it is probable that the nitrosimino imidacloprid, the dihydroxy imidacloprid, and the glucoside of 6-CPA are present in the potato tubers only at too low a level to confirm. Since potato vines are neither a livestock feed item nor a food commodity CBTS does not feel additional measures are necessary to identify any of the unidentified residues in potato vines. The metabolic profile determined in potato vines supports the metabolic profile determined in potato tubers.

For the foliar application the petitioner used pyridinyl-^{14}C-methyl imidacloprid that had a specific activity of 179.5 uCi/mg with 99.1% radiochemical purity. The radiolabeled imidacloprid was formulated as a 25 WP for use as a foliar spray on potatoes.

Potatoes (Hansa variety) were planted in 2 plant boxes at Bayer's Institute for Metabolism Research in Monheim, Germany. The potatoes were planted on April 29, 1987, emerged on May 12 and were sprayed on July 29 as a 0.2% spray at a rate of 134 grams/hectare or 0.12 lb/acre (30 mg per plant). The metabolism application rate approximates the proposed use rate of a maximum application of 0.2 lb a.i./a foliar spray. Each plant was sprayed individually one time and was wrapped in a box of plastic to prevent drift of the radiolabeled spray to adjoining potato plants. Samples were harvested 7, 28, and at maturity 64 days after application. The tubers were cleaned by washing.

Both the potato vines and tubers were extracted by successive blendings with methanol/water (1:1),methanol, and dichloromethane. The extracts of the vine samples were concentrated to the aqueous remainder, then this was extracted with hexane and methanol. The methanol/water and methanol extracts from the tubers was concentrated to the aqueous remainder and then extracted with dichloromethane. Aliquots of the water phase of the 64 day vine extract underwent enzymatic cleavage with cellulase, glucosidase, and esterase to free metabolites. The vines water extract was subjected to pH 1 HCl acid hydrolysis while the solids from the vines was subjected 70% H_2SO_4 at 10°C for 16 hours then heated to boiling for 4 hours.

The determination of the parent ^{14}C-imidacloprid and its metabo-lites was primarily by TLC using commercial plates from Merck pre-
coated silica gel 60 F_{254} on aluminum and four different mobile phases with one or two dimension development. The TLC plate were cleaned by dipping in a tank of IPA for 1 hour and then reactivated on a CAMAG plate heated II at 130°C over a stream of N_2. Visualization of the various compounds was by measurement of the UV absorption with a densitometer, linear analyzer scan, autoradiography as exposure on beta sensitive film, and quenching of the UV induced fluorescence. Co-chomatographed standards were visualized under UV light at 254 nm. The petitioner used two HPLC systems. One HPLC system was a Varian, model 5000 equipped with a RP 8, 10 um, 25 cm X 4 mm (id) column, gradient flow at 1 ml/min connected to Gilson, model 292 fraction collector, and a Ramona D radioactivity flow through detector. The other HPLC system is a HP 1090 A equipped with LiChrosphere 100 RP-18, 10 um, 25 cm X 4 mm (id) column, gradient mobile phase at 1 ml/min connected to a diode array detector and a Ramona 4 radioactivity detector. The ^{1}H-NMR spectra were run on Bucker AC 300. Samples were dissolved in CD_3OD and $CDCl_3$. The mass spectra were generated on a Finnigan 8230 with EI and CI ionization at 70 eV and a source temperature of 200°C. Isobutane or NH_3 were the CI reactant gases.
The ^{14}C-imidacloprid equivalents in the liquid extracts was determined by liquid scintillation counting (LSC) using a Philips PW 4700 or a LKB Rackbeta 1219. Solid samples were combusted in a Harvey OX 300 oxidizer. The $^{14}CO_2$ was trapped in a scintillation cocktail of Carbosorb plus Permafluor, then LSC.

The petitioner has presented an adequate amount of spectra, chromatograms, and copies of TLC plates to support his conclusions on the nature of the imidacloprid residue in potatoes from a foliar application.

The total ^{14}C-imidacloprid equivalents in potato tubers treated at the 134 grams/ha rate were 0.009 ppm from the foliar application. 0.007 ppm could be extracted from the potato tuber. 0.001 ppm was the parent imidacloprid and 0.003 ppm was 6-CNA. 0.001 ppm was bound, unextracted residue. The remaining 0.004-0.005 ppm was three other metabolites and diffuse radioactivity.

The residues detected in potato vines confirms the pathway found in tubers and the metabolic pathway found from the in furrow at planting granular application. In potato vines 0.51 ppm (37.9%) of the 1.35 ppm total residue was the parent imidacloprid. 5-hydroxy imidacloprid or WAK 4103 was 0.095 ppm (7%) and the olefin imidaclo-prid was 0.034 ppm (2.5%) of the residue. The major metabolite detected was guanidine imidacloprid at 0.17 ppm (12.6%). The nitro-simino imidacloprid was 0.03 ppm (2.2%) and the dihydroxy imidaclo-

prid was 0.036 ppm (2.7%) of the residue. 6-CNA was not detected; however the glucoside of 6-CPA was found at 0.025 ppm (1.9%). One metabolite was identified that has not been reported in other studies. It is the triazinone imidacloprid or BNF 4712B at 0.014 ppm. The petitioner has identified 0.901 ppm (66.8%) of the 1.35 ppm residue in potatoes vines. 0.19 ppm (14.1%) remained in the solid vines as unextracted, bound radioactive residue and 0.258 ppm (19.1%) was unidentified metabolites.

CBTS reiterates that it is probable that the imidacloprid metabolites detected in potato vines are also present in the potato tubers only at too low a level to confirm. The metabolic profile determined in potato vines supports the metabolic profile determined in potato tubers. We conclude that it is the same metabolic pathway for imidacloprid in potatoes for either in-furrow or foliar application.

The nature of the imidacloprid residue in potatoes is adequately understood. It follows the same metabolic pathway found in apples. Imidacloprid is metabolized by three pathways as follows:

1) hydroxylation of the dihydroimidazole ring of imidacloprid to form 4-hydroxy, 5-hydroxy, and dihydroxy imidacloprid followed by the loss of water to form the olefin imidacloprid,

2) reduction and loss of the nitro group on the dihydroimidazole ring to from the nitrosimine imidacloprid, then the guanidine imida-cloprid, and finally the urea imidacloprid, and

3) bridge cleavage of the C-N bond to form the 6-chloropicolyl alcohol (6-CPA) which rapidly forms the glucoside and 6-chloronico-
tinic acid (6-CNA) and dihydroimidazole.

The residues of concern in potatoes are imidacloprid and its metabolites containing the 6-chloropyridinyl moiety.

Plant cell suspension cultures (MRID# 425561-12)

The petitioner presented the results of imidacloprid metabolism in plant cell suspension cultures in a study titled "Comparative Metabolism of [pyridinyl-14C] NTN 33893 in Plant Cell Suspension Cultures" by J. Koester, dated February 22, 1990, and coded labor-atory project number M 1710181-9 and Miles report number 103213.

The purpose of this experiment was to determine the metabolism of imidacloprid in various heterotrophic plant cell suspension cultures. The plant cells used in study were from citrus (Keyline), potato (HH 258), tomato, soybean (Mandarin), tobacco, peanuts, parsley, wheat (Heines Koga II), cotton (Coker 310), and corn (Black Mexican sweet). The callus cultures were cultivated in about 50 ml of nutrient medium which was solidified with 8 gram/liter agar-agar. The temperature was maintained at 25°C $\pm$ 2°C with the heterotrophic cell cultures cultivated in the dark and the mixotrophic cell cultures maintained with cool white fluorescent lights. The suspension cultures were shaken on a rotary shaker at 130 cycles per minute. The imidacloprid application solution containing 511 ug/40 ml cell suspension, radioactivity at 2 uCi/40 ml cell suspension (specific activity of 3.91 uCi/mg) was applied directly to the plant cell after sterilization with cells from the beginning of the stationary

growth phrase. The cell cultures were grown for an additional seven days after application of ^{14}C-imidacloprid.

The cell cultures were harvested, processed, then extracted and fractionated with the instruments and determination steps as described for imidacloprid residues in apples, cottonseed, and potatoes. TLC was the primary identification technique with LSC as the quantifying technique.

The result of the study showed there were both qualitative and quantitative differences in cell suspension metabolism of imidaclo-prid. While imidacloprid was metabolized in all cell cultures the extent of metabolism varied widely. Imidacloprid, per se, was the dominant residue detected, except in apples, as it accounted for 77.1% to 98.5% of the recovered residue. In apples imidacloprid was only 39.4% of the residue recovered. There was very little metabolism of imidacloprid in corn, peanuts, and tomatoes. The major metabolites detected in all cell cultures were the 5-hydroxy imida-cloprid and olefin imidacloprid. The sum of identified residues ranged from 91.1% in soybean cells to 99.3% in peanut cells. Other metabolites identified, but not in all cell cultures were the dihydroxy, nitrosimino, urea, guanidine imidacloprid. Evidence for cleavage at the methylene bridge exists as 6-CNA, 6-CPA and its glucoside conjugate were recovered.

The results of the plant cell suspension cultures metabolism studies are complementary to the ^{14}C-imidacloprid metabolism studies conducted and reported in apples and potatoes. This supports the conclusion that imidacloprid is metabolized in three pathways as follows. First, there is the oxidation of the ethylene bridge in the imidazolidine ring; second is oxidation of the pyridinyl methylene group followed by cleavage and glucose conjugation; and third reduction of the nitro to the nitroso group.

Tomatoes (MRID# 425561-09)

The petitioner presented the results of an imidacloprid metabolism study in tomatoes in a document titled "Investigation on the Metabolism of NTN 33893 After Application to Tomatoes" by G. Drager, et al., dated October 12, 1989, and coded laboratory Project numbers M 173 0 237-3 and 238-4, and Miles report number 103212.

The petitioner used pyridinyl-^{13}C-methyl and ^{14}C-methyl imidacloprid. The ^{13}C-imidacloprid had a 99.3% degree of labeling and the ^{14}C-imidacloprid was 97.4% chemical purity and a specific activity of 150.68 uCi/mg. The radiolabeled imidacloprid was formulated as a 25 WP for use on tomatoes.

The petitioner conducted a two part metabolism study with one part for the metabolism after surface application and the other part after stem injection. The study was conducted at Bayer's metabolism research center in Monheim, Germany. For the surface application, the fruit on 2 tomato plants each with several tomatoes on each of 4 syncarpies were treated by brushing each tomato uniformally with the equivalent of a 0.05% a.i. solution. Samples of the tomatoes were harvested as they matured at days 4, 7, 14, and 21. For the stem injections 5 mg of ^{12}C-imidacloprid plus 5 mg of ^{13}C-imidacloprid plus 128 uCi of ^{14}C-imidacloprid in 1 ml of 50% ACN/water were injected in 10-20 ul portions into the stems and petioles of 2 tomato plants. After 10 days the plants were harvested for analysis.

The separation and identification plus quantitation of imidacloprid and its metabolites from tomatoes and tomato plants involved analytical techniques, reviewed above, similar to those used in the apple, cottonseed, and potato metabolism studies.

The total ^{14}C-imidacloprid equivalents at 0 day PHI were 1 ppm of which 0.95 ppm (95%) was imidacloprid, per se. Other residues identified were the guanidine imidacloprid at 0.011 ppm (1.1%), the urea imidacloprid at 0.009 ppm (0.9%), the olefin imidacloprid at 0.001 ppm (0.1%), the hydroxy imidacloprid at 0.011 ppm (1.1%), the nitrosimino imidacloprid at 0.003 ppm (0.3%), and the gentiobioside of 6-CPA. The petitioner has identified 0.986 ppm (98%) of the residues on tomatoes at 4 days PHI.

A similar residue profile was observed at 7 and 14 days PHI. At 7 days PHI the total imidacloprid equivalents were 0.84 ppm and at 14 days PHI the imidacloprid equivalents were 0.85 ppm. Of the 0.85 ppm at 14 days PHI 0.75 ppm (88%) of the residue was imidacloprid, per se. Other residues identified were the guanidine imidacloprid at 0.022 ppm (2.6%), the urea imidacloprid at 0.016 ppm (1.9%), the olefin imidacloprid at 0.004 ppm (0.5%), the hydroxy imidacloprid at 0.015 ppm (1.8%), the nitrosimino imidacloprid at 0.006 ppm(0.7%), the glucoside of 6-CPA at 0.0008 ppm (0.1%), and the gentiobioside of 6-CPA. The petitioner identified 0.82 ppm (96.4%) out of 0.85 ppm.

At 21 days PHI the total imidacloprid residue was 0.60 ppm of which 0.51 ppm (79.4%) was imidacloprid, per se. The same imidaclo-prid metabolites were detected at 21 days PHI as were detected at 14 days PHI, only the percentages increased slightly.

CBTS concludes that the nature of the imidacloprid residue in tomatoes is adequately understood. Imidacloprid is metabolized by the same 3 metabolic pathways identified for apples and potatoes. The residues of concern in tomatoes are imidacloprid and its metabolites that contain the 6-chloropyridinyl moiety.

Eggplant (MRID# 425561-10)

The petitioner presented the results of an imidacloprid in eggplant metabolism study in a document titled "Metabolism of NTN 33892 in Eggplant by Planting Hole Application" by H. Yoshids, dated February 5, 1991, and coded laboratory project ID 89054/ESR and Miles report number 103210. The objective of the study was to determine the adsorption, translocation, and degradation of imidacloprid in eggplants.

The petitioner used pyridinyl-^{14}C-methyl imidacloprid that had a specific activity of 155 mCi/mg and a radiopurity of > 99%. The radioactive imidacloprid was formulated as a 1% granular material for use on eggplants.

The petitioner used young eggplants (Solanum melongena L.) from a nursery and grew them until the 8 leaf stage. The test was conducted in a greenhouse in Nitokuno, Japan. The petitioner conducted a four part metabolism study. Part one was a balance study using 2 grams of ^{14}C-formulation per plant on 6 plants with 2 plants as non-treated controls in a planting hole application at transplanting. Part 2 was an identification of metabolites using 2 grams of the non-labeled formulation per plant on 2 plants as a plant hole application. Part three was at the 8 leaf stage in which the 2 eggplants were treated at an exaggerated rate of

10 grams (5X) ^{14}C-imidacloprid formulation per plant for plant hole application (30 kg of formulation /hectare, or 4.23 ounces a.i. imidacloprid per acre). Part 4 of the study was for identification of metabolites by application of an exaggerated rate of 10 grams (5X) of non-labeled formulation per plant as a plant hole application.

The sample preparation, and separation and identification plus quantitation of imidacloprid and its metabolites from eggplants involved analytical techniques, reviewed above, similar to those used in the apple, cottonseed, and potato metabolism studies. While the analytical instruments were different the residue results generated would be equivalent regardless of the instruments used. Tentatively, the petitioner has presented an adequate amount of spectra, chromatograms, and copies of TLC plates to support his conclusions on the nature of the imidacloprid residue in eggplants from a soil granular application.

The petitioner presented identification and quantitation of ^{14}C-imidacloprid residues in edible fruits from eggplants only from part one of the study and then from only 2 of the 6 treated plants. Data were presented for edible eggplant fruits harvested at PHIs of 49, 53, and 67. While CBTS would have preferred to review data from part three (5X exaggerated ^{14}C application) there is no proposed use for imidacloprid in the petition for eggplants, thus these imidacloprid on eggplants metabolism data are only supplementary for the proposed use and tolerances. If the petitioner proposes imidacloprid uses for eggplants or any member of the fruiting vegetables (except cucurbits) crop group, then the petitioner needs to present a complete imida-
cloprid metabolism study report for all four parts of the study. The data for part one alone are not fully sufficient to determine the nature of the imidacloprid residue in eggplants.

The total ^{14}C-imidacloprid equivalents in the edible eggplants ranged from 0.0315 ppm at PHI 53 days to 0.0534 ppm at PHI 67 days, averaging 0.0428 ppm. Since we are dealing with low levels of total radioactivity from the ^{14}C-imidacloprid application and the same metabolites were detected at all three PHIs we will review only the average residues reported. Imidacloprid, per se, or NTN 33893 was 0.0081 ppm (18.9%) of the residue. The major metabolites identified were the guanidine imidacloprid, or NTN 38014 at 0.0049 ppm (14.0%), 6-CNA at 0.0035 ppm (13.4%), and the 6-CPA glucoside, or RBN 114 at 0.0066 ppm (13.0%). Other metabolites detected were the 5-hydroxy imidacloprid, or WAK 4103 0.0015 ppm (3.2%), the olefin imidacloprid, or NTN 35884 at < 0.005 ppm (0.17%), and the nitrosimino imidacloprid, or WAK 3839 at < 0.005 ppm (0.093%). The urea imidacloprid was not detected. CBTS feels the report from part 3 where an exaggerated ^{14}C application would have better identified and quantitated the imidacloprid metabolites in eggplants.

Tentatively, CBTS concludes that the nature of the imidacloprid residue in eggplants is adequately understood. Imidacloprid is metabolized by the same 3 metabolic pathways identified for apples and potatoes. The residues of concern in eggplants are imidacloprid and its metabolites that contain the 6-chloropyridinyl moiety.

Corn (MRID# 425561-11)

The petitioner presented the results of an imidacloprid in corn metabolism study in a document titled "Metabolism of NTN 33893 in Corn After Seed Dressing" by K. Vogler, et al., dated January 14, 1992, and coded laboratory project number M 173 0211-5 and Miles study number 103217. The purpose of this study is to determine the metabolism of imidacloprid in corn after seed treatment.

The petitioner used pyridinyl-^{14}C-methyl imidacloprid that had a specific activity of 39.3 uCi/mg and a radiochemical purity of 99.9%. The radiolabeled imidacloprid was formulated as a WS 70 for use as corn seed treatment.

The corn seed was treated at a rate of 721 grams a.i. imidacloprid/100 kg of corn seed. This corresponds closely to a "proposed" application rate of 700 grams (24.7 ounces) per 100 kg of seed, or 105 grams per hectare (1.5 ounce/acre) assuming a corn seeding rate of 15 kg/ha. The study was carried out 1987-88 at Bayer's Institute for Metabolism Research in Monheim, Germany. 16 treated corn seeds (Variety Mutin D) were planted individually in various size pots containing a loamy silt soil.

Corn plants from four seeds were harvested at day 33 when the corn plants were at the 6-7 leaf stage, and another four plants were harvested at day 61 when the corn was at the 9 leaf stage. These samples were to determine the nature of the residue in green corn forage. The final 8 plants were harvested at maturity at day 134 and divided into fodder, husk, cob, and corn grain.

Sample preparation, extraction of imidacloprid from the different matrices, and identifying and quantitating the various residues
involved analytical techniques and instrumentation, reviewed above, similar to those used in the apple, cottonseed, and potato metabolism studies. The petitioner has presented an adequate amount of spectra, chromatograms, and copies of TLC plates to support his conclusions on the nature of the imidacloprid residue in corn from a seed treatment application.

At 33 days after planting the total ^{14}C-imidacloprid residue equivalents in immature corn forage were 5.84 ppm of which 3.81 ppm (65.2%) was imidacloprid, per se. Other residues identified in corn at 33 days PHI were the 5-hydroxy imidacloprid at 0.41 ppm (7%), the 6-CNA at 0.04 ppm (0.7%), the olefin imidacloprid at 0.26 ppm (4.5%), the nitrosimino imidacloprid at 0.1 ppm (1.7%), the dihydroxy imidacloprid at 0.03 ppm (0.5%), the ring opened guanidine imidacloprid at 0.04 ppm (0.6%), the guanidine imidacloprid at 0.33 ppm (5.7%), the 6-CPA at 0.03 ppm (0.05%), and the urea imidacloprid at a trace. The petitioner has identified 5.05 ppm (86.4%) of the 5.84 ppm. There are 7 other metabolites totaling 0.2 ppm and 0.44 ppm (7.6%) unextracted residue.

The same metabolites were identified in corn forage at 61 days PHI, only the amounts were different. At 61 days PHI the total ^{14}C-imidacloprid residues were 1.52 ppm. The petitioner also presented residue data for corn grain, husks, cobs, and fodder at 134 days PHI. The total ^{14}C-imidacloprid residue equivalents in corn husks were 0.21 ppm and in corn cobs the residues were 0.12 ppm.

In corn grain the total ^{14}C-imidacloprid equivalents were 0.04 ppm of which 0.01 ppm (25.2%) was imidacloprid. Other metabolites identified were 6-CNA at a trace level, the 5-hydroxy imidacloprid or WAK 4103 at 0.004 ppm (9.3%), the olefin imidacloprid or NTN 35884 at 0.005 ppm (13.1%), the dihydroxy imidacloprid or WAK 3772 at 0.002 ppm (4.4%), the guanidine imidacloprid or BEG 5322 at <0.001 ppm (2.0%), and 6-CPA or DIJ 9805 at 0.002 ppm (4.4%). The petitioner has identified 0.023 ppm (58.4%) of the 0.04 ppm residue in corn grain. Two other metabolites at < 0.002 ppm (4.6%) of the residue were not identified

and 0.01 ppm (26.2%) of the residue was unex-tractable residue.

In corn fodder the petitioner identified the same residues as were identified in corn forage at 33 and 51 days PHI only the amounts were different. In corn fodder only 0.68 ppm (22.2%) of the 3.08 ppm was imidacloprid. The petitioner identified 64.8% of the residue in corn fodder.

CBTS concludes that the nature of the imidacloprid residue in corn grain, fodder, and forage is adequately understood. Imidaclo-prid is metabolized by the same 3 metabolic pathways identified for apples and potatoes. The residues of concern in corn are imida-cloprid and its metabolites that contain the 6-chloropyridinyl moiety.

NATURE OF THE RESIDUE - LIVESTOCK

Ruminants (MRID#s 425561-14 and -15)

The petitioner presented the results of a caprine imidacloprid metabolism study in a document titled "[Pyridinyl-^{14}C-methylene] Imidacloprid: Absorption, Distribution, Excretion, and Metabolism in a Lactating Goat" by W. Karl, et al., dated December 18, 1991, and coded laboratory project ID M 184 0260-1 and Miles report number 103819. The petitioner also presented additional ruminant metabolism data in a document titled " [Methylene-^{14}C] Imidacloprid: Absorption,
Distribution, Excretion, and Metabolism in the Liver and Kidney of a Lactating Goat Addendum I" by O. Klein dated October 11, 1992, and coded laboratory project ID M184 0528-8 and Miles report number 103819-1.

The petitioner conducted the first part of the ^{14}C-imidacloprid ruminant metabolism study using a 1 1/2 year old lactating Bunte Deutsche Edelziege goat (Capra hirus). The goat was obtained from a local breeder in Baden-Wurttemberg. Metabolism testing was conducted at Bayer AG's Department for Structure Elucidation and Institute for Metabolism Research in Monheim, Germany in 1991.

The goat was acclimatized for 7 days to the facility prior to the start of the metabolism testing. Identification was by hide marking as well as an individual cage marking. Housing was in a stainless steel metabolism cage equipped with a variable restraining device and suitable for separate collection of feces and urine. Environmental conditions were for the temperature to range from 20 to 23°C, 18 hours of lighting, relative humidity to range from 48 to 90%. Since the study was conducted in a laboratory the air flow rate of change was 10-15 times per hour. The goat's weight did not change during the metabolism study as the initial weight was 29.7 kg and at sacrifice the weight was 29.8 kg.

The goat received approximately 500 grams hay and a commercial feed supplied by Soest, plus an apple at the daily milking. Water was drawn from the tap and given ad libitum. Considering that goats consumed about 5% to 11% of their body weigh in feed per day we consider the amount fed to be low.

The goat was dosed orally by intubation with ^{14}C-pyridinyl methyl imidacloprid at a rate of 10 mg/kg body weight per day for 3 consecutive days. Assuming a feed consumption of 5% body weight per day the dose is equivalent to 200 ppm. The specific activity of the

^{14}C-imidacloprid was 87 uCi/mg and its radiopurity was 99+%. The radio-labeled material was diluted with "cold" imidacloprid to a specific activity of 8.7uCi/mg for dosing.

The petitioner collected samples of blood, feces, and urine during the study. While these specimens are essential to understanding the distribution and elimination of imidacloprid from the test animal they are not germane to our understanding the nature of the residue in edible tissues and milk. Milk was collected daily prior to dosing, 8 hour later, and just before sacrifice. At sacrifice 2 hours after the last dose the petitioner collected the liver, both kidneys; loin, round, and flank muscle; and SC, omental, and perirenal fat.

The liver, kidney, and muscle tissue samples were minced with dry ice prior to determining total ^{14}C in samples. The high fat samples were solubilized without homogenization. Total radioactivity from ^{14}C in these samples was determined by combustion using an Oxidizer, model 306 from Packard Instruments Company and trapping the $^{14}CO_2$ in Carbosorb, then adding Permafluor and counting on a Beckman LSC, or a Philips PW 4700, or a LKB Rack Beta 1219. All LSCs used quench correction and care was taken to avoid chemiluminescence by adding approximately 0.1 ml glacial acetic acid.

Results of the total ^{14}C-imidacloprid equivalents in the tissues are 15.9 ppm in liver, 11.6 ppm in kidney, 3.8 to 4.0 ppm in the different muscle samples, 1.8 ppm to 2.2 ppm in the different fat samples, and a maximum in milk at 4.1 ppm.

The petitioner proceeded to extract, isolate, and identify the components of the radiolabeled residue from the tissues and milk. For a 5 gram milk sample the butterfat was separated from the coagulated protein by centrifugation. The fat was extracted 3 X 10 mls with acetonitrile, separating out the emulsion and combining the ACN extracts. The upper phase was evaporated under nitrogen and redissolved in water. The extracts were ready for analysis.

The 195 gram liver sample was exhaustively extracted first with water then the extract was separated out on an adsorber resin XAD-1180. The column was eluted with methanol, then water. The water phase was acidified to pH 2 with 0.05M heptanesulfonic acid plus 0.1 M citric acid. The precipitated protein was removed by pressurized filtration and centrifugation. The methanol extract proved to be too dirty for HPLC metabolite identification. Additional clean-up and separation were accomplished by using RP-18 in the batch mode. All of the radioactivity was successfully eluted off with rinses of CH_3OH, ACN, 1% acetic acid and a final acetonitrile rinse. Two of the methanol phases (no. 2 and 3) were recombined and were cleaned-up further using silica gel cartridges (Adsorbex 400).

The 30 gram kidney sample was extracted with 2 X 60 mls methanol and ethyl acetate (2:1). An aliquot was concentrated under a stream of nitrogen, redissolved in 5 ml of water and analyzed. 20 to 40 grams of muscle tissues were extracted with water 3 times using a Ultra-Turrax homogenizer. The water extracts were combined and mixed with ACN and the resulting emulsion was separated by centrifugation. The water phase was extracted 4 times with ACN and the extracts were combined for analysis. Fat samples were extracted three times in ACN using an Ultra-Turrax homogenizer. The extracts were combined and concentrated under a stream of N_2, then redissolved in water for analysis.

The HPLC determinations were carried out using either a Varian, 5060 LC, or a

Hewlett Packard 1090 LC both equipped with a variable wavelength detector. Radioactivity was measured with a flow-through Ramona D detector with solid scintillator beads. Thin layer Chromatography (TLC) was done with either silica gel 60 F_{254} plates or with RP-18_{254s} plates with various developing one and two dimension systems. Determination was with a TIC Linear Analyzer LB 2832 or with Hyper-film-Bmax. Mass spectrometry was with a Finnigan-MAT 8230 MS at 70eV for electron impact (EI) spectra. The GC part of the GC/MS was a Varian 3700 GC equipped with 30m DB-1 fused silica capillary column, He carrier gas, splitless injection. Fast Atom Bombardment (FAB) mass spectra were generated on the same system with Xe as the bombardment gas using thioglycerol as the matrix. ^1H NMR spectra were generated on a Bruker NMR spectrometer AM 360 at 360 MHz on a Brucker AMX at 500 MHz. When necessary derivatization was with ethereal CH_2N_2. Enzymatic cleavage was with beta-glucuronidase and arylsulfatase. The petitioner has provided extensive chromatographic supporting data for the TLC determinations, as well as numerous copies of MS and NMR spectra and HPLC chromatograms to support his conclusions.

The petitioner generated interesting data for milk. The initial extraction with acetonitrile recovered between 85 to 92% of the total radioactive residue which indicated a lack of protein binding. Total ^{14}C-imidacloprid equivalents were identified in milk at 8 hours after dosing and just before sacrifice. The levels were 1.7 ppm (81.1%) out of 2.09 ppm at 8 hours, 2.03 ppm (77.2%) out of 2.62 ppm at 32 hours and 3.33 ppm (81%) out of 4.10 ppm just before sacrifice. For the last milk sample with the highest total residue the petitioner identified the parent imidacloprid at 2.27 ppm (55.3%), the nitro-simino imidacloprid or WAK 3839 at 0.014 ppm (0.3%), the glycine conjugate of 6 CNA or WAK 3583 at 0.13 ppm (3.1%), the olefin imida-cloprid or NTN 35884 at 0.23 ppm (5.6%), the 4-hydroxy imidacloprid was at 0.4 ppm (9.7%), the 5-hydroxy imidacloprid or WAK 4103 was at 0.29 ppm (7.0%) for a total of 81% identified. The same six compounds were identified in all of the milk samples, only the amounts were different. The relative percentages in all milk samples were the same with the parent imidacloprid being the highest residue detected followed by the two mono hydroxy metabolites and the nitro-simino metabolite at the lowest level. The residue levels detected in milk also confirm that imidacloprid is rapidly cleared from the system as the total ^{14}C-imidacloprid residues in milk just before dosing were 0.17 ppm at 24 hours and 0.24 ppm at 48 hours into the study. In these two milk samples the petitioner identified only 27% of the total radioactivity.

In the goat kidney the petitioner identified a total of 4.4 (37.7%) of the 11.6 ppm. The major residue identified was the glycine conjugate of 6-CNA at 1.53 ppm (13.2%) followed by the parent imidacloprid at 0.68 ppm (5.9%) and the glucuronide conjugate of 5-hydroxy imidacloprid at 0.66 ppm (5.7%). The 5-hydroxy imidacloprid was detected in the goat kidney at 0.65 ppm (5.6%). The olefin imidacloprid was found at 0.49 ppm (4.3%). Nitrosimino imidacloprid was detected at 0.01 ppm (0.1%). CBTS does not consider identifying only 38% of the residue in kidney to adequately delineate the nature of the residue.

Significantly higher percentage of the total imidacloprid residues were identified in muscle tissues: in round 3.1 ppm (78.2%) out of 4.0 ppm, in flank 3.1 (80.2%) out of 3.8 ppm, and in loin 3.3 ppm (86.8%) out of 3.8 ppm. The same five compounds and at nearly the same percentages were identified in all three muscles tissue samples. In loin muscle imidacloprid, per se, was detected at 2.65 ppm (69.8%), followed by the olefin imidacloprid at 0.23 ppm (6.1%) and the 4-hydroxy imidacloprid at 0.27 ppm (7.1%). 5-hydroxy imida-cloprid was detected at 0.12 ppm (3.2%) and the nitrosimino imida-

cloprid was found at 0.02 ppm (0.6%). The petitioner has identified an acceptable amount of the imidacloprid residue in caprine muscle tissues.

The petitioner has also identified a significant amount of the total imidacloprid residue in various fat tissues. In perirenal fat the petitioner identified 1.58 ppm (86.7%) out of 1.81 ppm; 1.89 ppm (85.8%) out of 2.1 ppm in omental fat, and 1.91 ppm (90.9%) out of 2.20 ppm in subcutaneous (SC) fat. The same five compounds and at nearly the same percentages were detected in all three fat tissue samples. In SC fat the parent imidacloprid was detected at 1.54 ppm (73.5%), the olefin imidacloprid was at 0.17 ppm (7.7%), and 4-hydroxy imidacloprid was at 0.12 ppm (5.8%). The 5-hydroxy imidacloprid was found at 0.07 ppm (3.1%) and the nitrosimino was detected at 0.01 ppm (0.6%).

In caprine liver the petitioner only identified 2.3 ppm (14.4%) out of 15.9 ppm. The major compound identified was WAK 4126/NTN 38014 or picolylic guanidine at 1.59 ppm (10.0%). Other compounds identified were the glycine conjugate of 6-CNA at 0.29 ppm (1.8%), 6-CNA at 0.25 ppm (1.5%), urea imidacloprid or NTN 33519 at 0.01 ppm (0.4%), imidacloprid, per se, at 0.13 ppm (0.8%), and DIJ 9646-2, a primary amine at 0.04 ppm (0.2%). CBTS does not consider identifying only 14% of the residue in caprine liver to adequately delineate the nature of the residue.

The petitioner recognized that an insufficient amount of the total residue in the first part of the caprine imidacloprid metabolism study had been identified and conducted another study "to gain additional information on the metabolism of Imidacloprid in milk and edible tissues."

The second part of the ^{14}C-imidacloprid caprine metabolism study was conducted under conditions as closely as possible to the first part. The petitioner use the same strain/species as before only this lactating goat weighed 41 kg at the start of the study. The goat breeder, age (18 months), identification procedures (hide marking and cage tag), acclimation (one week), laboratory conditions and housing (stainless steel metabolism cages), type of feed, amount of feed (500 grams plus one apple per day) and water (ad libitum) were all the same. The dose was with ^{14}C-imidacloprid with a specific activity of 112 uCi/mg and a radiopurity of 99.3% at a rate of 10 mg/kg for a level of 200 ppm in the goat's diet. The manner of dosing was the same (oral by intubation).

Sample collection for milk was the same (5 milk samples total). At sacrifice the same tissues (liver, both kidneys, loin, round and flank muscle, perirenal, omental, and subcutaneous fat) were collected and prepared for analysis in the same manner (passed through a meatmincer). Sample preparation and determination for the total ^{14}C-imidacloprid equivalents in the different tissues collected were the same.

The total imidacloprid residue in milk at 8 hours after dosing was 3.16 ppm, 8 hours after the second was 2.77 ppm, and just before sacrifice was 3.65 ppm. The residues in milk just before dosing were 0.19 pm and 0.2 ppm. No new characterization and identification data of metabolites in milk were presented. The total imidacloprid in the fat composite was 1.07 ppm. The individual total imidacloprid equivalents were 0.92 ppm in perirenal fat, 1.19 ppm subcutaneous fat, and 0.94 ppm in omental fat. In the muscle composite there was 3.65 ppm imidacloprid equivalents. The total imidacloprid equivalents in round were 3.33 ppm, in flank were 3.62 ppm, and in loin were 3.68 ppm. No new data for characterizing and

identifying imidacloprid metabolite in muscle or in fat were presented. The residue levels reported in the second part of the caprine metabolism study using the same dose are in agreement with the data reported above. In general, the data presented in the first part of the metabolism study for these tissues are confirmed here.

For kidney and liver the petitioner used a different extraction, clean-up, and isolation scheme to characterize and identify a greater portion of the residue. 52 grams of liver were blended with 300 mls water in a Ultra-Turrax tissue homogenizer for 10 minutes. The liver was reextracted 6 times with 0.2% NaCl, then 2 times with 0.01M NaOH + 1% NaCl. The water plus the 0.2% NaCl were combined, concentrated, mixed with 50 grams of NaCl, taken to dryness, then reextracted 5 times with CH_3CN/CH_3OH (2:1). The extracts were combined and concentrated, then the extract was ready for analysis. For the NaOH-NaCl extraction solutions they were handled with minor variations in the same manner as were the water-NaCl extracts above.

25 grams of kidney were extracted in 200mls of water using a Ultra-Turrax for 8 minutes. The kidney was reextracted 5 times with 0.2% NaCl, then 2 times with 0.01M NaOH + 1% NaCl, and finally 2 times with 0.1N NaOH + 2% NaCl. The water plus NaCl solutions were combined, concentrated, then mixed with 25 grams and taken to dryness. The residue was extracted 5 times with 120 mls of CH_3CN/CH_3OH, the extracts were combined, and concentrated. The extract is ready for analysis.

Determination of total $^{14}CO_2$ from all of the imidacloprid and its metabolites was done using the same combustion techniques, trapping in the same binding agent and scintillator, and counting on the same LSCs. Other determination instruments; ie, HPLC, were the same as described as above. Enzymatic cleavage was accomplished with the same enzymes (beta-glucuronidase and arylsulfatase).

The extraction of the liver with water plus NaCl solutions recovered more then 99% of the ^{14}C-imidacloprid residue equivalents in the liver, thus there is an absence of bound imidacloprid residues. The total ^{14}C-imidacloprid residues in liver were 17.1 ppm. In liver the petitioner identified the glycine conjugate of 6-CNA at 0.16 ppm (0.96%), picolylic amine or GSE 1478 ([6-chloro-3-pyridinyl-methyl] amine) at 0.74 ppm (0.43%), picolylic urea or DJI 10739 at 0.22 ppm (1.3%), imidacloprid urea at 0.34 ppm (2.0%), olefin imidacloprid at 0.54 ppm (3.2%), aminoguanidine imidacloprid or WAK 3877/4 (1-([6-chloro-3-pyridinyl]methyl)-N-amino-2-imino-imidazolidine) at 0.26 ppm (1.5%), nitroguanidine or WAK 4230 (-({6-chloro-3-pyridinyl]methyl)-N-nitro-guanidine) at 0.06 ppm (0.35%), dihydroxy guanidine imidacloprid or WAK 5031 (1([6-chloro-3-pyridinyl] methyl)-N-nitro-2-imino-imidazolidine-4,5-diol) at 0.1 ppm (0.6%), guanidine imidacloprid at 2.8 ppm (16.4%), ring open guanidine imidacloprid or WAK 4126 (N-(6-chloro-3-pyridinyl-methyl)guanidine) at 1.2 ppm (7.2%). The petitioner has identified 5.8 ppm out of 17.1 ppm (34%). When the liver was analyzed with the common moiety method that detects all of the metabolites that can be converted to 6-CNA then 11.8 ppm are recovered (68.7%). An additional 6 ppm of 6-CNA containing metabolites are recovered with the common moiety method that the petitioner has not identified. CBTS would consider this is adequate characterization. Additional identification are not justified as the tolerance expression is based on a common moiety method and whatever new metabolite that could be identified will be determined as 6-CNA. While CBTS would like to have 90+% of the radioactive residue characterized and identified our review of the supporting chromatographic

data do not indicate additional work will improve our understanding of the nature of the residue in ruminants.

In the caprine kidney the petitioner detected a total of 13.5 ppm of ^{14}C-imidacloprid equivalents. Residues identified in the kidney are the glycine conjugate of 6-CNA at 2.3 ppm (16.8%), 6-CNA at 0.04 ppm (0.32%), picolylic amine or GSE 1478 at 0.25 ppm (1.8%), picolylic urea or DJI 10739 at 0.03 ppm (0.2%), urea imidacloprid at 0.99 ppm (0.7%), olefin imidacloprid at 2.4 ppm (17.7%), and nitro-
guanidine imidacloprid or WAK 4230 at 0.11 ppm (0.1%). 4-hydroxy imidacloprid, 5-hydroxy imidacloprid, and dihydroxy imidacloprid were determined at a total of 0.26 ppm (2.0%). The glucuronide conjugates of 5-hydroxy imidacloprid and 4-hydroxy imidacloprid were detected at 1.9 ppm (14.1%). Dihydroxy guanidine imidacloprid or WAK 5031 was detected at 0.08 ppm (0.6%), while guanidine imidacloprid was determined at 0.8 ppm (5.9%), and ring open guanidine or WAK 4126 was at 0.6 ppm (4.2%). Imidacloprid, per se, was detected at 0.84 ppm (6.2%). The petitioner has identified a total of 9.7 ppm (71.6%) of the 13.5 ppm.

When the kidney was analyzed with the common moiety method that detects all of the metabolites that can be converted to 6-CNA then a total of 10.6 ppm are recovered (77.9%). An additional 0.9 ppm of 6-CNA containing metabolites are recovered with the common moiety method that the petitioner has not identified. CBTS would consider this is adequate characterization of the residue in kidney. Additional identification are not justified as the tolerance expression is based on a common moiety method and whatever new metabolite(s) that could be identified will be determined as 6-CNA. While CBTS would like to have 90+% of the radioactive residue characterized and identified our review of the supporting chromatographic data do not indicate additional work will improve our understanding of the nature of the residue in ruminants.

The nature of the imidacloprid residue in ruminants is adequately understood. Imidacloprid is metabolized by three pathways as follows:

1) hydroxylation of the dihydroimidazole ring of imidacloprid to form 4-hydroxy, 5-hydroxy, plus the glucuronide conjugates of each monohydroxy metabolite, and dihydroxy imidacloprid followed by the loss of water to form the olefin imidacloprid,

2) reduction and loss of the nitro group on the dihydroimidazole ring to form the aminoguanidine imidacloprid, then the guanidine imidacloprid, and finally the urea imidacloprid, and

3) opening of the dihydroimidazole ring with loss of the ethyl group and subsequent oxidation. The first step is forming the nitroguanidine imidacloprid, next the ring open guanidine which can also form from both the guanidine imidacloprid and the dihydroxy guanidine imidacloprid. This metabolite can form picolylic urea, and picolylic amine which is oxidized to 6-chloronicotinic acid (6-CNA) and then conjugates with glycine.

The residues of concern in ruminants are imidacloprid and its metabolites containing the 6-chloropyridinyl moiety.

All of the identified metabolites contain the 6-chloropyridinyl moiety and all metabolites, except 6-CNA and its glycine conjugate, contain both rings of imidacloprid.

CBTS agrees that the proposed residue analytical method will recover the metabolites of concern in that 70 to 90% of the residue is identified and that the proposed common moiety method recovers this total residue. The question on whether or not the petitioner needs to do a double labeled metabolism study with a ^{14}C- label in the dihydroimidazole ring can be resolved by a logical review of the existing data. In ruminants the petitioner proposes dihydroimidazole ring opening, not a ring cleavage at the methylene bridge. Only in one of the caprine kidney samples does 6-CNA show a significant residue.

Poultry (MRID #s 425561-16 and -17)

The petitioner presented the results of a poultry metabolism study in a document titled "[Methylene-^{14}C] Imidacloprid Absorption, Distribution, Excretion, and Metabolism in Laying Hens" by O. Klein an A. Brauner dated September 17, 1990, and coded laboratory project ID M 185 0250-1 and Miles report number 102607. The petitioner also presented an additional poultry metabolism data in a study titled "[Methylene-14C] Imidacloprid: Absorption, Distribution, Excretion, and Metabolism in Laying Hens, Addendum I" by O. Klein and A. Brauner dated September 16, 1991 and coded laboratory project ID M71819017 and Miles report number 102607-1.

The petitioner conducted the first part of the ^{14}C-imidacloprid poultry metabolism study using 6 to 8 months old White Leghorn pullets (Gallus domesticus). The pullets were obtained from a local poultry breeder in Senden, Germany. Metabolism testing was conducted at Bayer's Institute for Metabolism Research in Monheim, Germany in 1988.

The pullets were acclimatized for 1 week prior to the start of the metabolism testing. Identification was by individual cage cards and wing tags. Each pullet was housed individually in a stainless steel metabolism cage which allowed complete collection of excreta. Housing was in air conditioned rooms at 18-20 °C with 18 hours of light and 10-15 air changes per hour. The weights of the pullets did not change significantly during the study ranging at the start of the metabolism study from 1.35 to 1.68 kg and at the end of the metabolism study ranging from 1.11 to 1.65 kg.

The pullets received about 150 grams per day of a commercial feed, LKV supplied by Soest. Tap water was available ad libitum. Both the feed and the drinking water were checked for contaminants.

The pullets were dosed orally via a syringe once a day with ^{14}C-pyridinyl methyl imidacloprid initially at a rate of 50 mg/kg, but the pullets showed signs of toxicity so the dose was reduced to 10 mg per kg for 3 consecutive days. Assuming the pullets consume approximately 10% of their body weight in their daily feeding the dose corresponds to 100 ppm in the feed. The specific activity of the ^{14}C-imidacloprid was 154 uCi per mg and radiopurity was > 99%. The radiolabeled material was diluted with "cold" imidacloprid at a ratio of 1:10.

The petitioner collected eggs, blood, and excreta during the 3 day metabolism study. While analysis of blood and excreta are essential to understand the distribution and elimination of imida-
cloprid from the pullets they are not germane to our understanding of the nature of the residue in eggs and edible poultry tissues.

Eggs were collected from the control pullets for 10 days prior to the start of the metabolism testing to be used as the control egg sample. Eggs were collected from the treated pullets during the acclimation period and were collected twice daily during the metabolism test (before dosing and 8 hours after administration of each oral dose). At sacrifice eggs were collected from the oviduct. The number of eggs produced per hen was recorded and the weight of each egg was also recorded. The yolks and white were separated from the shells, then thoroughly blended.

The pullets were sacrificed 2 hours after the last dose in the metabolism study. Samples collected were both kidneys, liver, gizzard (without lining and contents), skin (without SC fat), breast muscle, thigh muscle, and subcutaneous (SC) fat. All tissues, except fat, were thoroughly homogenized with dry ice using a meat mincer.

To determine the total ^{14}C-imidacloprid equivalents in the various poultry samples, three sub-samples of each of the tissues were freeze dried, combusted in an oxygen atmosphere using a Packard model 306 Oxidizer. Fatty samples were solubilized using a tissue solubilizer Beckman BTS 450 before determining the total ^{14}C-imidacloprid. The $^{14}CO_2$ was trapped in Carbosorb, then Permaflour V scintillator was added and counting was done on either a Beckman LS 7800, a Philips PW4700, or a LKB Rack Beta 1219 Spectral. All LSCs used quench correction and care was taken to avoid chemilluninesence by adding several drops of glacial acetic acid.

Results for the total ^{14}C-imidacloprid equivalents in the edible poultry tissues are 11.5 ppm in kidneys, 8.2 ppm in liver, 6.5 ppm in gizzard, 2.4 ppm in breast muscle, 1.5 ppm in thigh muscle, 3.2 ppm in poultry heart, 1.3 ppm in skin, 0.5 ppm in SC fat, and 0.6 ppm in eggs.

The petitioner proceeded to extract, isolate, and identify the components of the radiolabeled residue in eggs and poultry tissues. For eggs 168 grams were extracted 4 X 200 mls of acetonitrile (ACN)/ methanol (1:1). The extracts were combined, concentrated, and lyophilized, then redissolved in methanol and mixed with 18 grams of RP-18. The sample was extracted off the RP-18 with H_2O, H_2O/CH_3CN (1:1), H_2O/CH_3CN (1:3), and ACN/buffer pH 2 (1:1). The three extracts that contained water were combined and further purified by HPLC before metabolite identification. The acidified ACN extract was also cleaned-up on HPLC.

All of the tissue samples; liver, kidney, heart, and muscles, were extracted and cleaned-up the same way differing only in sample size and extracting volumes. Samples were extracted with water under ultrasonication, lyophilized, and reextracted with CH_3CN/CH_3OH (1:1) and further clean-up via prep HPLC.

The HPLC determination was carried out using a Hewlett-Packard 1090 LC equipped with a variable wavelength detector or a diode array detector, HP 1040; and a Ramona 6 radioactivity detector with solid scintillator cells. Mass spectrometry was with a Finnigan 8230 double focusing instrument for EI spectra, and for CI spectra NH_3 was the reagent gas. ^{1}H-NMR spectra were generated on a Bruker AM 330 NMR spectrometer with deuteromethanol being the solvent. IR spectra were recorded on a Bruker IFS 85 spectrometer with the samples being in KBr pellets. The petitioner has provided extensive chromatographic supporting data with numerous copies of MS and NMR spectra, and HPLC chromatograms to support his conclusions.

The extraction recovered approximately 82% of the radioactive residue from eggs. The only identified component in eggs was the olefin imidacloprid or NTN 35884 at 0.23 ppm (41% of 0.55 ppm).

The extraction recovered 66.2% of the radioactivity from liver. Only 24% of the radioactivity was identified. Three peaks were detected with the major compound being the cyanopicolylic metabolite or WAK 4613/3 at 16% of the initial 8.2 ppm or 1.3 ppm. The other identified is probably the olefin imidacloprid at 7.9% or 0.64 ppm. The third peak in the liver extract at 4% or 0.33 ppm was not identified.

The extraction recovered 86% of the 11.5 ppm ^{14}C-imidacloprid or 9.9 ppm from the poultry kidney. Two compounds were identified as the olefin imidacloprid or NTN 35884 at 0.7 ppm and the guanidine imidacloprid or NTN 33823 at 0.4 ppm.

The water extraction recovered 73 to 75% of the ^{14}C-imidacloprid from muscle. In thigh muscle 29% of the 1.48 ppm or 0.43 ppm was the olefin imidacloprid metabolite and 5.4% or 0.08 ppm was imidacloprid. In breast muscle imidacloprid was the major metabolite detected at 45.6% of the 2.35 ppm ^{14}C-imidacloprid or 1.07 ppm.

In gizzard the water extraction recovered 78% or 5.1 ppm of the 6.5 ppm ^{14}C-imidacloprid residue. Imidacloprid was identified at 53% or 3.4 ppm of the residue.

In poultry skin the olefin imidacloprid was 27.9% or 0.35 ppm of the 1.25 ppm ^{14}C-imidacloprid while imidacloprid, per se, was 7.6% or 0.1 ppm of the residue.

Poultry subcutaneous fat contained imidacloprid at 41% or 0.19 ppm of the 0.46 ppm ^{14}C-imidacloprid residue. CBTS does not consider that the petitioner has identified an adequate qualitative amount of the ^{14}C-imidacloprid residue in the various poultry tissues. Only 37% of the residue overall was characterized and identified. This is insufficient for delineating the nature of the imidacloprid residue in poultry. The petitioner apparently recognized that an insufficient amount of the total residue in the first part of the poultry metabolism study had been characterized and identified. Another poultry imidacloprid metabolism study was conducted to gain additional information of the metabolism of imidacloprid in eggs and edible poultry tissues; ie, muscle, liver, and fat.

The second part of the poultry ^{14}C-imidacloprid metabolism study was conducted under conditions as closely as possible to the first part. The petitioner used the same number and species (5 White Leghorn pullets), that were the same age (6 to 8 months), identified in the same manner (individual cage cards and wing tags), purchased from the same poultry breeder, housed in the same laboratory (Bayer's Institute for Metabolism Research in Monheim, Germany) under the similar conditions and acclimated for the same time (one week). The pullets received the same amount of feed per day (150 grams), but a different type, LA 55, supplied by Hoeveler. Tap water was given ad libitum. Both the feed and water were checked for contaminants as before. The pullets were dosed in the same manner (orally for three consecutive days using a syringe filled with ^{14}C-[methylene] imidacloprid) at a rate of 10 mg per kg body weight. The specific activity of this dose was 111 uCi/mg with a radiopurity of > 99%. If the pullets consume 10% of their weight per day in feed then the dose is 100 ppm, or assuming the pullets consume 6.4% of their weight per day in feed, then the dose becomes 158 ppm.

Sample collection for the eggs was the same (during acclimatization with twice daily during the test just before dosing and 8 hours after dosing). The pullets were sacrificed 2 hours after the last dose when the plasma levels of ^{14}C-imidacloprid were at their maximum. Tissues collected and analyzed were the kidney, liver, skin, subcutaneous fat, composite of thigh and breast muscle, and gizzard.

Sample preparation for the determination of total ^{14}C-imidaclo-prid equivalents was the same (oxidation with a Packard model 306 Oxidizer). The same LSCs (Beckman LS 7800,

Philips PW 4700, and LKB Rack Beta 1219) were used and the instrument parameters discussed above were the same; eg, addition of glacial acetic acid to prevent chemiluminescence.

Results for the total ^{14}C-imidacloprid equivalents in the edible poultry tissues for the second part of the study are 18.9 ppm in kidneys, 12.8 ppm in liver, 2.4 ppm in gizzard, 2.1 ppm in breast muscle, 2.3 ppm in thigh muscle, 3.0 ppm in skin, 1.5 ppm in SC fat, and 0.44 ppm in eggs.

In the second part of the poultry metabolism study the petitioner used different extraction procedures for eggs, liver, muscle, and fat. The extraction procedures improved the overall recovery of total imidacloprid.

The combined egg sample was suspended in water/NaCl, then exhaustively extracted with ACN. Nearly 97% of the total imidaclo-prid equivalents were recovered in this extract. The extracts were combined, concentrated to dryness and redissolved in ACN. Clean-up of the oily residues was by partition with n-heptane. Determination of imidacloprid and its metabolites was by HPLC. The petitioner identified a total of 0.385 ppm out of 0.44 ppm (87.5%) ^{14}C-imidaclo-prid in eggs. Residues identified in eggs are the picolylic urea or DIJ 10739 at 0.009 pm (2.0%), the olefin imidacloprid or NTN 35884 at 0.14 ppm (31.8%), 4-hydroxy imidacloprid or WAK 5839 at 0.028 ppm (6.4%), 5-hydroxy imidacloprid or WAK 4103 at 0.049 ppm (11.1%), the dihydroxy imidacloprid 0r WAK 3772 at 0.002 ppm (0.4%), the nitro-
guanidine imidacloprid or WAK 4230 at 0.087 ppm (19.8%), the picolylic amine or GSE 1478 at 0.019 ppm (4.3%), the dihydroxy guanidine imidacloprid or WAK 5031 at 0.004 ppm (0.9%), imidacloprid at 0.023 ppm (5.2%). and the ring open guanidine or WAK 4126 at 0.019 ppm (4.3%), and a mixture of the hydroxy imidacloprids at 0.005 ppm (1.1%).

The poultry liver sample was extracted with 0.2% NaCl, a mixture of 1% NaCl in 0.01 N NaOH, and 2% NaCl in 0.1 N NaOH which recovered more the 99% of the total ^{14}C-imidacloprid. Further purification was accomplished by extracting 2 times with CH_3CN/CH_3OH (2:1) prior to determining the residue on HPLC. Residues identified in poultry liver are the 6-CNA at 0.31 ppm (2.4%), the picolylic urea at 0.97 ppm (7.6%), the nitroguanidine imidacloprid at 1.12 ppm (8.8%), the olefin imidacloprid at 1.91 ppm (15.0%), a combination of the 4-hydroxy, the 5-hydroxy, and the dihydroxy imidacloprid at 1.06 ppm (8.4%), the picolylic amine at 0.22 ppm (1.9%), the dihydroxy guanidine at 0.27 ppm (2.1%), the ring open guanidine imidacloprid at 1.99 ppm (15.6%), and an isomeric form of the dihydroxy imidacloprid at 0.18 ppm (1.4%). The petitioner identified 8.1 ppm out of 12.8 ppm (63.3%) of the residue in poultry liver.

The combined poultry muscle sample was extracted with water and 0.2% NaCl which recovered 92% of the total ^{14}C-imidacloprid residues. The extracts were combined, then the pH was adjusted to 8.5 before reextraction with ACN. After concentration the solution was exhaustively extracted with CH_3CN/CH_3OH (1:2) followed by extraction with CH_3OH. After further concentration and extraction the residue was dissolved in CH_3OH and water. The oily residue was partitioned with n-heptane before determination with HPLC. Residues identified in poultry muscle are the picolylic urea at 0.08 ppm (3.7%), the nitro-guanidine at 0.15 ppm (6.7%), the olefin imidacloprid at 0.59 ppm (26.7 %), the 4-hydroxy imidacloprid at 0.1 ppm (4.6%), the 5-hydroxy imidacloprid at 0.19 ppm (8.6%), picolylic amine at 0.08 ppm (3.6%), the dihydroxy guanidine at 0.03 ppm (1.4%), imidacloprid at 0.14 ppm (6.3%), the ring open guanidine at 0.14 ppm (6.2%), a mixture of hydroxy imidacloprids at 0.03 ppm (1.5%), another olefinic metabolite at 0.04 ppm (1.8%), a diketo metabolite at 0.02 ppm (0.7%), and a

mixture of two compounds one of which is a N-acetyl picolylic amine at 0.05 ppm (2.4%). The petitioner has identified 1.6 ppm out of 2.1 ppm (77.8%) of the residue in poultry muscle.

Poultry fat was dissolved in n-heptane which recovered about 73% of the total ^{14}C-imidacloprid residue. Clean-up was accomplished by partitioning 5 times between H_20 and the n-heptane. After centrifugation to separate the layers the insoluble components were extracted with CH_3CN before determining the residues by HPLC. Residues identified in poultry fat are the picolylic urea at 0.02 ppm (1.4%), 6-CNA at 0.03 ppm (1.9%), the olefin imidacloprid at 0.35 ppm (22.6%), the 4-hydroxy imidacloprid at 0.04 ppm (2.4%), the 5-hydroxy imidacloprid at 0.15 ppm (9.7%), the nitroguanidine at 0.08 ppm (5.2%), picolylic amine at 0.02 ppm (1.5%), imidacloprid at 0.19 ppm (12.4%), and the ring opened guanidine at 0.07 ppm (4.2%). The petitioner has identified 0.94 ppm out of 1.51 ppm. (62.5%).

While CBTS would like to have 90+% of the radiolabeled residue characterized and identified our review of the supporting chromatographic data do not indicate additional work by the petitioner will improve our understanding of the nature of the imidacloprid residue in poultry.

The nature of the imidacloprid residue in poultry is adequately understood. Imidacloprid is metabolized by three pathways as follows:

1) hydroxylation of the dihydroimidazole ring of imidacloprid to form 4-hydroxy, 5-hydroxy, and dihydroxy imidacloprid followed by the loss of water to form the olefin imidacloprid,

2) reduction and loss of the nitro group on the dihydroimidazole ring to form the dihydroxyguanidine imidacloprid, and

3) opening of the dihydroimidazole ring with loss of the ethyl group and subsequent oxidation. The first step is forming the nitroguanidine imidacloprid, next the ring open guanidine which can also form from both the guanidine imidacloprid and the dihydroxy guanidine imidacloprid. This metabolite can form picolylic urea, and picolylic amine which is oxidized to 6-chloronicotinic acid (6-CNA).

The residues of concern in poultry are imidacloprid and its metabolites containing the 6-chloropyridinyl moiety.

CONFINED ACCUMULATION STUDIES ON ROTATIONAL CROPS (MRID # 425561-04)

The petitioner presented the results of a ^{14}C-imidacloprid confined accumulation study in rotational crops in a document titled "[Pyridinyl ^{14}C-methyl] NTN 33893 Residues in Rotational Crops" by K. Vogeler, et. al., dated August 10, 1992, and coded laboratory project ID M 130 0279-2 and Miles report number 103812.

The petitioner determined the total ^{14}C-imidacloprid in rotational crops following a singular spray application of 0.4 lb/A to sandy loam soil. The petitioner's 0.4 lb/A soil application is a 1X application as imidacloprid is applied to soil at planting for potatoes and cotton at a rate 0.3 lb a.i/A followed by several foliar applications. The ^{14}C-imidacloprid was sprayed onto a soil container with a surface area of 1.03 m^3. The petitioner used imidacloprid

labeled in the methylene bridge with a specific activity of 92.4 uCi/mg and a radiopurity of 99+%. The confined rotational crop study was conducted at Bayer's Research Center at Monheim Germany starting in March 1989 and ending in July 1992. Environmental conditions in this study were that the greenhouse temperature was about 68°F (20°C) during the day and about 59°F at night. When natural light dropped below 35 klux artificial light was added to insure optimum growth. Watering was to the soil at a rate sufficient to have optimum plant growth.

The leafy vegetable used in this study is Swiss Chard (beta vulgaris spp. vulgaris variety vulgaris). The root crop used is red beets (Beta vulgaris spp. vulgaris variety conditiva). The cereal grain crop was wheat (Triticum aestivum). The soil was aged 30 days after the single ^{14}C-imidacloprid application before it was thoroughly mixed at planted with the 3 crops for the first rotation. Following the harvest of the first crop the soil was mixed again, then replanted with the same crops 120 days after the single imidacloprid application. The second rotation crops were grown to maturity and harvested. The soil was mixed a third time and replanted again with the same crops for the third crop rotation at 271 days after the single imidacloprid application. The wheat was sampled at an appropriate growth stage for wheat forage and at maturity wheat was separated into grain and straw. At harvest the red beets were separated into leaves and the red beet roots. All harvested samples were homogenized in liquid N_2 then stored at -18°C until analysis.

Soil sample were taken at the day of application (0 day), at each of the rotations (30, 120, and 271 days after application), and at the end of the study (412 days after imidacloprid application). Control crops were also grown under the same conditions at the same time as the ^{14}C- rotational crop study.

The residue analytical methods used to determine imidacloprid and its metabolites in the rotational crops are essentially the same as the methods used in other 14C-imidacloprid metabolism studies. The methods have been reviewed above.

In summary, the total radioactivity was determined by taking an aliquot with combustion in a Harvey OX 300 oxidizer. The $^{14}CO_2$ was trapped in a scintillation cocktail of carbosorb plus permafluor and the determination was on a Philips PW4700. Radioactivity on the TLC plates was determined by scrapping off the zones, then counting them in a scintillation cocktail, or using a linear analyzer. Peak resolution of the linear analyzer was checked by autoradiography.

To identify the individual components of the residue homogenized aliquots of the plants were extracted in CH_3OH/H_2O. Two different cleanup steps were used; the first was taking the aqueous remainder to the XAD-4 resin clean-up column, and the other using a partition against ethyl acetate with cleanup of the aqueous phase on XAD-4 . Determination was by TLC using Kisselgel 60 F_{254} precoated plates from Merck in one of 5 different solvent systems. Determination of radioactivity zones was by a linear analyzer, or exposure to X-ray film. Co-chromatography of pure reference standard was by visualization in 254 nm light. HPLC was with a Hewlett Packard HP 1090 equipped with a LiChrosorb RP 8 (7um), 25 cm X 4 mm (id). The solvent system was methanol/water, gradient at a flow rate of 1 ml/minute. The HPLC detectors were the diode array and radioactivity flow-through. The petitioner has presented an adequate amount of spectra, chromato-grams, and copies of TLC plates to support his conclusions on the nature of the imidacloprid residue in rotational crops.

To be certain all of the radioactive residues were extracted from the plants the petitioner conducted exhaustive extraction of the wheat grain and straw and beet leaves. Samples were extracted 6 hours each in boiling methanol, then in boiling 6N HCL/methanol, and finally in 2N NaOH/methanol. Released metabolites were extracted from these solution with ethyl acetate. The water phase of the beet leaves and the wheat straw were subjected to enzymatic hydrolysis using beta-glucosidase and cellulase.

Total imidacloprid residues in wheat grain were 0.07 ppm (120 days), 0.06 ppm (271 days), and 0.03 ppm (408 days). The same magnitude of imidacloprid residue was detected in beets roots at 0.07 ppm (120 days), 0.03 ppm (271 days), and 0.04 ppm (384 days). Higher residues were detected in beet leaves and Swiss chard. In beet leaves imidacloprid residues were 0.26 ppm, 0.2 ppm, and 0.17 ppm while in Swiss chard imidacloprid residues were 0.13 ppm (93 days), 0.24 ppm (201 days), and 0.09 ppm (345 days). Imidacloprid residues in wheat forage were 0.48 ppm, 1 ppm, and 0.26 ppm. The highest imidacloprid residues were detected in wheat straw at 2.5 ppm, 2.38 ppm, and 0.96 ppm.

The petitioner reported the identification of imidacloprid in samples from all 3 crop rotations in red beet roots and leaves, Swiss chard, and in wheat forage, grain, and straw. The same metabolites were identified in all 3 rotations differing only slight in the amount reported. At the first rotation in beet roots imidacloprid was at 0.0041 ppm. Metabolites identified were the 4-hydroxy/5-hydroxy at 0.0.0034 ppm (4.9%), the dihydroxy or 6-CPA at 0.004 ppm (0.5%), the olefin at 0.009 ppm (1.3%), the nitrosimino at 0.002 ppm (0.3%), the glucoside at 0.001 ppm (00.2%), 6-CNA at 0.01 ppm (13.5%), the ring open guanidine at 0.004 ppm (5.4%). In red beet leaves imidacloprid was 0.02 ppm (3.7%). Metabolites identified were the dihydroxy at 0.003 ppm (1.3%), the 4-hydroxy/5-hydroxy at 0.021 ppm (8%), the olefin at 0.01 ppm (4%), the glucoside at 0.02 ppm (6.1%), 6-CNA at 0.004 ppm (1.7%), the ring open guanidine a t0.008 ppm (3.1%), and the guanidine at 0.02 ppm (6.2%).

In Swiss chard samples from the first rotation imidacloprid was 0.031 ppm (23.5%). Other metabolites were the dihydroxy at 0.002 ppm (1.2%), the 4-hydroxy/5-hydroxy at 0.011 ppm (8.1%), the olefin at 0.006 ppm (4.6%), the nitrosimino at 0.008 ppm (0.6%), 6-CNA at 0.012 ppm (9.3%), the glucoside at 0.003 ppm (2%), the ring opened guanidine at 0.002 ppm (1.3%), and guanidine at 0.02 ppm (11.2%).

Wheat forage samples from the first rotation contained imidacloprid at 0.2 ppm (42.1%). Other metabolites were the dihydroxy at 0.004 ppm (0.9%), the 4-hydroxy/5-hydroxy are 0.17 ppm (14%), the olefin at 0.019 ppm (4%), the nitrosimino at 0.013 ppm (2.7%), 6-CNA at 0.004 ppm 0.9%), the glucoside at 0.011 ppm (2.2%), the ring opened guanidine at 0.007 ppm (1.5%), and the guanidine at 0.057 ppm (11.9%).

At the first rotation wheat grain contained imidacloprid at 0.001 ppm (1.9%). Other metabolites were the dihydroxy at 0.003 ppm (3.6%), the 4-hydroxy/5-hydroxy at 0.007 ppm (10.3%), the olefin at 0.004 ppm (5.3%), the glucoside at 0.001 ppm (1.8%), and the guanidine at 0.003 (3.7%).

Wheat straw samples contained the highest total imidacloprid residues harvested from each of the 3 rotations. Imidacloprid at the first rotation was 0.12 ppm (4.7%). Other metabolites were the 6-chloropicolyl alcohol at 0.005 ppm (0.2%), the dihydroxy at 0.07 ppm

(2.6%), the 4-hydroxy/5-hydroxy at 0.17 ppm (6.8%), the olefin at 0.16 ppm (6.3%), the nitrosimino at 0.03 ppm (1%), 6-CNA at 0.02 ppm (0.6%), the glucoside at 0.01 ppm (4.4%), the ring open guanidine at 0.13 ppm (5%), and the guanidine at 0.46 ppm (18.5%).

The total extractability of imidacloprid residues from rotational crops ranged from 45% in wheat grain to 91% in Swiss chard. We note that at least 9% of the radioactivity in wheat grain had been reincorporated into the glucose.

The nature of the identified residue in rotational crops is nearly identical to that identified in the primary crops. Imidaclo-prid is metabolized by the same three metabolic pathways as described for apples and potatoes. The petitioner identified around 45% of the residue in the different rotational plant matrices. When the same matrices were analyzed by the common moiety method for 6-CNA, then 91-96% of the residue was recovered. This characterizes the additional components of the residues as containing the 6-chlorpyridinyl moiety. The petitioner has adequately characterized and identified the nature of the imidacloprid residue in rotational crops.

All 3 rotational crops in the confined study had imidacloprid residues when planted 1, 4, and 9 months (or at the first, second, and third crop rotations) after imidacloprid soil application. The total imidacloprid residues were all greater then 0.01 ppm from a 1X application. CBTS concludes there is sufficient potential for inadvertent imidacloprid residues to occur in non-target crops planted in rotation. Limited field rotation crop studies are necessary for a representative crop at 2 sites per crop for the following 3 crop groups: root and tuber vegetables, leafy vegetables, and cereal grains. At least a total of 6 field trials are necessary all at the 1X application rate.

Based on the data presented from the confined imidacloprid accumulation studies CBTS anticipates that the petitioner will need to propose rotational imidacloprid tolerances. A final decision on the need for tolerances and more extensive field trials will be based on the results of the limited field trials.

RESIDUE ANALYTICAL METHODS (MRID #s 425561-18 through -28)

The imidacloprid residues analytical methods and their supporting validation data plus the pre-review by the Analytical Chemistry Laboratory for the proposed Tolerance Method Validations were the subject of a separate memorandum by F.D. Griffith, Jr., on June 18, 1993. All discussions on the various methods and deficiencies in the write-up and supporting validation data noted are incorporated herein by reference. Since the petitioner has not responded, these deficiencies remain outstanding and continue unresolved.

STORAGE STABILITY (MRID #s 425561-35 through -38)

The petitioner presented the results of several imidacloprid frozen storage stability studies. One study involved ^{14}C-imidacloprid in corn, lemon, and lemon in a study titled "Storage Stability Study of NTN 33898 (Imidacloprid) and its Five Metabolites in Corn, Lemon, and Lettuce" by N. Morishima dated August 12, 1992, and coded laboratory project ID 91040/ESP and Miles report number 103820 (MRID # 425561-36). Another storage

stability study was titled "Determination of Storage Stability of the 'Total Residue' of Imidacloprid and Major Metabolites in Fortified Samples During Frozen Storage" by Y. Ishii and F.J. Placke dated September 2, 1992, and coded P6420 4524/P 6420 4525 P6420 4526/P6420 4530 and Miles report number 103831 (MRID #425561-37). The petitioner presented the partial results from 3 months of a long term frozen storage stability study for imidacloprid and some of its metabolites in potatoes, apples, cottonseed and wheat and their processed commodities in a report titled "Imidacloprid and its Metabolites - Freezer Storage Stability Study in Crops" by P. Noland dated September 17, 1992, and coded ABC Laboratories Project ID Final Report # 40100 (MRID # 425561-35). The petitioner presented an overview and summary of frozen storage stability data including data from the plant metabolism studies in a document titled "Review of the Storage Stability Data of NTN 33983 and its Metabolites in Plant Matrices" T. Clark dated October 1, 1992, and coded Miles report number 103847 (MRID # 425561-38).

In the frozen storage stability study for corn, lemons, and lettuce the petitioner used ^{14}C-methylene labeled imidacloprid, the olefin, the guanidine, 5-hydroxy, the nitrosimino, and 6-CNA that were at least 97% radiopurity and had the specific activity ranging from 104 to 180 uCi/mg. These compounds were dissolved in methanol and stored in brown glass bottles prior to fortification. The petitioner spiked individual aliquots of lemons, lettuce, corn forage, corn fodder, and corn grain with a mixture of the 6 radio-labeled compounds at a concentration of 1 ppm each. The samples were stored frozen at -20°C until analysis in duplicate at 3 and 6 months; and at 9 months for lemons. The samples were analyzed in triplicate at the time of fortification for a 0-day residue value.

The sample extraction and clean-up steps followed in general the procedures discussed above. Determination of the residues was by TLC using 20 X 20 cm plate coated with 0.25 mm silica gel (Art.5715 from E. Merck). Application to TLC was with a Linimet IV. The developing solution was a mixture of dichloromethane/ACN/tetrahydrofuran/acetic acid (110:60:30:0.5). The radioactive compounds were located with X-ray film, then quantitation was by a TLC linear analyzer, RITA IV; and LSC counting. The petitioner has presented an adequate number of chromatographic profiles of the samples which show complete separation of the six compounds and quantitation of results.

In lettuce recoveries at 3 and 6 months ranged from 85% for the nitrosimino to 107% for the guanidine imidacloprid. In lemons recoveries at 3 and 6 months ranged from 65/67% for the nitrosimino to 123/128% for the guanidine imidacloprid. However, at 9 months the recoveries for all 6 compounds from lemons were all within the 90-110% range. For corn forage and corn fodder at 3 and 6 months all imidacloprid and its 5 metabolites recoveries ranged from 90 to 110%. In corn grain imidacloprid and its metabolites recoveries at 3 and 6 months ranged from 79% for the nitrosimino to 112% for the guanidine imidacloprid.

The petitioner has presented data to show that residues of imidacloprid and its major metabolites are stable in frozen storage for at least 6 to 9 months in lemons, lettuce, and corn. CBTS considers these storage stability data acceptable and supplementary for storage stability of total imidacloprid residues in apples, potatoes, and cottonseed.

The petitioner presented preliminary results from 3 months of a proposed 36+ months frozen storage stability study. The petitioner has spiked homogenized rac samples of whole apples and whole potatoes; and the homogenized processed commodities apple juice, wet

apple pomace, dry apple pomace, cottonseed hulls, cottonseed oil, cottonseed soapstock, wheat bran, wheat flour, wheat shorts, and wheat grain dust with imidacloprid and its guanidine, olefin, 4-hydroxy, and 6-CNA metabolites at a concentration of 0.5 ppm as a mixture. Individual sample were stored frozen in glass jars covered with aluminum foil at -20°C until analysis at 3 months. Other proposed analysis dates are to be at 6, 12, 18, 24, and 36 months or until the residue concentration drops to < 70% of the initial spiking level.

The sample extraction and clean-up steps in general followed the residue analytical methods reviewed above where the petitioner used 20 to 50 grams of sample extracted with 300 ml of methanol/1% H_2SO_4, clean-up on an Amberlite XAD-4 resin column, then oxidation with NaOH plus $KMnO_4$ under reflux to form the common moiety, extraction of the 6-CNA common moiety with methyl t-butyl ether, and derivatization with methyl-N-trimethylsilyl-trifluro acetamide (MSTFA) before determination by SIM capillary CG/MS at m/z 214. The petitioner presented extensive supporting chromatographic data including standard curves for all samples including the frozen control and spikes as well as the concurrent control and spiked samples. The petitioner validated the method at 0.1 ppm with each compound individually, then with a mixture of the compounds totaling 0.5 ppm. We feel that 0.1 ppm is the probable LOQ with a MDL around 0.001-0.005 ppm. Recoveries overall ranged from 71% to 117%. The petitioner has adequately validated the method to gather the frozen storage stability data for imidacloprid and its metabolites.

After 3 months 118% of the total imidacloprid in potatoes was recovered. Recoveries of total imidacloprid in apples and processed apple commodities ranged from 91% to 106% after 3 months. The range of recoveries was wider in processed wheat commodities ranging from 77% in wheat bran to 110% in wheat grain dust after 3 months. In cottonseed processed commodities total imidacloprid recoveries ranged from 86% to 116%. While the frozen storage stability data show no decline in total imidacloprid residues in potatoes, apples and apple processed commodities, cottonseed processed commodities, and in wheat processed commodities at 3 months, CBTS defers judgement on this data to support the magnitude of the crop field trial data until the petitioner has completed the study and submitted the final report. In the interim the petitioner is encouraged to submit an additional interim report that includes the storage stability data for 6, 12, and possibly 18 months.

In another frozen storage stability study, which CBTS considers to be supplementary information, the petitioner provided imidacloprid recovery data for imidacloprid and its guanidine, 5-hydroxy, and olefin metabolites spiked as a mixture into sugar beet roots and tops, barley grain forage and straw, sunflower seeds, and green and dried hop cones at 1 ppm, and frozen at -20°C for 24 months with sampling at 1, 2, 3, 6-8, 12, and 24 month intervals. The residue data were gathered with plant residue method, Bayer method 0200, reviewed above. At each sampling interval analyses were conducted with the control samples and concurrent recovery samples along with the stored spiked samples,

Recovery of total imidacloprid over the 24 months storage from sugar beet roots ranged from 76 to 91% averaging 83.35% with concurrent recoveries of 84.7%, and from sugar beet leaves imidacloprid recoveries ranged from 67% to 88 %, averaging 82% with concurrent recoveries of 83%. Recovery of total imidacloprid residues over the 24 months of frozen storage in barley grain ranged from 64% to 99%, averaging 89% with concurrent recoveries of 91%, from barley forage ranged from 79% to 96%, averaging 88% with concurrent recovery of 91%, and from barley straw ranging from 69% to 91%, averaging 78%

with concurrent recovery of 79%. From frozen sunflower seeds imida-cloprid recoveries over the 24 month interval ranged from 71% to 88% averaging 81% with 89% concurrent recoveries. With one year only of frozen storage stability data available for hop samples the total imidacloprid recoveries are consistent with the other data presented.

In summary, the results show that total imidacloprid residues in a 1 ppm spiking mixture of imidacloprid plus the guanidine, monohydroxy, and olefin metabolites spiked into sugar beet tops and roots, barley forage, straw, and grain, and sunflower seeds are stable for at least 24 months of frozen storage. These storage stability data are supplementary as there are no tolerance proposals for these commodities in this petition.

As part of the plant metabolism studies the petitioner also provide some additional storage stability data. Potato tubers were analyzed at 2 intervals; ie, at 169 and 379 days. In the organic phase there was no change in the imidacloprid, the 5-hydroxy, and olefin residues; and in the aqueous phase there was no change in guanidine and 6-CNA residues. There were more potato leaves remaining in the ^{14}C-imidacloprid potato metabolism study, thus more sample intervals were tested. Residues were determined at 5, 203, 342, 502, and 747 days. There was no significant change in residues of imidacloprid, 5-hydroxy, olefin, guanidine, and 6-CNA in potato leaves for the 342 days. The increase in residues at 502 and 747 days is attributed to the leaves drying out during frozen storage.

The immature corn, corn fodder, and corn grain from the ^{14}C-imidacloprid metabolism study were stored and reanalyzed at different time intervals. Immature corn was analyzed at day 43 and 541 for imidacloprid and its guanidine metabolite and showed no change in residue results. Corn fodder was analyzed at day 42, 426, and 719 for imidacloprid and its guanidine metabolite and there was no change in residue results over this time period. The same results are reported for corn grain.

Whole apples were stored frozen and analyzed at 6 different intervals (1, 192, 274, 332, 590, and 729 days) for ^{14}C-imidacloprid and its olefin metabolite. There were 2 runs per sample analysis which were in agreement and showed there was no loss of ^{14}C-imida-cloprid and its olefin metabolite during frozen storage.

Analysis for the 6-CNA metabolite from ^{14}C-imidacloprid in cottonseed at 21 and 279 days showed that while there is no loss of residue due to frozen storage there was a conversion of 6-CNA to its methyl ester.

Analysis of samples from the ^{14}C-imidacloprid plant metabolism studies for corn, cotton, apples, and potatoes showed there is no loss of imidacloprid and its major metabolites (olefin, guanidine, 5-hydroxy, and 6-CNA) during a period of 2 years frozen storage.

MAGNITUDE OF THE RESIDUE - CROP FIELD TRIALS

Cottonseed (MRID # 425561-29 and 427678-02)

The petitioner presented imidacloprid magnitude of the residue on cottonseed in a study titled "Imidacloprid (2.5GR and 240FS) - Magnitude of the Residue on Cotton" by R.N. Burger and C.A. Lenz dated October 1, 1991, and coded Miles report number 103824. In an

amendment received on June 23, 1993, the petitioner submitted additional imidacloprid on cotton crop field trial residue data in a study titled "Imidacloprid (2.5GR & 240FS) - Magnitude of the Residue on Cotton (Addendum I)" by Burger and Lenz dated January 21, 1993 and coded Miles report number 103824-1.

The petitioner presented imidacloprid magnitude of the residue data from nine field trials in six states; ie, Mississippi (2), California (2), Georgia (2), Texas, Arkansas, and South Carolina for the 1991 crop year. The petitioner presented additional crop field trial residue data for the 1992 crop year from California (2), Georgia (2), Mississippi (2), and Texas. A total of 16 crop field trial were conducted. Field trials from these states represent cotton harvested from 8,613,500 acres out of a national cotton harvest on 11,708,000 acres (73.6%) [see Ag. Stat. 1991].

The field trials received 4 applications of imidacloprid as 240 FS (which is equivalent to 2 lbs per gallon) and or 2.5 GR (2.5% granular). Cottonseed was treated with 240 FS at a rate of 250 grams a.i./100 kg seed or 0.0025 lb a.i./lb seed. The petitioner has not proposed a cottonseed treatment prior to planting. At planting the cottonseed received a narrow banded in-furrow treatment at a rate of 0.33 oz (0.02 lb) a.i per 1000 foot row, or 4.3 to 4.8 ozs (0.28 lb) a.i. per acre, depending on row spacing. The in-furrow application for the field trials is the same as proposed in the directions for use. Cotton received 2 foliar applications at a rate of 3.8 ozs (0.24 lb) a.i. per acre in either 2 gallons aerial application or 8-10 gallons per acre ground application for a total foliar application of 0.48 lb a.i. per acre per season. The foliar applications were 6-7 days apart and the PHIs were 7-8, 13-15, and 21-22 days. The in-furrow application matches the proposed use while the foliar applications are at a 5X exaggerated application rate with only 2 applications vs. a proposed maximum of 6 applications at a rate of 0.05 lb ai per application. The cotton field trials received a maximum application of 0.76 lb (1.51X) a.i. per acre per season vs. a proposed label maximum use of 0.5 lb a.i. per acre per growing season. All 7 of the 1992 cotton field trials were conducted with the plots being divided in half with 1/2 receiving imidacloprid mixed with the spray adjuvant Silwet L-77 at a concentration of 1 pt/100 gallons of imidacloprid spray solution and the other half receiving an imidacloprid spray with no adjuvant.

Cotton samples were harvested from at least 12 separate areas of each plot, ginned, and the undelinted cottonseed frozen at -20°C until sample preparation. Samples were analyzed by the analytical method for plant residues reviewed above and currently undergoing a Tolerance Method Validation in EPA labs. Concurrent recoveries were with a mixture of imidacloprid and its guanidine metabolite at levels of 0.1 to 3 ppm in cottonseed and 0.1 ppm to 50 ppm for the green cotton forage. Recoveries in 1991 ranged from 75 to 109% (n = 5) from cottonseed and from 76% to 93% (n = 6) in cotton forage. The 1992 concurrent recoveries ranged from 93% to 119% (n = 9) from cottonseed and ranged from 71% to 119% (n = 13) from cotton forage. The petitioner has adequately validated the method to gather the magnitude of the imidacloprid residue data reported in this study. All of the 1991 cotton samples, except one were harvested in October and analyzed between April 20 and August 12, 1992 (6-8 months of frozen storage). The 1992 cotton field trial were harvested between August 12 and November 2 and analyzed by January 4, 1993 (2-4 1/2 months of frozen storage).

Total 1991 imidacloprid residues in cottonseed at 7 days PHI ranged from 0.11 ppm to 0.87 ppm. At 14 days PHI total imidacloprid residues ranged from 0.15 ppm to 1.32 ppm,

averaging 0.45 ppm (n = 9) while at 21 days PHI the total imidacloprid residues in cottonseed ranged 0.17 ppm to 2.51 ppm, averaging 0.71 ppm (n = 9). The average total imidacloprid residue for the 14 and 22 day PHI cottonseed was 0.58 ppm (n = 18). In the 1991 cotton field trials, all treated without a spray adjuvant, we note that maximum residues were approximately 2X higher at 7 days PHI, 3X higher at 14 days PHI, and 6X higher in field trials that received a granular in-furrow at planting application as opposed to those trials that received an in-furrow spray application. The amount of imidacloprid applied was the same whether it was a granular application or a spray application.

The 1992 cottonseed total imidacloprid residue data at 7 days PHI ranged from 0.12 ppm to 1.28 ppm (n = 7) for cotton treated with imidacloprid but without the spray adjuvant. At 14 days PHI total imidacloprid residues on cottonseed ranged from 0.12 ppm to 1.49 ppm averaging 0.59 ppm $\pm$ 0.46 ppm, while at 21 days PHI total imidaclo-prid residues on cottonseed without a spray adjuvant application ranged from 0.19 ppm to 2.0 ppm averaging 0.6 ppm $\pm$ 0.66 ppm (n = 7). The average residues for both 14 and 21 days PHI cottonseed samples were 0.59 ppm $\pm$ 0.54 ppm (n =14). Similar differences in residues that result from a granular application and a spray application were confirmed in the 1992 trials only this time there was 2X higher total imidacloprid residues in cottonseed from trials that received a granular in-furrow at planting application than from the trials that received only the spray applications. The total imidacloprid residues on/in cottonseed at 7 days PHI for cotton treated with imidacloprid plus the spray adjuvant ranged from 0.19 ppm to 3.33 ppm (n = 7). At 14 days PHI total imidacloprid residues ranged from 0.24 ppm to 4.27 ppm averaging 1.02 ppm $\pm$ 1.45 ppm, while at 21 days PHI total imidacloprid residues on cottonseed with the spray adjuvant ranged from 0.23 ppm to 5.12 ppm averaging 1.1 ppm $\pm$ 1.8 ppm (n = 7). The average residues for 14 and 21 day PHI cottonseed samples were 1.06 ppm $\pm$ 1.56 ppm (n = 14). There is a 5X difference between residues from field trials that received a granular in-furrow at planting application from the trials that received only the spray at planting application with the samples from the granular application having higher residues. The was little if any difference in residues from the plots that received the spray adjuvant and from those plots that did not receive spray adjuvant. There was a 2.5X difference in residues between plots, both receiving the spray adjuvant, which received the granular application and the others receiving the spray application.

Total 1991 imidacloprid residues in cotton green forage at 7 days PHI ranged from 2.52 ppm to 25.53 ppm. At 14 days PHI total imidacloprid residues ranged from 1.24 ppm to 10.25 ppm, averaging 4.83 ppm (n = 9) while at 21 days PHI total imidacloprid residues in green cotton forage ranged from 0.92 ppm to 13.03 ppm, averaging 3.65 ppm (n = 9). The average total imidacloprid residue for the 14 and 21 day PHI green cotton forage was 4.55 ppm (n = 18). The differences noted above in total imidacloprid residue data between a granular application and a spray application were also apparent in the residue data in cotton forage, except they were less pronounced ranging from 1.5X at 7 days PHI, to 2X at 14 days PHI, and 2.5X at 21 days PHI. Higher total imidacloprid residues were detected in cotton forage from the granular imidacloprid in-furrow at planting application than was detected from the in-furrow spray application.

Total 1992 imidacloprid residues in cotton green forage at 7 days PHI ranged from 5.79 ppm to 38.23 ppm for trials that did not receive the spray adjuvant and ranged from 5.63 ppm to 54.29 ppm for samples from trials that did receive the spray adjuvant. At 14 days PHI total imidacloprid residues on samples that did not receive the spray adjuvant ranged from

2.71 ppm to 15.91 ppm averaging 7.1 ppm ± 5.9 ppm and on samples from plots that did receive the spray adjuvant residues ranged from 2.64 ppm to 24.84 ppm averaging 9.1 ppm ± 9.3 ppm. For cotton forage samples that received no spray adjuvant at 21 days PHI residues ranged from 0.71 ppm to 10.9 ppm averaging 4.74 ppm ± 3.8 ppm and on samples that did receive the spray adjuvant residues ranged from 1.37 ppm to 11.37 ppm averaging 5.12 ppm ± 3.8 ppm. The differences noted in the cottonseed samples having higher residues in samples from the granular application than from the spray application is not confirmed in the 1992 cotton forage samples that did not receive the spray adjuvant. The maximum residues were approximately 1.5X higher from the spray and foliar applications in cotton forage.

The petitioner's crop field trial data for imidacloprid on cotton green forage is helpful. While cotton forage is currently listed in Table 2 as a rac and cattle feed item CBTS does not feel this is a significant or viable feed item at this time. Information we have received indicates cotton forage is not a feed item; and if it is, then cotton forage has only a very limited, if any use. CBTS will not require any additional crop field trial residue data for imidacloprid on cotton forage. We suggest the petitioner submit a revised label prohibiting the possible use of cotton forage as a feed item, and also propose a revised section F deleting the proposed total imidacloprid tolerance on cotton forage.

The petitioner's cotton crop field trial residue data do not support the proposed 6 ppm tolerance on cottonseed. None of the imidacloprid on cotton crop field trial residue data have been generated at the proposed use of an in-furrow at planting application (granular or spray) and up to 6 foliar applications at a rate of 0.05 lb a.i. per application with a spray adjuvant for a maximum of 0.5 lb a.i. imidacloprid per acre per season. The petitioner has the options of either generating all new cotton field trial residue data at the proposed use, or proposing a new set of directions which accurately reflect the use pattern for generating the magnitude of the residue data, namely adding a use for treating cotton seed, and having only 2 foliar applications at a rate of 0.24 lb a.i., 7 day repeat application interval and with a 14 day PHI plus the use of the spray adjuvant Silwet L-77. For this use the petitioner needs to present additional crop field residue data from the Texas/Oklahoma/ New Mexico region to improve geographical representation.

If the petitioner wishes to keep the current set of directions for use on the proposed label, then he needs to present at least 12 new geographically representative cotton field trials showing the residues on/in cottonseed at the maximum 0.5 lb a.i. proposed use; eg, an in-furrow application (preferably with the granular formulation as higher residues are reported from the use of the granular formulation) followed by up to 6 foliar applications at 0.05 lb per application all with the spray adjuvant Silwet L-77, 7 day repeat application interval, 14 day PHI with no imidacloprid seed treatments.

Apples (MRID # 425561-33 and 427678-01)

The petitioner presented imidacloprid magnitude of the residue on apples in studies titled "Imidacloprid (240FS) - Magnitude of the Residue on Apples" by R.N. Burger and C.A. Lenz dated September 28, 1992, and coded Miles report number 103234; and in another study titled "Imidacloprid (240FS) - Magnitude of the Residue on Apple" by Burger and Lenz dated December 10, 1992, and coded Miles report 103234-1.

The petitioner presented imidacloprid magnitude of the residue data on apples from 12

field trials in seven states; ie, Washington (3), California (3), Pennsylvania, New York, Michigan, Indiana (2), and Virginia on 8 varieties for the 1991 and 1992 crop years. Field trials from these states represent apple production of 8,037 million pounds out of a national apple production of 9,078.4 million pounds (88.5%) [see Ag. Stat. 1991].

The apple field trials received 5 foliar ground applications of imidacloprid as 240 FS (which is equivalent to 2 lbs per gallon) at a rate of 0.67 oz (0.04 lb) a.i. per 100 gallon per application as a dilute spray. On a per acre basis or as a concentrated spray apply 1 to 2.8 ozs (0.063 to 0.17 lb) a.i. in 25 to 50 gallons ground application. Total imidacloprid foliar application is 0.845 lb a.i. (a 1.625X exaggerated application from the proposed maximum use rate of 0.5 lb) per acre per season. The foliar applications were a minimum of 10 days apart and the PHIs were 6-7, 13-14, 20-21, and 29-30 days. Generally, the applications were made at the pink bud, pedal fall stages, and as three separate cover sprays later in the season. One field trial in Washington received a 0.5X application at a rate of 0.33 oz a.i. imidacloprid in 50 gallons per acre. The use of imida-cloprid in the apple field trials approximates the proposed directions for use.

At least 16 apples were proportionally harvested from each tree and frozen at -20°C until sample preparation. Apple samples were analyzed by an earlier version of the analytical method for plant residues reviewed above and currently undergoing a Tolerance Method Validation in EPA labs. The differences between the method used to gather the residue data and the method undergoing the TMV are as follows: 1) the method used as an extracting solvent methanol:water (3:1) without acid, 2) no XAD-4 resin clean-up column, and 3) a larger amount of permanganate for oxidation. Concurrent recoveries were with a mixture of an equal amount of imidacloprid and its guanidine metabolite at levels of 0.1 to 0.5 ppm in apples. The 1991 recoveries in apples ranged from 74 to 119%, averaging 97% $\pm$ 18% (n = 10). The 1992 concurrent recovery data were generated with an equal mixture of imidacloprid and its guanidine metabolite at levels of 0.1 to 0.8 ppm. The 1992 recoveries ranged from 92% to 113%. The petitioner has adequately validated the method to gather the magnitude of the imidacloprid residue data on apples reported in these studies. All of the 1991 apple samples were harvested in September-October and analyzed between May 12 and June 9, 1992 (8-9 months of frozen storage). The 1992 apple sample were harvested in September-October and analyzed between November 3 and 19, 1992 (2-3 months of frozen storage from harvest to analysis).

No total imidacloprid residues were detected in any of the control apples to a level of < 0.05 ppm. Total imidacloprid residues in apples at 7 days PHI ranged from < 0.05 ppm to 0.74 ppm, averaging 0.158 ppm $\pm$ 0.2 ppm, n = 11. At 14 days PHI total imidacloprid residues in apples ranged from < 0.05 ppm to 0.46 ppm, averaging 0.112 $\pm$ 0.12 ppm (n = 11). The combined 7 and 14 day PHI samples residues averaged 0.135 ppm $\pm$ 0.16 ppm (n = 22). While at 20-21 days PHI the total imidacloprid residues in apples ranged from < 0.05 ppm to 0.23 ppm, averaging 0.072 ppm $\pm$ 0.06 ppm (n = 11). Total imidacloprid residues at 29-30 days PHI ranged from <0.05 ppm to 0.24 ppm, averaging 0.059 ppm $\pm$ 0.043 ppm. The average total imidacloprid residue for the 20-21 and 29-30 day PHI residue data in apples was 0.062 ppm (n = 16). The one field trial that was treated at 0.5X application rate had all results at all PHIs at < 0.05 ppm. These data are acceptable since there are other residue data at the 1X application rate which are also at < 0.05 ppm.

The petitioner has presented an adequate amount of geographically representative

and varietal crop field trial data to show that total imidacloprid residues are not expected to exceed the proposed 1.0 ppm tolerance on apples when Confidor® is used as directed.

However, since the Agency sets tolerances no higher then necessary, the petitioner needs to submit a revised Section F proposing a lower total imidacloprid tolerance for apples at 0.5 ppm. The maximum residue was 0.74 ppm, only on 1 sample from a 1.62X exaggerated use, thus when this is extrapolated to a 1X use the expected residue is under 0.5 ppm. This is supported by a majority of the field trial residue data as well as the average residues being at the 0.1-0.2 ppm level.

Potatoes (MRID # 425561-31)

The petitioner presented imidacloprid magnitude of the residue on/in potatoes in a study titled "Imidacloprid (2.5 GR and 240FS) - Magnitude of the Residue on Potato" by R.N. Burger and C.A. Lenz dated September 22, 1992, and coded Miles report number 103235.

The petitioner claims to have presented imidacloprid magnitude of the residue data on potatoes from 19 field trials in 12 states; ie, Washington, California (2), Wisconsin, Colorado, Minnesota, North Dakota, Michigan (2), Indiana (2), Maine (2), Idaho (2), Florida (2), and Kansas (2) on 9 varieties for the 1991 crop year. Review of the field trial locations and plots sites reveals that the double field trial in Kansas, Florida, Indiana, Michigan, and California are actually 2 plots at the same site/test facility which are not separated a large distance apart. Thus, we conclude the petitioner has presented multiple sampling sites (and samples) at these test plots, not separate field trials. CBTS concludes that the petitioner has presented 14 field trial from 12 states. We consider that separate field trials were conducted in Maine and Idaho as the trials were conducted at different locations. Field trials from these states represent potato production of 338,058,000 cwt out of a national potato production of 393,867,000 cwt (85.8%) [see Ag. Stat. 1991].

The potato field trials received an in-furrow narrow banded at planting application of either the 2.5 GR or 240 FS formulation at a rate of 0.03 gram a.i. per meter row. Depending on the width of the potato row spacing this is equivalent to 4.2 to 5.7 oz or 0.263 lb to 0.356 lb a.i. per acre vs. the proposed use of 0.18 to 0.3 lb a.i. for the in-furrow at-planting application use. The in-furrow application was followed by 4 foliar ground applications at a rate of 0.74 a.i. (0.046 lb) in 5, 20 or 50 gallons per application as a dilute spray. Total imidacloprid foliar application is 0.2 a.i. (1X) application; the same as the proposed maximum foliar use rate per season for potatoes. The sum of the foliar applications plus the in-furrow application for the 14 field trials is 0.5 lb a.i. per season which is a 1.67X exaggerated application rate. The foliar applications were 6 to 9 days apart and the PHIs were 7-8, 14, 21-22, and 28-29 days. The use of imidacloprid in the potato field trials reasonably approximates the proposed directions for use.

At least 16 whole potato tubers were harvested from each vine and frozen at -20°C until sample preparation. Potato samples were analyzed by the same method as was used for apple samples and is an earlier version of the analytical method for plant residues reviewed above and currently undergoing a Tolerance Method Validation in EPA labs. The differences between the method used to gather the residue data and the method undergoing the TMV are as follows: 1) the method used as an extracting solvent methanol:water (3:1) without acid, 2) no XAD-4 resin clean-up column, and 3) a larger amount of permanga-nate

for oxidation. Concurrent recoveries were with a mixture of an equal amount of imidacloprid and its guanidine metabolite at levels of 0.1 to 0.5 ppm in potatoes. Recoveries in potatoes ranged from 77 to 116%, averaging 101.4% ± 9.97% (n = 25). The petitioner has adequately validated the method to gather the magnitude of the imidacloprid residue data on potatoes reported in this study. All potato samples were harvested in July-October 1991, prepared in January-February 1992, and analyzed at ABC Laboratories between July 23 and September 23, 1992 (6 to 11 months of frozen storage between harvest and analysis).

11 of the 14 field trials showed total imidacloprid residues ≤ 0.05 ppm. Total imidacloprid residues in potatoes at 7-8 days PHI ranged from < 0.05 ppm to 0.16 ppm, with 3 samples having residues above 0.1 ppm, and averaging 0.046 ppm ± 0.042 ppm, n = 19. At 14 days PHI total imidacloprid residues in potatoes ranged from < 0.05 ppm to 0.28 ppm, with 3 samples having residues above 0.1 ppm, and averaging 0.054 ± 0.065 ppm,. The combined 7 and 14 day PHI samples residues averaged 0.05 ppm ± 0.054 ppm. While at 21-22 and 29-30 days PHI the total imidacloprid residues in potatoes ranged from < 0.05 ppm to 0.15 ppm, averaging 0.044 ppm (n = 38). The average total imidacloprid residue for the 7-8, 14, and 21-22 day PHI residue data in potatoes was 0.045 ppm ± 0.041 ppm (n = 57). We could not detect any significant differences in residues on plots treated only with the 240 spray material from the plots that were treated with the granular material plus spray material.

The petitioner has presented an adequate amount of geographically representative and varietal crop field trial data to show that total imidacloprid residues are not expected to exceed the proposed 0.4 ppm tolerance when Confidor® is used as directed.

However, since the Agency sets tolerances no higher then necessary, the petitioner needs to submit a revised Section F proposing a lower total imidacloprid tolerance for potatoes at 0.2 ppm. The maximum residues was 0.28 ppm from a 1.67X exaggerated use, thus when this is extrapolated to a 1X use the expected residue is under 0.2 ppm. This is supported by a majority of the field trial residue data as well as the average residues being at the 0.05 ppm level.

MAGNITUDE OF THE RESIDUE - PROCESSED FOOD/FEED

Cottonseed (MRID # 425561-30 and 427678-03)

The petitioner presented imidacloprid magnitude of the residue data on processed cotton commodities in a study titled "Imidacloprid (2.5GR and 240FS) - Magnitude of the Residue on Processed Cotton Commodities" By Burger and Lenz dated September 29, 1992 and coded Miles report number 103246.

One additional cotton field trial in Mississippi from the 1991 crop year was treated with 3 applications of imidacloprid at an exaggerated rate to ensure there were sufficient residues for a cottonseed processing study. The cotton seed was treated one time in-furrow at planting at a rate of 22.9 ozs a.i. per acre or 1.43 lb (5.1X) and with 2 foliar application at a rate of 18.8 oz a.i per acre per application or 1.18 lbs. (23.5X) for a total imidacloprid

application of 3.79 lbs a.i. (7.58X). The foliar applications were 7 days apart and the cotton forage and seeds were harvested from 12 separate areas 14 days later on October 18, 1991, for the cottonseed processing study.

The control and treated cottonseed samples were frozen and shipped to Texas A and M University's Engineering Biosciences Research Center for processing. The control sample was processed first followed by the treated sample. Sample processing was in February 1992. The processing simulated commercial cottonseed processing and was a material balance study. Starting with 47 lbs of cottonseed the seed were ginned (i.e.; saw delinted) to separate out the lint and produce 38.4 lbs of mostly delinted cottonseed with the balance of the residue as linters and linter motes. The delinted seed was mechanically cracked and screened to dehull the seed and produce 8.7 lbs of cottonseed hulls and 29.4 lbs of kernels which was heated, flaked, expanded into collects, and then solvent extracted to yield 18.4 lbs of desolventized cottonseed meal and 1961 grams of crude cottonseed oil. With the additional of NaOH this produced 501 grams of cottonseed oil (soapstock) and 2538 grams of refined cottonseed oil. All processed commodities were frozen at -20°C and returned of Miles, then shipped frozen to ABC Laboratories for analysis in April 1992. Samples were analyzed during July 1992 or 9 months after harvest and 5 months after processing.

The analytical method used to generate the residue data on cottonseed, cottonseed meal and hulls is reviewed above. Minor modifications were made to the extraction step for cottonseed crude and refined oil, and soapstock prior to the XAD-4 resin column clean-up. The permanganate oxidation, methyl t-butyl ether extraction, derivatization step, and the GC-MS SIM determination were basically unchanged. Recovery data were generated for imidacloprid and several of its metabolites for fortifications at 0.05 ppm, 0.25 ppm, and 3 ppm with recoveries ranging from 96% to 113%. Cottonseed hulls, meal, crude and refined oil were fortified with a mixture of imida-
cloprid and its guanidine metabolite each at 0.5 ppm or 8 ppm. Recoveries from cottonseed meal ranged from 77 to 79%, from soapstock ranged from 75 to 81%, from crude oil ranged from 71 to 107%. and from refined oil ranged from 84 to 106%. The methods were adequately validated to gather the magnitude of the residue data for the imida-cloprid cottonseed processing study.

No total imidacloprid residues were detected in the control cottonseed to 0.05 ppm and no total imidacloprid residues were detected in cottonseed hulls, meal, soapstock, crude and refined cottonseed oil to 0.5 ppm. The raw agricultural commodity (rac) cottonseeds contained 2.88 ppm total imidacloprid. When cottonseed containing these residues were processed into hulls, meal, crude oil, refined oil, and soapstock the total imidacloprid residues were 1.07 ppm (0.37X conc. factor) in hulls, <0.5 ppm (<0.17X) in crude oil, refined oil, and soapstock, and 4.21 (1.46X conc. factor) in cottonseed meal. Total imidacloprid residues were shown to concentration only in cottonseed meal, thus a FAT is required.

The petitioner has conducted a cotton processing study using cottonseed bearing detectable total imidacloprid residues following an exaggerated 7.58X total imidacloprid application. While an imidacloprid FAT is required for cottonseed meal, judgement is deferred as there are insufficient crop field trial data available from the proposed imidacloprid use to determined the proper imida-
cloprid tolerance on cottonseed, and thus the FAT for cottonseed meal.

Apples (MRID # 425561-34)

The petitioner presented imidacloprid magnitude of the residue data on processed apple commodities in a study titled "Imidacloprid (2.5GR and 240FS) - Magnitude of the Residue on Processed Cotton Commodities" By Burger and Lenz dated September 29, 1992 and coded Miles report number 103246.

One additional apple field trial in Indiana from the 1991 crop year was treated with 5 foliar applications of imidacloprid at an exaggerated rate to ensure there were sufficient residues for an apple processing study. The apple trees were treated five times by ground application at a rate of 3.35 ozs a.i. (0.21 lb [2X]) per 100 gallons or at a spray rate of 150 gallon per acre this is equivalent to 5.02 oz (0.31 lb [3X]) ai per acre for a total imidacloprid application of 1.57 lbs a.i. (3.14X) per acre per season. The foliar applications were at least 10 days apart with the first application being on April 17 and the last of five applications on August 20. 200 lbs of apples were harvested from 4 quarters of each tree in the plot 14 days after the last imidacloprid application on September 3, 1991, for the apple processing study.

The control and treated apples samples were frozen and shipped to Miles Research Park for processing. The control sample was processed first followed by the treated sample. Sample processing was from September 4 to 16, 1991. The processing simulated commercial apple processing and was a material balance study. Starting with 87,947 grams of thawed quartered apples the apples were placed in a Goodnature Products apple press and produced 67,192 grams of apple juice and 26,332 grams of wet apple pomace. 8,226 grams of wet pomace was placed in a tared porcelain dish and dried in a forced air oven at 77°C for 6 + hours or until dry. All processed commodities were frozen at -20°C, then shipped frozen to ABC Laboratories for analysis in April 1992. Samples were analyzed during May-June 1992 or 8-9 months after harvest and processing.

The analytical method used to generate the residue data on apples, apple juice, and wet and dry apple pomace is reviewed above. Minor modifications were made to the method which were the same as those described above for the method for generating data for the magnitude of the residue on apple crop field trials. Recovery data were generated for imidacloprid and several of its metabolites at 0.05 ppm, 0.1 ppm, 0.25 ppm, 0.5 ppm, and 1 ppm fortifications with recoveries ranging from 74% to 116%. Whole apples, apple juice, wet and dry apple pomace were fortified individually with imidacloprid, its olefin, guanidine, 5-hydroxy, and 6-CNA metabolites, or a mixture of imidacloprid and its guanidine metabolite. Concurrent recoveries were from a mixture of imidacloprid and the guanidine metabolite at 0.1 ppm fortification ranging from 82% to 119%. Recoveries from whole apples ranged from 88 to 103%, from apple juice ranged from 74 to 116%, from wet apple pomace ranged from 75 to 96% and from dry apple pomace ranged from 74 to 115%. The method was adequately validated to gather the magnitude of the residue data for the imida-cloprid apple processing study.

No total imidacloprid residues were detected in the control apples and apple processed commodities to 0.05 ppm. The raw agricultural commodity (rac) apples contained 0.13 ppm total imidacloprid. When apples containing these residues were processed into juice, wet and dry pomace the total imidacloprid residues were 0.1 ppm (0.77X conc. factor) in apple juice, 0.21 ppm (1.6X conc. factor) in wet apple pomace, and 0.82 ppm (6.3X conc. factor) in dry apple pomace. Total imidacloprid residues were shown to concentrate only in apple pomace (wet and dry), thus a FAT is required.

The petitioner has conducted an apple processing study using apples bearing

detectable total imidacloprid residues following an exaggerated 3.14X total imidacloprid application. While an imida-
cloprid FAT is required for wet and dried apple pomace, CBTS prefers the petitioner propose one total imidacloprid tolerance for apple pomace (wet and dried) using a 6X concentration factor to avoid a proliferation of tolerances. The petitioner needs to propose in a revised Section F a total imidacloprid tolerance for apple pomace (wet and dried) at 3 ppm.

Potatoes (MRID # 425561-32)

The petitioner presented imidacloprid magnitude of the residue data on processed potato commodities in a study titled "Imidacloprid (2.5GR and 240FS) - Magnitude of the Residue on Processed Potato Commodities" By Burger and Lenz dated September 24, 1992 and coded Miles report number 103238.

One additional potato field trial in California from the 1991 crop year was treated with 5 applications of imidacloprid at an exaggerated rate to ensure there were sufficient residues for an apple processing study. The potatoes were treated first as an in-furrow at planting (April 19, 1991) application at a rate of 21 ozs a.i. (1.313 lbs. [4.38X]) per acre using the 2.5GR formulation, then with 4 foliar ground applications using the 240FS formulation at a spray rate of 3.8 ozs a.i per acre (0.238 lb a.i. per acre [4.75X]) at a 7 day repeat application interval. This is equivalent to 2.27 lbs. [7.56X]) ai per acre total imidacloprid application for the potato rowing season. The last of 4 foliar applications was on July 29, 1991 with potato harvest 14 days later for the potato processing study. A total of 125-165 lbs of potatoes were harvested. Samples were collected from 4 quarters of each vine relative to the tuber load.

The control and treated potatoes samples were immediately delivered to the National Food Laboratory for processing. The potato samples were stored in a 70°C incubator prior to processing. The control sample was processed first followed by the treated sample. Sample processing was from August 16 to October 1, 1991. The processing simulated commercial potato processing and was a material balance study. Starting with 161 lbs. of control potatoes and 125 lbs of treated potatoes the potatoes were washed in 250 lbs. of water in a stainless steel tank and with hand brushes. The weight of the washed potatoes was recorded and a subsample was taken for analysis. The potatoes were peeled with a rotary hand peeler (Apple Mate 3) or with a hand peeler. A subsample of wet potato peels were taken for analysis, and the remaining wet potato peels were placed in drying trays for dehydrating in a Harvest Maid Dehydrator, model FD 5000, to produce dry potato peels. A 10 lbs. subsample of peeled and rinsed potatoes were sliced in a Hobart Vegetable Slicer then blanched in 40 lbs of water in a steam jacketed kettle and then fried in 15 lbs of oil in a Wells Auto Fry (deep fat fryer) to produce potato chips. Another 10 lbs. subsample was diced and cooked in 20 lbs of water, then mashed with a Hobart Mixer to produce mashed potatoes. These potatoes were frozen, then later processed into a dried mash potatoes (standard laboratory pasta dryer). The dried potatoes were ground in Buhler-Miag Grinder and granules from the treated potatoes were passed through a 30 mesh screen. All processed potato commodities were frozen at -10°C, then returned to Miles frozen on November 5, 1991. The frozen processed potato commodities were shipped frozen to ABC Laboratories for analysis in March 1992. Samples were analyzed during May-June 1992 or 10 months after harvest and processing.

The analytical method used to generate the residue data on potatoes, washed potatoes, wet and dry potato peels, potato chips and potato granules is reviewed above.

Minor modifications were made to the method which were the same as those described above for the method for generating data for the magnitude of the residue on apple pomace and apple juice. Recovery data were generated for imidacloprid and its guanidine, hydroxy, and olefin metabolites at 0.05 ppm, 0.1 ppm, 0.25 ppm, 0.5 ppm, and 1 ppm fortifications with recoveries ranging from 75% to 123%. Whole potatoes, potato chips, wet and dry potato peels, and potato granules were fortified individually with imidacloprid, its olefin, guanidine, 5-hydroxy, and 6-CNA metabolites, or a mixture of imidacloprid and its guanidine metabolite. Concurrent recoveries were from a mixture of imidacloprid and the guanidine metabolite at 0.1 ppm fortification ranging from 86% to 105%. Recoveries from whole potatoes ranged from 75 to 123%, from potato granules ranged from 83 to 96%, from dry potato peels ranged from 75 to 114%. The method was adequately validated to gather the magnitude of the residue data for the imidacloprid potato processing study.

No total imidacloprid residues were detected in the control potato and the potato processed commodities to <0.05 ppm. The raw agricultural commodity (rac) potatoes contained 0.26 ppm total imidacloprid. When these potatoes containing these residues were processed into washed potato tubers, potato granules, wet and dry potato peels, and potato chips the total imidacloprid residues were 0.24 ppm (0.92X conc. factor) in potato granules, 0.21 ppm (0.81X conc. factor) in washed potatoes, 0.17 ppm (0.65X conc. factor) in wet potato peels, 0.76 ppm (2.9X conc. factor) in dry potato peels, and 0.35 ppm (1.3X conc. factor) in potato chips. Total imidacloprid residues were shown to concentrate in potato chips and in dry potato peels, thus a FAT is required.

The petitioner has conducted a potato processing study using potatoes bearing detectable total imidacloprid residues following an exaggerated 7.56X total imidacloprid application. Residues concentrated at 1.3X in potato chips and 2.9X in dry potato peels. While an imidacloprid FAT is required for dry potato peels and potato chips, the Agency sets tolerances no higher then necessary. We prefer the petitioner propose one total imidacloprid tolerance for processed potato waste, not dry potato peels, to avoid a proliferation of tolerances and use a 3X concentration factor for potato wastes. The petitioner needs to propose in a revised Section F a total imidacloprid tolerance for processed potato waste at 0.5 ppm and for potato chips at 0.25 ppm.

MAGNITUDE OF THE RESIDUE - MEAT, MILK, POULTRY, EGGS

Ruminant (MRID # 425561-39)

The petitioner presented the results of a ruminant imidacloprid feeding study in a document titled "NTN 33893 - Cattle Feeding Study" by U. Heukamp dated September 10, 1992, and coded laboratory project ID P 67315000 and Miles report number 103833.

The petitioner conducted a bovine feeding study using 12 lactating dairy cows of mixed breed (German black and white X HF). The cows were in mid lactation, but not breed. CBTS notes that the age and the source of the cows were not provided. The cows were acclimated to "normal dairy housing practices" for 13 to 16 days depending on the test sequence. Since the feeding study was conducted at Bayer's Research Center in Monheim, Germany the petitioner needs to more completely define normal dairy housing practices so we can compare these to dairy housing practices in the USA. The cows were in individual

stalls with tethered housing and were divided randomly into 4 test groups. The cows receive a daily veterinary inspection with a more intensive weekly inspection.

Each cow in the study was identified by two different numbers. One number was on a metal ear tag given by the supplier. The other number was on a plastic ear tag provided on the day the cows arrived at the test facility and were randomized. The cows were adequately identified.

The cows were expected to weigh between 550 and 650 kg. For the cows assigned to the control group body weights ranged from 583 kg to 610 kg at the start of the dosing and all 3 cows showed a loss of weight in the control group ranging from a loss of 15 to 28 kgs. In the 5 kg/mg dose cows at the start of the dosing weighed from 555 kg to 632 kg and at the end of the dosing one cow gained 3 kg while the other 2 cows lost 36 and 7 kgs. For the 15 mg/kg dose the cows at the start of dosing ranged in weight from 527 kg to 575 kg and at sacrifice 2 cows had lost 11 and 22 kg while 1 cow actually gained 7 kg. At the 50 mg/kg dose the cows ranged in weight from 515 kg to 556 kg at the start of the test and at sacrifice the cows had all lost weight ranging from 14 to 32 kg. We do not consider the weight loss significant as the cows in the control group weight loss was in the same amount loss range as was the weight loss for the cows in the different test groups.

Water and a salt lick were provide ad libitum. The feed consisted of 8 kg of hay and 20 kg of corn silage plus 8 kg of "high energy dairy concentrate." This amount of feed corresponds to approximately 18-20 kg of dry matter. The type of hay needs to be defined as well as what is a high energy dairy concentrate. The petitioner needs to provide a sample label for the concentrate (in English), and the label should list the amount of protein, fat, fiber, and major ingredients that are in the feed. The petitioner carefully measured out the amount of feed given each cow, then recorded the amount of feed not consumed each day for each cow and provided an average feed consumption over the course of the feeding study. We were unable to locate any data showing the feeds were free for other potentially interfering heavy metals, aflatoxins, and other pesticides. The petitioner need to provide these data.

The cows were dosed with technical imidacloprid, 97.6% a.i. The doses were 5 mg/kg, 15 mg/kg, and 50 mg/kg of feed. The doses were prepared in gelatine capsules daily just before dosing with a balling gun. Saturday and Sunday doses being prepared on Friday. Dosing was for 28 consecutive days. The control cows received empty gelatine capsules. Duplicate capsules were analyzed on different days to confirm the doses administered.

The choice of dose levels was based not only on residue data from the feed commodities in this petition, but also on residue data from grape, cereal grains, corn and rice livestock feed items. The ruminant feeds items associated with this petition are cull potatoes (20% dry matter) at up to 40% of sheep diets, up to 75% of beef cattle diets, up to 50 % of dairy cattle diets and hog diets. Processed potato wastes (12% dry matter); ie, wet and dry potato peels are also a ruminant feed item at up to 25% of sheep diets, up to 75% of beef cattle diets, up to 50 % of dairy cattle diets, and up to 25% of breeding hog diets. The potential dietary burden from feeding cull potatoes or potato waste is up to 0.4 ppm in sheep, up to 0.75 ppm in beef cattle, up to 0.5 ppm in dairy cattle, and up to 0.1 ppm in hog diets. Another ruminant feed item is apple pomace (21% dry matter in wet pomace and 90% dry matter in dried apple pomace) and the potential dietary exposure from wet apple pomace for sheep is up to 30% of the diet or 4.3 ppm, in beef cattle at up to 40% of the diet or 5.7 ppm, in dairy cattle at up to 20% of the diet (2.9 ppm), and in breeding swine at up to 25% of the diet

(0.75 ppm).

From cotton, feed items are cottonseed meal (89% dry matter) at up to 10% of sheep and beef cattle diets, up to 25% in dairy cattle and hog diets; cottonseeds (88% dry matter) at up 25% of the sheep and cattle diets; cottonseed hulls (90% dry matter) at up to 20% for sheep and beef cattle diets, and 15% of dairy cattle diets; cotton oil or soapstock (99% dry matter) at 5% of sheep, cattle and hog diets; and cotton gin by-products or gin trash (90% dry matter) at up to 20% of sheep and beef cattle diets. The potential dietary burden from cottonseed and its processed commodities can not be ascertained as there are insufficient crop field trial residue data available to determine the magnitude of the residue.

The petitioner's worst case diet, while highly improbable, but which he claims none-the-less maximizes potential imidacloprid exposure, includes for grape pomace at 40% (2.8 ppm), raisin waste at 10% (0.7 ppm), potatoes at 30% (0.75 ppm), and cottonseed at 20% (0.14 ppm). We agree with the petitioner that 100% of the ruminant diet can be treated with imidacloprid. While the petitioner's worst case dietary burden at 4.4 ppm is slightly lower then we would expect from the total imidacloprid residues on bovine feed items (calculated on a % dry mater basis) in this petition we agree that 5 ppm or mg/kg feed is a reasonable 1X dose. The petitioner calculated his bovine diet on a dry matter basis. The petitioner used 20% dry matter for apples, grapes, citrus, and potatoes and their associated processed feed items in his calculations for a probable diet that is exposed to imidacloprid. While CBTS does not agree with using 20% dry matter across the board for all of the possible feed items in the petition-er's bovine diet, we feel changes in moisture based on our data for % dry matter in these bovine and ovine feed items will not change our 5 ppm estimate for a reasonable 1X feeding dose from the petitioner calculated 4.4 ppm proposed 1X dose.

Milk samples were collected twice daily using a bucket milking machine (ie, Westfalia Separator). Milk samples from different cows were not combined, but the morning and evening samples were combined then frozen immediately at -18 to -20°C. The milk production from the control group cows ranged from 23.8 kg to 26.8 kg, in the 5 mg/kg dose milk production was from 18.5 kg to 27.5 kg, in the 15 mg/kg dose milk production ranged from 18.8 to 23.8 kg, and at the 50 mg/kg dose milk production ranged from 21.6 to 27.2 kg. The amount of milk produced does not appear to be affected by the imidacloprid dose. Sacrifice was 14-18 hours after the last dose. At sacrifice there were no morphological or pathological findings noted. The whole liver without the gall bladder, both kidneys, sufficient samples of round, flank, and loin muscle; and subcutaneous (SC), renal, and mesenterial fat were collected, then cut in 2 cm cubes and immediately frozen at -20 to -24°C.

The residue analytical method used to generate the imidacloprid ruminant feeding study residue data was Bayer method 00191. This method has been previously reviewed and is currently undergoing a TMV in EPA labs. In summary, 10 grams of milk or tissues samples were extracted with methanol/water, then concentrated to an aqueous remainder, while fat samples were partitioned against hexane. Samples were cleaned-up on a XAD-4 resin column, residues were eluted off the column in methanol, oxidized with permanganate to 6-CNA, neutralized, then the 6-CNA was extracted out with t-butylmethyl ether, derivatized with trimethylsilyl ester, and determined by capillary GC-MS selective ion monitoring at 214 m/z. The limit of quantitation (LOQ) is 0.02 ppm for both tissues and milk.

The petitioner has provided additional concurrent method validation data. Milk samples were spiked individually with imidacloprid and its hydroxy and olefin metabolites at 0.02 ppm and 0.1 ppm plus at mixtures of all 3 compounds at 0.033 ppm each. Total

imidacloprid recoveries from milk ranged from 71.4% to 87.5% (N = 6 to 10). Fat samples were spiked individually with imidacloprid, its olefin and hydroxy metabolites at 0.02 and 0.3 ppm plus a mixture of all 3 at 0.1 ppm each. Total imidacloprid recoveries from fat ranged from 93.7% to 111.3% (n = 5 to 7). Muscle samples were spiked individually with imidacloprid and its hydroxy and olefin metabolites at 0.02 ppm and 0.6 ppm plus a mixture of all 3 at 0.2 ppm each. Total imidacloprid recoveries from muscle ranged from 72.8% to 102.2% (n = 3). Bovine kidney samples were spiked with imidacloprid at 0.02 ppm, 0.5 ppm and 2 ppm, with the olefin, hydroxy, 6-CNA metabolites at 0.02 ppm and 0.5 ppm plus a mixture of all of these compounds individually at 0.25 ppm. Total imidacloprid recoveries from kidney ranged from 70% to 86.3% (n = 3). Bovine liver was spiked with imidacloprid at 0.02, 0.05, 0.25, 0.5 and 2.5 ppm. Imidacloprid recoveries ranged from 70.5 to 79.9% The liver samples were also spiked individually with the olefin, 6-CNA, hydroxy, and guanidine at 0.02 and 0.5 ppm plus a mixture of imidacloprid and the metabolites at 0.2 ppm each. Recovery for the imidacloprid metabolites ranged from 72.1% to 89.2% with recovery from the mixture at 80.8%. Recoveries for the guanidine metabolite were 76.3% and 83.4%. Adequate supporting chromatographic data were presented. The petitioner has adequately validated the method to gather the magnitude of the residue data for the ruminant imidacloprid feeding study. The petitioner did not correct for recovery in this study.

In the control milk no imidacloprid equivalents were detected to <0.02 ppm. At the 5 mg/kg dose total imidacloprid residues ranged from <0.02 ppm to 0.023 ppm with 2 samples above 0.02 ppm (X = 0.012 ppm $\pm$ 0.004 ppm, n = 18). At the 15 mg/kg dose total imidacloprid residues ranged from 0.02 ppm to 0.055 ppm with 5 samples above 0.05 ppm (X = 0.034 ppm $\pm$ 0.010 ppm, n = 30). From the high dose of 50 mg/kg total imidacloprid residues ranged from 0.088 ppm to 0.177 ppm with 18 samples above 0.15 ppm (X = 0.132 $\pm$ 0.025, n = 64).

In fat sample no imidacloprid residues were detected in the control fat, and in fat from the 5 and 15 mg/kg doses. Imidacloprid was detected only from the 50 mg/kg dose ranging from 0.05 ppm to 0.079 ppm averaging 0.064 ppm $\pm$ 0.013 ppm, n = 6.

Total imidacloprid residues were not detected in the control muscle or in muscle from the 5 mg/kg dose. In muscle from the 15 mg/kg dose total imidacloprid residues ranged from <0.02 ppm to 0.033 ppm averaging 0.024 ppm $\pm$ 0.011 ppm, n = 6. At the 50 mg/kg dose total imidacloprid residue in muscles ranged from 0.097 ppm to 0.192 ppm averaging 0.121 $\pm$ 0.035 ppm, n = 6.

The control kidney samples did not have any imidacloprid equivalent to <0.02 ppm. From the 5 mg/kg dose total imidacloprid residues ranged from 0.023 ppm to 0.032 ppm, averaging 0.028 $\pm$ 0.004 ppm, n = 6. At the 15 mg/kg dose residues ranged from 0.053 ppm to 0.106 ppm averaging 0.085 $\pm$ 0.024 ppm, n = 6. From the high dose of 50 mg/kg total imidacloprid residues ranged from 0.201 ppm to 0.384 ppm averaging 0.286 ppm $\pm$ 0.067 ppm, n = 6.

In the control liver no imidacloprid residues were detected to <0.02 ppm. At the 5 mg/kg dose total imidacloprid residues in liver ranged from 0.041 ppm to 0.054 ppm averaging 0.05 ppm $\pm$ 0.007 ppm, n = 6, and from the 15 mg/kg dose total imidacloprid residues ranged from 0.084 pm to 0.168 ppm averaging 0.0.132 ppm $\pm$ 0.033 ppm, n = 6. From the high dose at 50 mg/kg dose total imidacloprid residues ranged from 0.384 ppm to 0.566 ppm averaging 0.486 ppm $\pm$ 0.077 ppm, n = 6.

Based on the results of the imidacloprid bovine feeding study CBTS concludes that finite residues will actually occur in milk and meat from feeding of imidacloprid treated racs or their processed feed commodities when Confidor® is used as directed. Since this situation falls under 40 CFR 180.6(a)(1) secondary imidacloprid tolerances are required in meat and milk. However, judgement is deferred on the study supporting the proposed 0.05 ppm tolerance in milk and 0.2 ppm in meat, fat, and meat by-products until the petitioner has supplied the following information to allow CBTS to complete its review of the bovine feeding study and additional crop field trial data for cottonseed.

CBTS reiterates that since the feeding study was conducted at Bayer's Research Center in Monheim, Germany the petitioner needs to more completely define normal dairy housing practices so we can compare these to dairy housing practices in the USA. The type of hay needs to be defined as well as what is a high energy dairy concentrate. The petitioner needs to provide a sample label for the concentrate (in English), and the label should list the amount of protein, fat, fiber, and major ingredients that are in the feed. And finally we were unable to locate any data showing the feeds were free for other potentially interfering heavy metals, aflatoxins, and other pesticides. The petitioner needs to provide these data.

Poultry (MRID # 425561-40)

The petitioner presented the results of a poultry imidacloprid feeding study in a document titled "NTN 33893 - Poultry Feeding Study" by U. Heukamp dated September 10, 1992 and coded laboratory project ID P 67315001 and Miles report number 103832.

The petitioner conducted a poultry feeding study using 50 laying pullets, SLS purchased from a local poultry producer in Senden. The petitioner needs to further identify the poultry breed used in the feeding study so that we may ascertain whether this is a commercially accepted breed. The pullets were 24 weeks old, in good health and egg production. The poultry feeding study was conducted at Bayer' Research Center in Monheim, Germany between October 7 and November 26, 1991. Environmental conditions at the center for the poultry feeding study were the temperature at 20-23°C, humidity at 30-60%, lighting for 12 hours at 15 lux, and air flow at 900 m^3 per hour. The pullets were acclimated to the facility for 16 days prior to the start of the study. The pullets were in individual cages containing a wide mesh floor to prevent fecal contamination. Each cage had an individual feeding bowl. The pullets were divided randomly into 4 test groups of 12 each just before the start of the feeding study. The pullets receive a daily veterinary inspection after a more intensive inspection on arrival. The pullets were housed in acceptable conditions for a poultry feeding study which approximated normal poultry housing practices.

Each pullet in the study was identified by a number between 1 and 50. The number was on a metal ring around each leg as well as on the cage and the feeding bowl. The pullets were adequately identified.

The pullets assigned to the 4 test groups weighed between 1.11 kg and 1.68 kg at the start of the study, and ranged between 1.20 to 1.75 kg at sacrifice. For the pullets assigned to the 2 mg/kg dose at the start of the dosing weighed from 1.11 to 1.56 kg and at the end of the dosing 5 pullets gained while 4 other pullets lost between 0.03 kg to 0.16 kg. For the 6 mg/kg dose the pullets at the start of dosing ranged in weight from 1.19 kg to 1.54 kg and at

sacrifice 1 pullet had lost 0.1 kg while 10 pullets actually gained from 0.004 kg to 0.16 kg. At the 20 mg/kg dose the pullets ranged in weight from 1.20 kg to 1.68 kg at the start of the test and at sacrifice the 8 pullets had gained weight ranging from 0.03 to 0.25 kg. We do not consider the weight changes significant as a few of the pullets in the different groups showed weight loss while most of the pullets in the same test groups showed the weight gains.

Water and feed were provided ad libitum throughout the study. The feed was a commercially available feed, LA 55 from Hoveler Feed. The petitioner needs to provide a sample label for the poultry feed (in English), and the label should list the amount of protein, fat, fiber, and major ingredients that are in the feed. The petitioner carefully measured out 200 grams of feed each morning for each pullet. The amount of feed not consumed from the previous day was recorded before the fresh feed was provided. The amount of feed consumed by each pullet was displayed in graphic form. An average feed consumption over the course of the feeding study was provided. We were unable to locate any data showing the feeds were free from other potentially interfering heavy metals, aflatoxins, and other pesticides. The petitioner needs to provide these data.

The pullets were dosed with technical imidacloprid, 97.6% a.i. The doses were 2 mg/kg, 6 mg/kg, and 20 mg/kg imidacloprid in the feed. The doses were prepared by dissolving the tech. imidacloprid in peanut oil, then thoroughly mixing this into the feed (twofold premixing). The control feed was prepared by blending with blank peanut oil. The fortified feed was prepared in 25 kg batches on a weekly basis. Each batch was feed for only 7 days after fortification. Any unused feed was discarded and replaced with a fresh batch of fortified material. The petitioner conducted tests on the different batches to show homogeneity of the test feeds. For the 2 mg/kg feed the expected imidacloprid ranged from 102% to 113% and for the 20 mg/kg feed the expected imidacloprid ranged from 106% to 116%. The imidacloprid is adequately mixed into the poultry feed. The petitioner also conducted storage stability tests on the fortified feeds. For the 2 mg/kg feed that was 109% of expected dose at day 0 the dose was 96% at day 8 and 15 while for the 20 mg/kg dose that at day 0 was 112% of the expected dose the values dropped to 104% at day 8 and to 92% at day 15. CBTS agrees that the fortified feed be used only for 7 days after preparation, then discarded.

The choice of dose levels was based not only on residue data from the feed commodities in this petition, but also on residue data from grape, cereal grains, corn and rice livestock feed items. The poultry feeds items associated with this petition are cull potatoes at up to 20% of laying hens diets and up to 7% of turkey and broiler diets. Potato wastes; ie, wet and dry potato peels are also a laying hen feed item at up to 10% of the diets. The potential dietary burden from feeding cull potatoes and potato waste is up to 0.09 ppm in laying hens and up to 0.014 ppm in turkey and broiler diets. Another poultry feed item is apple pomace and its potential dietary exposure for turkeys and broiler is up to 5% of the diet or 0.15 ppm. Apple pomace is not a laying hen feed commodity. From cotton, feed items are cottonseed meal at up to 10% of turkey and broiler diets, and 3% of laying hen diets; cotton oil (soapstock) at 5% of laying hens and turkey and broiler diets. The potential poultry dietary burden from cottonseed and its processed commodity cotton oil (soap-stock) can not be ascertained as there are insufficient crop field trial residue data available to determine the magnitude of the residue.

The petitioner's worst case poultry diet, while highly improbable, but none-the-less maximizing potential imidacloprid exposure, included grape pomace at 8% (0.56 ppm), spring cereal grains (not specified) at 50% (0.025 ppm), grain dust at 4% (0.002 ppm), potatoes at

30% (0.75 ppm), cotton oil (soapstock) at 5% (0.175 ppm), and cottonseed meal at 3% (0.105 ppm). We agree with the petitioner that 100% of the poultry diet can be treated with imidacloprid. While the petitioner's worst case dietary burden at 1.617 ppm is slightly higher than we would expect from the total imidacloprid residues on feed items in this petition we agree that 2 ppm or mg/kg feed is a reasonable 1X dose.

Eggs were collected twice daily and immediately frozen at -20 to -24°C. 3 egg samples from same dose group that had nearly the same feed consumption were thawed; then cracked, opened, and homogenized. The number of eggs produced in all four test groups does not appear to be affected by the imidacloprid dose. Sacrifice was after the last dose. At sacrifice no morphological or pathological finding were noted. The whole liver, composite thigh leg, and breast muscle, and abdominal fat were collected, then cut roughly and immediately frozen at -20 to -24°C.

The residue analytical method used to generate the imidacloprid poultry feeding study residue data was Bayer method 00191. This method has been previously reviewed and is currently undergoing a TMV in EPA labs. In summary, 10 grams of eggs or tissues samples were extracted with methanol/water, then concentrated to an aqueous remainder, while fat samples were partitioned against hexane. Samples were cleaned-up on a XAD-4 resin column, residues were eluted off the column in methanol, oxidized with permanganate to 6-CNA, neutralized, then the 6-CNA was extracted out with t-butylmethyl ether, derivatized with trimethylsilyl ester, and determined by capillary GC-MS selective ion monitoring at 214 m/z. The limit of quantitation (LOQ) is 0.02 ppm for both tissues and eggs.

The petitioner has provided additional concurrent method validation data. Egg samples were spiked individually with imidacloprid and its olefin metabolites at 0.02 ppm and 0.1 ppm plus with mixtures of the 2 compounds at 0.05 ppm each. Total imidacloprid recoveries from eggs averaged 74.6 $\pm$ 11, n = 15 from the 0.02 spike and averaged 89.5% $\pm$ 3.3%, n = 5 from the 0.1 ppm fortification. Egg samples spiked with 0.05 ppm imidacloprid plus the olefin had an average recovery of 73.9% during method validation and 77.5% during sample analysis. Fat samples were spiked individually with imidacloprid and its olefin metabolite at 0.02 and 0.1 ppm plus a mixture of the 2 at 0.05 ppm each. Total imidacloprid recoveries from fat averaged 78.4% from the 0.02 ppm spike and 79.6% from the 0.1 ppm spike. Recovery of the mixture from abdominal poultry fat averaged 74.6% during method validation and 86.5% during sample analysis. Muscle samples were spiked individually with imidacloprid and its olefin metabolite at 0.02 ppm and 0.1 ppm plus a mixture of the 2 at 0.05 ppm each. Total imidacloprid recoveries from muscle averaged 73.5% from the 0.02 ppm spike and 76.9% from the 0.1 ppm spike. Recovery of the mixture from muscle average 67.8% during method validation and 68.8% during sample analysis. Poultry liver was spiked with imidacloprid at 0.02, 0.5 and 2 ppm. Imidacloprid recoveries averaged 70.5 to 79.9% The liver samples were also spiked individually with the olefin, 6-CNA, and guanidine at 0.02 and 0.5 ppm plus a mixture of imidacloprid and the metabolites at 0.125 ppm each. Average recovery for the imidacloprid metabolites ranged from 85% to 102.8% with an average recovery from the mixture at 93.1%. Average recoveries for the guanidine metabolite were 86.6% from the 0.02 ppm spike and 89.5% for the 0.5 ppm spike. Adequate supporting chromatographic data were presented. The petitioner has adequately validated the method to gather the magnitude of the residue data for the poultry imidacloprid feeding study.

In the control eggs no imidacloprid equivalents were detected to < 0.02 ppm. At the 2

mg/kg dose total imidacloprid residues were also < 0.02 ppm. At the 6 mg/kg dose total imidacloprid residues ranged from 0.021 ppm to 0.056 ppm with 5 samples above 0.05 ppm (X = 0.037 ppm ± 0.009 ppm, n = 40). From the high dose of 20 mg/kg total imidacloprid residues ranged from 0.034 ppm to 0.149 ppm with 29 samples above 0.1 ppm (X = 0.103 ± 0.027, n = 72).

In poultry fat samples no imidacloprid residues were detected in the control fat, and in fat from the 2, 6, and 20 mg/kg doses to the LOQ of < 0.02 ppm.

Total imidacloprid residues were not detected in the control poultry muscle or in muscle from the 2 mg/kg dose. In muscle from the 6 mg/kg dose total imidacloprid residues ranged from <0.02 ppm to 0.022 ppm. At the 20 mg/kg dose total imidacloprid residue in muscles ranged from 0.031 ppm to 0.072 ppm averaging 0.048 ± 0.019 ppm, n = 6.

In the control poultry liver no imidacloprid residues were detected to < 0.02 ppm. At the 2 mg/kg dose total imidacloprid residues in liver ranged from 0.035 ppm to 0.042 ppm averaging 0.04 ppm ± 0.003 ppm, n = 6, and from the 6 mg/kg dose total imidacloprid residues ranged from 0.121 pm to 0.16 ppm averaging 0.0.14 ppm ± 0.015 ppm, n =6. From the high dose at 20 mg/kg dose total imidacloprid residues ranged from 0.235 ppm to 0.448 ppm averaging 0.346 ppm ± 0.076 ppm, n = 6.

Based on the results of the imidacloprid poultry feeding study CBTS concludes that finite residues will actually occur in eggs and meat from feeding of imidacloprid treated racs or their processed feed commodities when Confidor® is used as directed. Since this situation fall under 40 CFR 180.6(a)(1) secondary imidacloprid tolerances are required in eggs and poultry. However, judgement is deferred on the study supporting the proposed 0.02 ppm tolerance in eggs and in poultry meat, fat, and meat by-products until the petitioner has supplied the following information to allow CBTS to complete its review of the poultry feeding study and additional crop field trial data for cottonseed.

CBTS reiterates that the petitioner needs to provide a sample label for the poultry feed (in English), and the label should list the amount of protein, fat, fiber, and major ingredients that are in the feed. And finally we were unable to locate any data showing the feeds were free for other potentially interfering heavy metals, aflatoxins, and other pesticides. The petitioner needs to provide these data.

Bridging Data for the Feeding Studies (MRID # 425561-41)

During presubmission conferences CBTS expressed concerns to Miles on the use of fortified controls for quantitation, and the modification in the method's extraction step with the addition of acid to improve the recovery of the guanidine type metabolites. The petitioner has generated and presented additional recovery data to show the feeding studies data are valid.

Eggs from days 15 and 16, milk from day 16, bovine liver, and bovine muscle were reanalyzed. With these samples a control and recovery samples were concurrently analyzed. The spike consisted of imidacloprid, the guanidine metabolite and 6-CNA each at 0.05 ppm.

When quantification of residues was done using the standards in the control sample extract vs. the preferred procedure of using a solvent, percent recoveries and residues in the

test samples were slightly higher when the standards were in the control extracts. While a positive bias probably exists in the results from using a standard in the control extract the answers are within the expected reproducibility and repeatability of the method. Since the guanidine type metabolites are a low percentage of the total imidacloprid residues in ruminants and poultry the addition of acid to improve recovery does not significantly change the results reported. The petitioner reports consistently higher total imidacloprid residues using acid in the extraction step in the different animal matrices. CBTS would like to have the results of the livestock feeding studies reported using the method currently undergoing a TMV. However, there will be no improvement or changes in the final results if the studies are rerun. The same tolerance levels will have to be proposed. The petitioner has presented sufficient bridging data to validate the results of the livestock feeding studies and resolve CBTS concerns on the analytical residue method use to gather the residue data. Fortuitously there were canceling errors in the original method.

HARMONIZATION OF TOLERANCES

In International Residue Limit Status Sheet (IRLS) is attached to this review. Since there are no Canadian, Mexican, or Codex MRLs/tolerances compatibility is not a problem at this time.

cc:R.F.,Circu.,Reviewer(FDG),PP#3F4169.

H-7509C:CBTS:Reviewer(FDG):CM#2:Rm804Q:305-5826:FDG:7/13/93:edit:fdg:9/10/93.

RDI:SecHd:RSQuick:9/13/93:BrSrSci:RALoranger:9/15/93.