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

JUN 18 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: **PP# 3F4169 - IMIDACLOPRID (CONFIDOR®) ON APPLES, COTTONSEED, POTATOES, MEAT, MILK, POULTRY, AND EGGS.**
Review of the Analytical Method and Petitioner's February 5, 1993 Letter.
(MRID #s 425561-18 thru -28)[CBTS # 12027]{DP Barcode D192343}

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Robert D Quick for

EXECUTIVE SUMMARY OF RESIDUE CHEMISTRY DEFICIENCIES

- RESIDUE ANALYTICAL METHOD
  - ADDITIONAL MRM RECOVERY DATA
  - ADDITIONAL INDEPENDENT LABORATORY VALIDATION DATA
  - REVISED CONFIRMATORY PROCEDURE NEEDED
  - ADDITIONAL VALIDATION DATA FOR IMIDACLOPRID AND ITS METABOLITES
  - ADDITIONAL SUPPORTING CHROMATOGRAPHIC DATA
  - ADDITIONAL RECOVERY DATA USING AGED RADIOLABELED RESIDUES
  - REVISE METHODS

NOTE: THIS REVIEW DOES NOT ADDRESS OTHER RESIDUE CHEMISTRY STUDIES; IE, NATURE OF THE RESIDUE OR MAGNITUDE OF THE RESIDUE.

CONCLUSIONS

CBTS Conclusions on **Residue Analytical Methods**

1. The petitioner has conducted an adequate interference study which shows that positive interference from only one of 281 pesticides; ie, clopyralid is possible, and the interference is not expected to be a problem in determining imidacloprid residues.

2. The petitioner has presented adequate multiresidue method (MRM) recovery data for imidacloprid and its olefin, hydroxy, guanidine, and 6-chloronicotinic acid (6-CNA) metabolites through FDA Protocols A through E. These data will be forwarded to FDA for more review and will be printed in FDA'S PAM Vol I, Appendix I in a future update. Additional MRM recovery data should be presented for the urea and nitrosimino imidacloprid metabolites through Protocols A through E, as appropriate.

3. The petitioner presented two common moiety methods for total imidacloprid and its metabolites containing the 6-chloropyridine moiety in plants and animal tissues using a permanganate oxidation, silyl derivatization, and capillary GC-MS selective ion monitoring at m/z 214.

4. The confirmation procedure in both the plant and animal tissue methods use only one additional ion for identification of the common moiety. Monitoring with less then 3 ions for confirmation can lead to misidentification. The methods should state criteria for the relative response ratios of sample ions compared with relative ratios for analytical standards. The petitioner needs to provide an acceptable ratio value for the selected ions used for mass spectrometric quantitation as an index for the determination of interference when encountered with either ion.

5. CBTS concludes that the petitioner has not presented adequate imidacloprid confirmatory procedures for both the residue plant and animal methods. Since the primary detection system is GC/MS the confirmatory procedure should use an alternative detection system. The petitioner needs to have a different confirmatory procedure than that proposed in which only another ion is measured. CBTS suggests that a different imidacloprid confirmatory procedure be presented which has enhanced specificity using different extraction and clean-up techniques, derivatization reagents, and alternate GC columns. The confirmatory method should be at least semi-quantitative, though we would prefer the confirmatory method be quantitative. In either case additional petitioner generated validation data as well as ILV data are necessary. An additional TMV may be requested for the confirmatory procedure.

6. **THE PETITIONER HAS INFORMED CBTS THAT IMIDACLOPRID IS THE FIRST OF A NEW CLASS OF INSECTICIDES. WE HAVE CONCLUDED THE CONFIRMATORY METHOD NEEDS TO PRECISELY IDENTIFY IMIDACLOPRID AND ITS MAJOR METABOLITES, AS WELL AS BE AT LEAST SEMI-QUANTITATIVE, THOUGH OUR CHOICE WOULD BE TO HAVE THE**

CONFIRMATORY METHOD BE QUANTITATIVE. CBTS SUGGESTS THAT THE PETITIONER DIRECT HIS EFFORTS TOWARD DEVELOPING A CONFIRMATORY METHOD THAT CAN ADEQUATELY IDENTIFY RESIDUES OF IMIDACLOPRID AND ITS METABOLITES, NOT JUST MEASURING ANOTHER ION FROM THE SPECTRUM OF A DERIVATIZED COMMON MOIETY ENTITY.

7. The recovery data presented do not adequately validate the imidacloprid plant residue method to gather the magnitude of the residue data, or to enforce the proposed tolerances. The petitioner has not presented any recovery data for imidacloprid fortifications at the proposed tolerance levels in apples; cottonseed, cottonseed meal, and cotton forage; and potatoes, potato chips and potato flakes. The petitioner needs to present imidacloprid recovery data at levels appropriate to the proposed tolerances, including ILV data requirements and at levels that encompass the residue data reported.

8. The olefin imidacloprid and hydroxy imidacloprid metabolite standards are not listed as being available in the write-up of the methods. Standards for which we have requested and received MRM recovery data as well as petitioner generated recovery data are to be supplied to the EPA laboratories and the EPA Repository as appropriate. CBTS requests that the petitioner note in the revised method that standards for the olefin, hydroxy, urea, and nitrosimino imidacloprid metabolites are also available.

9. In addition the petitioner needs to generate imidacloprid recovery data for the imidacloprid olefin and the 5-hydroxy metabolites in apples at the 0.05-0.5 ppm level (the level where most of the residue data are reported), and at levels appropriate to the proposed tolerances, including ILV data requirements. Complete imidacloprid metabolite recovery data for the olefin, guanidine, 5-hydroxy, and urea metabolites are needed from cottonseed, cotton forage, and potatoes.

10. The petitioner needs to present additional supporting chromatographic data for the plant residue method showing recovery of 6-CNA at levels appropriate to the proposed tolerances, including ILV data requirements in each raw agricultural commodity and processed commodity for which a tolerance is proposed.

11. Additional recovery data for the plant method are required. The petitioner has presented his recovery data showing only the range of recoveries and the mean recovery. The raw data showing each individual recovery datum point, as well as the total number of analyses that went into determining the mean recovery were not presented. These individual recovery datum points for imidacloprid and its metabolites are required.

12. The ILV data for both the plant method and the animal tissue method from Germany appear to have been generated at the same testing facility as were the petitioner's original method validation data. CBTS cannot ascertain from the material presented whether or not the same facilities, equipment/ instrumentation, reagents, and personnel were used to generate the method validation data and the ILV data. The petitioner needs

to provide proof the ILV data were generated separately in every respect from the petitioner's method validation data.

13. The German ILV data were generated using the original version of the Bayer plant residue method No. 00200. Since there were major changes to the method, none of these ILV recovery data can be used as ILV data for the enforcement method. Only the ILV data on apples can be used from this study and only to give further confidence on the magnitude of the residue data.

14. CBTS defers judgement on the ^{14}C-imidacloprid recovery data using the proposed enforcement and residue gathering method to support the method as adequate for recovery of total imidacloprid residues from crop field trials and to enforce the proposed imidacloprid tolerances. We would prefer recovery data of aged radiolabeled residues be presented using the methanol/1% H_2SO_4 instead of the methanol/water extracting solvent and that the recovery data be from ^{14}C-imidacloprid treated apples, potato tubers, cottonseeds and cottonseed forage, not from other commodities for which there are no tolerance proposals.

15. Additional petitioner generated animal tissues method recovery data are required. Again, the petitioner has presented his recovery data showing only the range of recoveries and the mean recovery. The raw data showing each individual recovery datum point were not presented. These individual recovery datum points for imidacloprid and its metabolites are required.

16. These recovery data presented do not adequately validate the imidacloprid animal tissues residue method to enforce the proposed secondary tolerances. The petitioner has not presented any recovery data for imidacloprid, per se, and its significant metabolites fortifications at the proposed tolerances in milk, eggs, liver, kidney, fat, and various muscle from ruminants and poultry. The petitioner needs to present imidacloprid, per se, and its significant metabolites recovery data at all proposed meat, milk, poultry, and egg tolerance levels.

17. In addition, based on the ruminant and poultry metabolism studies the petitioner needs to generate imidacloprid metabolite recovery data for the imidacloprid metabolites listed in the following conclusion, in addition to the data already presented for the olefin, hydroxy, and 6-CNA metabolites. Recovery data are also needed for 6-CNA in milk before the TMV can be started.

18. The petitioner has not presented acceptable ILV data for the proposed imidacloprid animal tissues enforcement method. ILV data are required for imidacloprid, <u>per se</u>, and its olefin, hydroxy, urea, WAK 3583, nitrosimino, and 6-CNA in ruminant liver, kidney, fat, muscle, and milk at the proposed tolerances and 2-5 times the proposed tolerances. ILV data are also required for imidacloprid, <u>per se</u>, and its olefin, hydroxy, dihydroxy, DIJ 10739, WAK 4126, 6-CNA, and WAK 4230 in eggs, poultry liver, and muscle tissues at levels appropriate to the proposed tolerances, including the ILV data requirements. The petitioner is reminded that the TMV for milk cannot be completed without

these additional ILV data. Based on the new recovery ILV data the milk and tissues TMV may be modified.

19. None of the additional ILV data recently generated in the USA are suitable to support the proposed enforcement method for imidacloprid and its metabolites containing the 6-chloropyridine moiety because these data were not generated at the proposed tolerance levels and at levels above the proposed tolerances. These recovery data are suitable to provide further confidence in the petitioner's method to generate magnitude of the residue data.

20. CBTS concludes the petitioner has provided acceptable ^{14}C-imidacloprid recovery data from aged ^{14}C-imidacloprid caprine tissues to show the Bayer method 00191 can gather the magnitude of the residue data from poultry and ruminant feeding studies, and to enforce tolerances.

RECOMMENDATION

CBTS recommends that all of the imidacloprid residue analytical methods be remanded to the petitioner for revisions and additional validation data (including supporting data) as described in Conclusions 2, 4, 5, 7 through 12, and 14 through 19. CBTS can not recommend for any of the proposed imidacloprid tolerances in this petition without a successful TMV, nor can we fully accept any of the magnitude of the residue data until all residue analytical method concerns are resolved. While CBTS is completing the rest of our review of this petition, the petitioner has the opportunity to resolve the analytical method deficiencies. If the petitioner fails to respond to our method concerns before the full review is completed, then the discussion and deficiencies on the imidacloprid residue analytical methods noted here will be incorporated into that review by reference. The deficiencies will remain unresolved and continue outstanding.

DETAILED CONSIDERATIONS

BACKGROUND

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 byproducts of cattle, goats, hogs, horses, and sheep at 0.2 ppm, eggs at 0.02 ppm, and the meat, fat, and meat byproducts 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 on wet apple pomace at 2 ppm, dry apple pomace at 7 ppm, and cottonseed meal at 9 ppm.

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 other imidacloprid petitions or special local need registrations for permanent food tolerance have been submitted as of April 1, 1993. One Emergency Exemption (Section 18) request from Arizona for use of imidacloprid on cotton received a favorable CBTS recommendation (see memorandum by F.D. Griffith, Jr., dated June 1, 1993 for 93AZ0003).

Tolerance method validations (TMVs) have been requested for both residue analytical methods (see memorandum from F.D. Griffith, Jr. to D.A. Marlow dated March 3, 1993). The plant method is to be validated by the EPA laboratories for imidacloprid, per se, and its olefin, guanidine, and hydroxy metabolites in apples at 0.5 and 1 ppm, in cottonseed at 3.5 and 7 ppm and in cotton forage at 30 ppm. The animal tissues method is to be validated by the EPA laboratories for imidacloprid, per se, and its guanidine, hydroxy, and 6-CNA metabolites in milk at 0.05 and 0.1 ppm, and in liver at 0.2 and 0.5 ppm. Successful TMVs are necessary before CBTS can recommend in favor of the proposed tolerances.

The Analytical Chemistry Laboratory (ACL) of the Analytical Chemistry Branch (ACB) has completed their pre-review of both methods. The results and discussion of data deficiencies were reported to CBTS in a memorandum by H.K. Hundley dated April 15, 1993. Since most of the deficiencies noted by ACL are the same as CBTS found we have decided to write one consolidated report on the residue analytical methods so that the petitioner will not have to respond piecemeal, but can respond once in a timely manner to all method deficiencies.

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

Interference Study

The petitioner presented the results of an interference study in a document titled "Interference Study of Imidacloprid Total Residue Method for Crops and Animals" By F.J. Placke dated September 4, 1992 and coded Miles report number 103828.

281 compounds with established tolerances on a variety of commodities were tested through the plant method, Bayer Method 00200, and the animal method, Bayer Method 00191. The various pesticides were grouped together and spiked into the different commodities at

the established tolerances. Samples were analyzed through the entire original method including all clean-up and derivatization steps. Determination was by SIM GC/MS using the ion 214 m/z and two different temperature programs. One spiking mixture showed a significant response at 0.152 ppm imidacloprid equivalents. When each of the individual components in that mixture was tested separately only clopyralid spiked at 500 ppm showed a positive imidacloprid equivalent interference in Bayer's methods.

The petitioner claims a 0.05 ppm limit of quantitation (LOQ) for the plant method. Thus, other mixtures did not show imidacloprid equivalents interference above this value. However, the minimum detection limit (MDL) is less then 0.05 ppm and three other mixtures responded with imidacloprid equivalents at 0.002 ppm, and 0.004 ppm in 2 mixtures. CBTS concludes the petitioner has conducted an adequate interference study which shows that positive interference from only one of 281 pesticides is possible, and the interference is not expected to be a problem in determining imidacloprid residues.

MULTIRESIDUE METHOD RECOVERY DATA

The petitioner presented the results of the testing of imidacloprid, per se, through the FDA multiresidue methods (MRM) in a study titled "NTN 33893 Multiresidue Method Testing" by M.E. VerHey dated August 17, 1989, and coded laboratory project ID Mobay 1093. The study was conducted by Colorado Analytical Research and Development Corporation in Colorado Springs, Colorado. The study for imidacloprid, per se, was conducted using the FDA decision tree for Protocols A through E. Imidacloprid was recovered through the GC columns and the EC and N/P detectors listed in Protocol C. There was no recovery through the florisil clean-up columns, thus no further work was done on Protocol E (MOG method). Imidacloprid was not recovered through Protocol D (Luke method). We did note that the chromatography is not good and it is difficult to ascertain whether or not imidacloprid is present. Recovery data were not required through Protocol A as the compound is not a N-methyl carbamate, and through Protocol B as the compound does not have an acid or phenolic structure. These data will be forwarded to FDA for more review and will be printed in FDA'S PAM Vol I, Appendix I in a future update. Unless FDA finds a problem with these MRM recovery data CBTS concludes the petitioner has presented the results of MRM testing for imidacloprid and that no further data are required.

The petitioner presented the results of testing the imidacloprid metabolites through the FDA multiresidue methods (MRM) in a study titled "NTN 33893 Metabolites - Multiresidue Method Testing" by W.F. McCullough and B.B. Williams dated September 18, 1992, and coded Miles - N3161602 ABC - 40082. The study for the imidacloprid metabolites was conducted by ABC Laboratories in Columbia, Missouri. The FDA decision tree was used for Protocols A through E. No recovery data were required for Protocol A as none of the metabolites were N-methyl carbamates. The hydroxy, guanidine, and the olefin imidacloprid metabolites were recovered through the columns and the EC and N/P detectors listed in Protocol C; however the response was quite variable, low and multiple trailing peaks. 6-chloronicotinic acid also gave broad tailing, non-linear peaks; however the response for

methyl ester of 6-CNA for Protocol B was good, only it eluted very early and could not be easily separated for the solvent peaks. As with imidacloprid the metabolites could not be recovered through florisil using either series of eluting solvents, thus they were not recovered through Protocol E. With poor chromatographic response for the metabolites recovery was not possible with a 0.5 ppm fortification. These data will be forwarded to FDA for more review and will be printed in FDA'S PAM Vol I, Appendix I in a future update.

Additional MRM recovery data should be presented for the urea and nitrosimino imidacloprid metabolites through Protocols A through E, as appropriate.

RESIDUE ANALYTICAL METHOD - PLANTS

The petitioner presented a residue analytical method to gather the total imidacloprid residues in plants and enforce the proposed tolerances in a study titled "Method for the Determination of Total Residues of Imidacloprid in Plant Materials and Drinking Water (Bayer Method 0200 - Reformatted)" by M.E. Krolski dated September 15, 1992, and coded Miles report number 102624-R.

The petitioner presented a common moiety method for total imidacloprid and its metabolites containing the 6-chloropyridine moiety in plants using a permanganate oxidation, silyl derivatization and capillary GC-MS selective ion monitoring at m/z 214.

For samples containing little oil or wax such as apples and potatoes and samples that are relatively dry such as cotton forage 50 grams of sample are soaked in 300 mls of methanol/1% H_2SO_4 for 30 minutes, then blended for 3 minutes using a Polytron blender. The mixture is filtered through 10 grams of 545 Celite using a Whatman 541 filter paper. The filtrate is brought to a 500 ml volume with CH_3OH. 100 mls (10 gram aliquot) is concentrated on a rotary evaporator to about 10 mls, then proceed to the column clean-up step.

The extraction steps for samples that are high in oil, such as cottonseeds are the same as above with the addition of a partition clean-up step. The 10 mls aqueous extract from the rotary evaporator is transferred to 500 ml separatory funnel with 100 mls of water and partitioned 3 X 100 mls hexane; discarding the hexane. The instructions for emulsions are to carry the emulsion along with the hexane, allowing the layers to separate as completely as possible. No hexane is to be carried forward to the resin column clean-up step.

There are instructions for extracting the total imidacloprid residues from other commodities such as hops, rapeseed, cucumbers, eggplants, oil, beverages, and water. While varying the sample size from 10 grams in rapeseed to 250 mls for water the petitioner follows the same general extraction using CH_3OH/1% H_2SO_4 soak, blending with a polytron, filtering through celite/541 Whatman filter paper, concentration on a rotary evaporator and partitioning between hexane.

The plant samples are cleaned-up on a 10 gram XAD-4 resin column. The total imidacloprid residues are eluted off the column in 100 mls of CH_3OH. The petitioner notes that this is a convenient

over night stopping point in the procedure. Note; if the analyst decides to proceed to the oxidation step, then the oxidation step must be carried through to completion. The methanol is concentrated to about 1-2 mls, then to dryness and the residue is transferred into water. The petitioner cautions that no methanol is to be carried forward as methanol will interfere with the permanganate oxidation step.

Using 32% NaOH the pH is adjusted to $\geq$ 14 using pH paper. Oxidation of the total imidacloprid residues is accomplished by adding 50 mls of a 50 gram/liter aqueous $KMnO_4$ to the mixture. Add a magnetic stirring bar and connect to a reflux condenser. Rapidly bring to a boil (10 minutes or less) and reflux for only 5 minutes once the solution has been brought to a boil to convert imidacloprid and all of its metabolites that contain the 6-chloro pyridine common moiety to 6-chloronicotinic acid (6-CNA). The petitioner notes that if the solution is refluxed longer, then 6-CNA will start to decompose. The flask is removed from heat and the condenser is rinsed with 50 mls of water. The solution is cooled with agitation until the temperature is less than 15°C, then acidified with 10% H_2SO_4. Sodium bisulfite is added a gram at a time until the color changes from purple (permanganate) to chocolate brown (MnO_2) to clear and colorless. Check the solution to be sure it is less then pH 1; adjust if necessary.

Extract the 6-CNA from the solution with 3 X 50 mls of t-butyl methyl ether (MTBE) drying each extract through 30 grams of anh. Na_2SO_4, rinsing the Na_2SO_4 with an additional 30 ml of MTBE. The solution is evaporated to almost dryness using a rotary evaporator, then to dryness under a gentle stream of N_2. The residue is dissolved in 2.00 mls of derivatizing grade acetonitrile (ACN). The petitioner notes this is also a convenient over night stopping point. In fact, the sample may be stored for up to two weeks in a refrigerator before derivatization.

250 ul of the solution is placed in a reaction vial and 250 ul of N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) is added, the vial is sealed and contents are mixed. The samples are allowed to react 1 hour at ambient temperature before GC analysis.

Determination is by GC/MS using a Hewlett-Packard 5890 GC equipped with 7673 autosampler. The column is a 12 m quartz capillary, 0.2 mm i.d., HP ULTRA 1 (dimethyl silicone), 0.33 um film thickness. Sample injection was in the splitless mode and the column temperature was programmed. The detector is a HP 5970 mass specific detector in the single ion monitoring (SIM) mode for detecting ions at m/z 214 and confirmation at m/z 170.

The confirmation procedure uses only one additional ion for identification of the common moiety. According to standard references monitoring with less then 3 ions for confirmation can lead to misidentification. In addition, the method should state criteria for the relative response ratios of sample ions compared with relative ratios for analytical standards. The petitioner needs to provide an acceptable ratio value for the selected ions used for mass spectro-

metric quantitation as an index for the determination of interference when encountered with either ion.

Since the primary detection system is GC/MS the confirmatory procedure should use an alternative detection system. The petitioner needs to have a different confirmatory procedure than that proposed when only another ion is measured; ie, at m/z 170 from the same extract, following the same cleanup and derivatization steps and using the same GC capillary column and the same MS detector. CBTS suggests that a different imidacloprid confirmatory procedure be presented which has enhanced specificity using different extraction and clean-up techniques, derivatization reagents, and alternate GC columns. CBTS concludes that the petitioner has not presented an adequate imidacloprid confirmatory procedure. The confirmatory method should be at least semi-quantitative, though we would prefer the confirmatory method be quantitative. In either case additional petitioner generated validation data as well as ILV data are necessary. An additional TMV may be requested for the confirmatory procedure

NOTE: THE PETITIONER HAS INFORMED CBTS THAT IMIDACLOPRID IS THE FIRST OF A NEW CLASS OF INSECTICIDES. WE HAVE CONCLUDED THAT THR CONFIRMATORY METHOD NEEDS TO PRECISELY IDENTIFY IMIDACLOPRID AND ITS MAJOR METABOLITES, AS WELL AS BE AT LEAST SEMI-QUANTITATIVE, THOUGH OUR CHOICE WOULD BE TO HAVE THE CONFIRMATORY METHOD BE QUANTITATIVE. CBTS SUGGESTS THAT THE PETITIONER DIRECT HIS EFFORTS TOWARD DEVELOPING A CONFIRMATORY METHOD THAT CAN ADEQUATELY IDENTIFY RESIDUES OF IMIDACLOPRID AND ITS METABOLITES, NOT JUST MEASURING ANOTHER ION FROM THE SPECTRUM OF A DERIVATIZED COMMON MOIETY ENTITY.

Quantitation is by peak area from a standard curve. Standards were prepared in ACN and the petitioner presented reasonable "shelf life" stability for the standards in solution. Conversion factors to correct for the molecular weight difference between 6-CNA and the analyte of interest, whether it is imidacloprid or a metabolite, are listed.

The petitioner has presented recovery data for imidacloprid spiked at 0.05 ppm and 0.5 ppm in or on a number of commodities such as barley, oats, and wheat forage, grain, and straw, corn grain and forage, sugarbeets, field beans, pea seed and pod, eggplant, cucumber, paprika, lettuce, tomato, pears, oranges, sunflower seed, rapeseed, hops and beer, tobacco, and drinking water. Over all imidacloprid recoveries in these commodities appear acceptable in these low level fortifications ranging from around 70% to near 120%. The olefin imidacloprid and hydroxy imidacloprid metabolite standards are not listed as being available in the write-up of the method. Standards for which we have requested and received MRM recovery data as well as petitioner generated recovery data are to be supplied to the EPA laboratories and the EPA Repository as appropriate. CBTS requests that the petitioner note in the revised method that standards for the olefin, hydroxy, urea, and nitrosimino imidacloprid metabolites are also available.

Potato tuber were fortified at 0.05 and 0.5 ppm imidacloprid with recoveries ranging from 93% to 103%. Apples were fortified with

imidacloprid at 0.05 and 0.5 ppm with recoveries ranging from 87% to 109%. Imidacloprid recoveries from apple juice spiked at 0.05 ppm were a mean of 106%. Cottonseed and cottonseed oil fortified with 0.05 ppm imidacloprid had recoveries ranging from 82% to 98%.

The petitioner presented recovery data for the imidacloprid metabolites guanidine, dihydroxy, urea, and nitrosimino fortified at 0.05 ppm in corn straw, sugarbeet roots, cottonseed and apples. The guanidine imidacloprid metabolite was also spiked in sugarbeet roots, corn straw, and apples at 0.5 ppm. In apples the guanidine metabolite recoveries ranged from 79% to 100%. The dihydroxy metabolite recoveries from apples ranged from 104% to 107%. The nitrosimine metabolite recoveries from apples ranged from 78% to 90% and the mean urea metabolite recovery from apples was 106%. The mean guanidine metabolite recovery from cottonseed was 65%. The mean dihydroxy imidacloprid recovery from cottonseed was 88% and the urea imidacloprid metabolite recovery from cottonseed was 80%. The mean nitrosimine metabolite recoveries from cottonseed was 69%. 6-CNA recovery from potatoes was 104% following a 0.05 ppm fortification.

The recovery data for the imidacloprid metabolites from eggplants, cucumbers, corn straw and sugarbeet roots are useful if we are investigating possible imidacloprid misuse on these commodities, but they are not germane to validating the method to enforce total imidacloprid tolerance in cottonseed, apples, and potatoes.

Additional recovery data are required. The petitioner has presented his recovery data showing only the range of recoveries and the mean recovery. The raw data showing each individual recovery datum point, as well as the total number of analyses that went into determining the mean were not presented. These individual recovery datum points for imidacloprid and its metabolites are required.

These recovery data presented do not adequately validate the imidacloprid plant residue method to gather the total imidacloprid magnitude of the residue data, or to enforce the proposed tolerances. The petitioner has not presented any recovery data for imidacloprid, per se, and its metabolites fortifications at the proposed tolerances in apples; cottonseed, cottonseed meal, and cotton forage; and potatoes, potato chips and potato flakes. The petitioner needs to present imidacloprid, per se and its metabolite recovery data at levels appropriate to the proposed tolerances, including ILV data requirements and at levels that encompass the residue data reported.

In addition, based on the plant metabolism studies the petitioner needs to generate imidacloprid recovery data for the imidacloprid olefin and the 5-hydroxy metabolites in apples and cottonseed at the 0.05-0.5 ppm level (the levels where most of the residue data are reported), and at levels approporiate to the proposed tolerances, including ILV data requirements. Complete imidacloprid metabolite recovery data for the olefin, guanidine, 5-hydroxy, nitrosimino, and urea metabolites are needed from cotton forage and potatoes.

The petitioner presented 40 copies of supporting chromatograms showing the recovery of 6-CNA from a number of commodities; however only 6 of these chromatograms are germane to this petition. Crop co-

extractives as illustrated by the chromatograms varied depending on the matrix. The unidentified analytical responses (UARs) did not present an interference problem for determining 6-CNA in the commodities of interest. The petitioner needs to present additional supporting chromatographic data showing recovery of the 6-CNA at and above the propose tolerance in each raw agricultural commodity and processed commodity for which a tolerance is proposed.

INDEPENDENT LABORATORY VALIDATION DATA AND RADIOLABELED RECOVERIES - PLANTS

The petitioner presented the results of an independent laboratory validation (ILV) in a study titled "Outside Laboratory Validation of the Analytical Residue Method No. 00200 for the Determination of the Total Residues from Imidacloprid in Plant Sample Materials and Drinking Water" by H. Allmendinger dated August 6, 1991, and coded Miles report number 103214. After revising the method to conform to the requirements in the Residue Chemistry Guidelines the petitioner presented additional ILV data in a study titled "Outside Laboratory Validation of the Analytical Residue Method No. 00200 for the Determination of Total Residues of Imidacloprid in Plant Materials and Drinking Water - Additional Validation Data" by R.R. Gronberg dated October 21, 1992, and coded Miles report number 103214-1. The petitioner also presented recovery data using radiotreated samples from the metabolism studies to validate the plant residue method in a study titled " Validation of the Residue Analytical Method for the Total Residue of Imidacloprid in Plant Materials Based on Radioactive Aged Residues" by E. Weber dated September 2, 1992, and coded Miles report number 103827.

It appears from our review of the title pages that the ILV data from Germany were generated at the same testing facility as were the petitioner's original method validation data. CBTS cannot ascertain from the material presented whether or not the same facilities, equipment/instrumentation, reagents, and personnel were used to generate the method validation data and the ILV data. The petitioner needs to provide proof the ILV data were generated separately in every respect from the petitioner's method validation data.

The German ILV data were generated using the original version of the Bayer plant residue method No. 00200 where the residues were extracted using water/methanol, not methanol/1% H_2SO_4. The petitioner also used a control fortified blank to generate the standard curve for the calculations. Since there were major changes to the method to overcome the low guanidine recoveries and to avoid using a control blank none of these ILV recovery data can be used as ILV data for the enforcement method. Recovery data from three separate fortification experiments were presented for imidacloprid and the dihydroxy, guanidine, urea, and nitrosimine metabolites in sunflower seeds at 0.05 ppm. Imidacloprid recovery data were also presented at 0.05 ppm and 0.5 ppm fortifications in apples and wheat grain and straw. Since most of the ILV and petitioner's method validation data are at the LOQ of 0.05 ppm it is difficult to determine whether these data are in agreement. CBTS points out that a 20% difference at 0.05 ppm is 0.01 ppm; a very good agreement between laboratories in any case at this low residue value. However, if the recoveries were 20% different at 30 ppm; ie, 24 to 36 ppm we would have some concerns.

Only the ILV data on apples can be used from this study and only to give further confidence on the magnitude of the residue data. Since this is the first food tolerance we are not willing to translate any data; ie, substitute sunflower seeds recovery data for cottonseeds recovery data (both are high lipid samples).

After consulting with EPA chemists Miles decided to conduct additional ILV data in the USA. These ILV data were conducted at the Miles facilities in Stilwell, Kansas and at Analytical BioChemistry Laboratories in Columbia, Missouri, thus questions relating to use of common facilities, equipment/instrumentation, personnel, and reagents is not an issue with these ILV data. All of the ILV data were generated with the revised residue analytical method that uses dilute acid in the extraction step and does not use a fortified control blank sample to generate the standard curve. All of the recovery data were generated at 0.1 ppm; a level slightly above the LOQ. No ILV recovery data were generated at any of the proposed tolerances. Recovery data were presented for imidacloprid, per se, and the guanidine, olefin, 5-hydroxy, and 6-CNA metabolites. No new ILV data were presented for the urea or the nitrosimine metabolites. ILV data generated by the petitioner's laboratory in Kansas for imidacloprid and four metabolites spiked at 0.1 ppm in cottonseeds ranged from 75% to 93%. ILV data generated by ABC Laboratories for imidacloprid and four metabolites in potatoes ranged from 75% (olefin) to 108% (guanidine), in apples from 89% (5-hydroxy) to 115% (6-CNA), and in cotton hulls, oil, and soapstock from 72% (6-CNA and guanidine) to 114% (5-hydroxy).

None of the additional ILV data recently generated in the USA are suitable to support the proposed enforcement method for imidacloprid and its metabolites containing the 6-chloropyridine moiety because these data were not generated at the proposed tolerance levels and at levels above the proposed tolerances. These recovery data are suitable to provide further confidence in the petitioner's method to generate magnitude of the residue data.

CBTS reiterates (see memorandum by F.D. Griffith, Jr., dated September 25, 1992) that recovery data generated by the petitioner as well as ILV data are required for the parent pesticide and all metabolites that are to be regulated in the tolerance expression. Since the petitioner has proposed a common moiety enforcement method that measures the parent and all metabolites as one analytical entity, then recovery data are required for each component of the tolerance expression. Again, the petitioner may not combine components in a common moiety method recovery study to improve the overall recovery to obtain a value of 70% to 120% to meet the Agency requirements. The petitioner is reminded that the Residue Chemistry Guidelines very clearly state that "recoveries should be at fortification levels appropriate to the proposed tolerance." The petitioner is also reminded that the PR Notice 88-5 clearly states that ILV data are required at the proposed tolerance and 2-5 times the proposed tolerance. These petitioner and ILV recovery data have not been presented. There is no way recovery data at 0.05-0.1 ppm can validate a method to enforce tolerances at 1 ppm (apples), 3.5 ppm (cottonseed), or 30 ppm (cotton forage). CBTS reiterates that we

consider the addition of acid to improve recoveries to be a major significant change to the proposed enforcement method.

The petitioner presented the results of a study to determine the efficiency of the Bayer method 00200 to recover aged radiolabeled residues of imidacloprid. Samples of ^{14}C-imidacloprid treated corn straw (fodder?), forage, and grain; apples, and potato vines were extracted by maceration with 300 mls of methanol/water (3/1, v/v) following by filtration through a Buchner funnel with fast filter paper. The retained solids were washed and dried. The radioactivity was determined in both the retained solids and in the extracts. In apples only 5% of the radiolabeled imidacloprid residue remained in the solids and 95% was in the methanol/water extract. For potato vines and green corn forage 13% of the radiolabeled residue was not extracted from the matrix with 87% recovered in the extract. In corn fodder 26 to 31% of the radiolabeled residues was not extracted from the matrix. In corn grain only 52 to 62% of the radiolabeled residue was recovered in the methanol/water extract. CBTS concluded the water/methanol extraction would not be satisfactory for recovering the radiolabeled imidacloprid residues for corn grain.

The petitioner has provided recovery data for the ^{14}C-imidacloprid equivalents through the clean-up and the oxidation steps of the Bayer method 00200. In summary, the samples were partitioned/ cleaned-up with hexane, then through a XAD-4 resin column, followed by basification with NaOH and oxidation by $KMnO_4$ to 6-CNA . The ^{14}C-6-CNA residues were determined by thin layer chromatography (TLC). The Rf of 6-CNA as well as the Rfs of imidacloprid and other 6-chloropyridine containing metabolites of imidacloprid were determined on silica 60 F254 plates using 2 different polarity mobile phases. For the radioactivity in the extracts 87% of the ^{14}C-imidacloprid equivalents in apples were recovered. 67 to 75% of the ^{14}C-imidacloprid equivalents were recovered through the clean-up and oxidation steps from potato vines extracts. Recoveries of total ^{14}C-imidacloprid equivalents following the clean-up and oxidation steps for extracts of corn fodder, forage, and grain ranged from 73% to 80%. Results of the TLC analysis showed that > 95% of the residue on the TLC plates was 6-CNA.

CBTS defers judgement on the ^{14}C-imidacloprid recovery data using the proposed enforcement and residue gathering method to support the method as adequate for recovery of total imidacloprid residues from crop field trials and to enforce the proposed imidacloprid tolerances. We would prefer recovery data of aged radiolabeled residues be presented using the methanol/1% H_2SO_4 rather than using methanol/water extracting solvent and that the recovery data be from ^{14}C-imidacloprid treated apples, potato tubers, cottonseeds and cottonseed forage, not from other commodities for which there are no tolerance proposals.

RESIDUE ANALYTICAL METHOD - LIVESTOCK

The petitioner presented a residue analytical method to gather the total imidacloprid residues in tissues, milk, and eggs and enforce the proposed meat, milk, poultry, and egg tolerances in a study titled "Method for the Determination of Total Residues of Imidacloprid in Animal Materials (Bayer Method 00191 M001 - Reformat-

ted)" by E. Weber and U. Heukamp dated September 18, 1992, and coded Miles report number 103848-R.

The petitioner presented a common moiety method for total imidacloprid and its metabolites containing the 6-chloropyridine moiety in animal tissues, milk, and eggs using a methanol/water extraction, resin column clean-up, permanganate oxidation, silyl derivatization and capillary GC-MS selective ion monitoring at m/z 214.

For samples of muscle, kidney, liver, and eggs 10 grams are blended with 50 ml of methanol/water (3/1, v/v) using a Polytron for at least 30 seconds. The mixture is centrifuged and the supernatant is poured into a 1 liter boiling flask. The extraction is repeated, the supernatants are combined, and concentrated using a rotary evaporator in a 60°C bath to approximately a 20 ml aqueous remainder. The petitioner cautions that no methanol can be carried forward to the resin column as methanol will interfere with the adsorption of imidacloprid and its metabolites. Muscle and kidney samples are diluted with 25 mls water before proceeding to the resin clean-up column. Liver samples are also diluted with 25 mls of water plus 2.5 mls of 10% H_2SO_4, and egg samples are diluted with 70 mls of water before proceeding to the resin column clean-up.

10 grams of fat are blended with 100 mls of methanol/water (3/1, v/v) for at least 30 seconds. The suspension is centrifuged and the supernatant is concentrated on a rotary evaporator to about 10 mls. The sample is diluted to 50 mls with water, transferred to a separatory funnel, and partitioned 3 X 50 mls hexane, discarding the hexane. The aqueous remainder is concentrated by rotary evaporation. The resin column clean-up is omitted and fat samples are taken directly to the oxidation step.

Milk samples (10 grams) are blended 30 seconds with 100 mls of methanol, centrifuged, and the supernatant is concentrated by rotary evaporation to approximately 10 mls. The sample is diluted with 25 mls of water, transferred to a separatory funnel, and partitioned 3 X 50 mls hexane, discarding the hexane. The aqueous remainder is concentrated to 10-15 mls and the sample is taken directly to the oxidation step.

Muscle, liver, kidney, and eggs samples are cleaned-up on an Amberlite XAD-4 resin column. The aqueous samples are transferred to the column and the interfering matrix compounds are eluted off with a water wash. Imidacloprid and its 6-chloropyridine containing metabolites are eluted off the column with 125 mls methanol. The petitioner notes this a convenient over night stopping point in the procedure. Note: if the analyst decides to proceed to the oxidation step, then the oxidation step must be carried through to completion. The methanol is concentrated to 1-2 mls on a rotary evaporator, then to dryness as the petitioner cautions that the methanol must be removed before the permanganate oxidation step as methanol will interfere with the oxidation of imidacloprid and its metabolites to 6-chloronicotinic acid.

Using 32% NaOH the pH is adjusted to > 14 using pH paper. Oxidation of the total imidacloprid residues to 6-CNA is accomplished

by adding 50 mls of 50 grams/liter aqueous $KMnO_4$ to fat, muscle, eggs, milk, and kidney; and 150 mls to liver samples. Add a magnetic stirring bar and connect to a reflux condenser. Rapidly bring to a boil (10 minutes or less). Reflux for only 5 minutes once the solution has been brought to a rapid boil as this will convert total imidacloprid to 6-CNA. The petitioner cautions that if the solution is refluxed longer, then 6-CNA will start to decompose. The flask is removed from heat and the condenser is rinsed with 50 mls. The solution is cooled with agitation until the temperature is less then 15°C, then acidified with 50 mls of 10% H_2SO_4. Sodium bisulfite is added a gram at time until the color changes from purple (permanganate) to opaque chocolate brown (MnO_2), clear and colorless. Check the solution to be sure it is less then pH 1; adjust if necessary.

Extract the 6-CNA from the solution with 3 X 25 mls of t-butyl methyl ether (MTBE) drying each extract through 30 grams of anh. Na_2SO_4, rinsing the Na_2SO_4 with an additional 30 ml of MTBE. The solution is evaporated to almost dryness using a rotary evaporator, then to dryness under a gentle stream of N_2. The residue is dissolved in 1.00 mls of derivatizing grade acetonitrile (ACN). The petitioner notes this is also a convenient over night stopping point. In fact, the sample may be stored for up to two weeks in a refrigerator before derivatization.

250 ul of the solution is placed in a reaction vial and 250 ul of N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) is added, the vial is sealed and contents are mixed. The samples are allowed to react 1 hour at ambient temperature before GC analysis.

Determination is by GC/MS using a Hewlett-Packard 5890 GC equipped with 7673 autosampler. The column is a 12 m quartz capillary, 0.2 mm i.d., HP ULTRA 1 (dimethyl silicone), 0.33 um film thickness. Sample injection was in the splitless mode and the column temperature was programmed. The detector is a HP 5970 mass specific detector in the single ion monitoring (SIM) mode for detecting ions at m/z 214 and confirmation at m/z 170 and 140 m/z.

The confirmation procedure uses only one additional ion for identification of the common moiety. According to standard references monitoring with less then 3 ions for confirmation can lead to misidentification. In addition, the method should state criteria for the relative response ratios of sample ions compared with relative ratios for analytical standards. The petitioner needs to provide an acceptable ratio value for the selected ions used for mass spectrometric quantitation as an index for the determination of interference when encountered with either ion.

As with the imidacloprid plant residue method, since the primary detection system is GC/MS the confirmatory procedure should use an alternative detection system. The petitioner needs to have a different confirmatory procedure than that proposed when only another ion is measured; ie, at m/z 170 and 140 from the same extract, following the same cleanup and derivatization steps and using the same GC capillary column and the same MS detector. CBTS suggests that a different imidacloprid confirmatory procedure for residue in meat, milk, poultry, and eggs be presented which has enhanced specificity

using different extraction and clean-up techniques, derivatization reagents, and alternate GC columns. CBTS concludes that the petitioner has not presented an adequate imidacloprid confirmatory procedure for residues in meat, milk, poultry, and eggs. The confirmatory method should be at least semi-quantitative, though we would prefer the confirmatory method be quantitative. In either case additional petitioner generated validation data as well as ILV data are necessary. An additional TMV may be requested for the confirmatory procedure.

Quantitation is by peak area from a standard curve. Standards were prepared in ACN and the petitioner presented reasonable "shelf life" stability for the standards in solution. Conversion factors to correct for the molecular weight difference between 6-CNA and the analyte of interest, whether it is imidacloprid or a metabolite are listed.

The petitioner presented recovery data for imidacloprid and its olefin and hydroxy metabolites in milk spiked at 0.02 and 0.1 ppm. Recoveries ranged from a mean of 71.4% $\pm$ 8.2% imidacloprid, n = 6, to a mean of 89% $\pm$ 13.4% hydroxy imidacloprid, n = 9. From a mixture of imidacloprid, the olefin and hydroxy metabolites all spiked at 0.03 ppm the mean recovery was 87.5% $\pm$ 7.3%, n = 6.

Liver samples were spiked at 0.02 ppm, 0.05 ppm, 0.25 ppm, 0.5 ppm, and 2.5 ppm imidacloprid. Mean recoveries range from 70.5% $\pm$ 2.4%, n = 5, for the 0.02 ppm spike to a mean of 80.0 $\pm$ 2.7%, n = 3, for the 0.5 ppm fortification. Additional recovery data for the imidacloprid olefin, hydroxy, guanidine, and 6-CNA metabolites were presented from 0.02 and 0.5 ppm fortifications. Mean recoveries ranged from 72.1% $\pm$ 2.2% for 6-CNA, n = 3, to a mean of 89.2% $\pm$ 8.2% for olefin imidacloprid, n = 3. A mixture of approximately 0.2 ppm each of imidacloprid, and its olefin, hydroxy, guanidine, and 6-CNA metabolite were spiked in liver with a mean recovery of 80.8% $\pm$ 3.3%, n = 3.

Kidney samples were spiked with imidacloprid at 0.02 ppm, 0.5 ppm, and 2 ppm; with 0.02 ppm and 0.05 ppm 6-CNA, olefin, and hydroxy imidacloprid; and with a mixture of these compounds each at 0.25 ppm. Fat samples were fortified with imidacloprid and its olefin and hydroxy metabolites at 0.02 ppm and 0.3 ppm. A mixture of 0.1 ppm of each of these compounds was also used as a spiking solution. Muscle samples were fortified with imidacloprid and its olefin and hydroxy metabolite at 0.02 and 0.6 ppm. Muscle samples were also spiked with a mixture of imidacloprid and its olefin and hydroxy metabolites at 0.1 ppm each. Only mean recovery values were reported along with one standard deviation. Generally recoveries were lower in kidney samples and somewhat higher in fat samples with more spread in muscle samples.

Egg and poultry muscle samples were fortified with imidacloprid and its olefin at 0.02 ppm and 0.1 ppm; plus a mixture of each at 0.05 ppm. Mean recoveries of imidacloprid were in the 70% range and mean recoveries of the olefin were in the 60% range. Mean recovery of the mixture was 73.9% in eggs and 67.8% in poultry muscle. Poultry fat spiked with imidacloprid and its olefin at the same

levels as in eggs had mean recoveries from 72.6% to 79.6%. Poultry liver samples were fortified with imidacloprid and its olefin and guanidine metabolites at 0.02 ppm and 0.5 ppm. A higher imidacloprid fortification level at 2 ppm was also reported. Recovery data for 6-CNA was reported from fortifications of 0.02 ppm and 0.3 ppm. Overall mean recoveries from poultry liver for imidacloprid and its metabolites ranged from 85% to 110.8%, a higher percentage recovery than for any other poultry tissue.

The petitioner presented 48 copies of supporting chromatograms showing the recovery of 6-CNA from eggs, kidney, liver, milk, and muscle. Chromatographic data were presented showing blank or control samples, standards of 6-CNA, and recoveries from of individual standards and standard mixture fortifications. The chromatograms show few UARs and none interfering with the determination of 6-CNA.

Additional petitioner generated animal tissues method recovery data are required. Again, the petitioner has presented his recovery data showing only the range of recoveries and the mean recovery. The raw data showing each individual recovery datum point were not presented. These individual recovery datum points for imidacloprid and its metabolites are required.

These recovery data presented do not adequately validate the imidacloprid animal tissues residue method to enforce the proposed secondary tolerances. The petitioner has not presented any recovery data for imidacloprid, per se, and its significant metabolites fortifications at the proposed tolerances in milk, eggs, liver, kidney, fat, and various muscle from ruminants and poultry. The petitioner needs to present imidacloprid, per se, and its significant metabolites recovery data at all proposed meat, milk, poultry, and egg tolerances. Specifically, petitioner generated recovery data are required for imidacloprid, <u>per se</u>, and its olefin, hydroxy, urea, WAK3583, nitrosimino, and 6-CNA in ruminant liver, kidney, fat, muscle, and milk at the proposed tolerances. Petitioner generated recovery data are also required for imidacloprid, <u>per se</u>, and its olefin, hydroxy, dihydroxy, DIJ 10739, WAK 4126, 6-CNA, and WAK 4230 in eggs, poultry liver, and muscle tissues at levels appropriate to the proposed tolerances, including the ILV data requirements.

In addition, based on the ruminant and poultry metabolism studies the petitioner needs to generate imidacloprid metabolite recovery data for the imidacloprid metabolites such as the dihydroxy, DIJ 10739, WAK 4126, and WAK 4230, in addition to the data already presented for the olefin, hydroxy, and 6-CNA metabolites.

Again CBTS reiterates (see memorandum by F.D. Griffith, Jr., dated September 25, 1992) that recovery data generated by the petitioner as well as ILV data are required for the parent pesticide and all metabolites identified in the animal metabolism studies containing the 6-chloropyridine moiety that are to be regulated in the tolerance expression. The recovery of the parent imidacloprid and the hydroxy, olefin, and 6-CNA metabolites may or may not be the same as for all of the imidacloprid metabolites identified in the livestock metabolism studies. Recovery data for these few metabolites does not validate the proposed common moiety enforcement method to

determine imidacloprid and its metabolites containing the 6-chloropyridine moiety. Specifically, the petitioner needs to generate acceptable recovery data as well as ILV data for the significant imidacloprid metabolites identified in the livestock metabolism studies. Again, the petitioner may not combine components in a common moiety method recovery study to improve the overall recovery to obtain a value of 70% to 120% to meet the Agency requirements.

The petitioner is reminded that the Residue Chemistry Guidelines very clearly state that "recoveries should be at fortification levels appropriate to the proposed tolerance." The petitioner is also reminded that the PR Notice 88-5 clearly states that ILV data are required at the proposed tolerance and 2-5 times the proposed tolerance. We reiterate that in our October 1989 Overview of the Residue Chemistry Guideline we stated that the residue analytical method is to be validated by the petitioner on each matrix for which "crop field trial" data are generated and on each matrix for which tolerances are proposed.

INDEPENDENT LABORATORY VALIDATION DATA AND RADIOLABELED RECOVERIES - LIVESTOCK

The petitioner presented the results of an independent laboratory validation (ILV) for the meat, milk, poultry, and egg method in a study titled "Outside Laboratory Validation of the Analytical Residue Method No. 00191 for the Determination of Total Residue from Imidacloprid in Materials of Animal Origin" by W. Blass dated August 28, 1992, and coded Miles report number 103830. The petitioner also presented recovery data using radiotreated samples from the caprine and poultry imidacloprid metabolism studies to validate the animal tissues method in a study titled "Validation of the Residue Analytical Method for the Total Residue of Imidacloprid in Animal Tissues Based on Radioactive Aged Residues" by E. Weber dated September 2, 1992, and coded Miles report number 103829.

It appears from our review of the title pages that the ILV data for the meat, milk, poultry, and egg method from Germany were generated at the same testing facility as were the petitioner's original method validation data. CBTS cannot ascertain from the material presented whether or not the same facilities, equipment/ instrumentation, reagents, and even personnel were used to generate the method validation data for recovery of total imidacloprid residues from meat, milk, poultry, and eggs and the corresponding ILV data. The petitioner needs to provide proof the ILV data were generated separately in every respect from the petitioner's method validation data.

The ILV data for the animal tissues method was conducted in Germany using the Bayer method 00191 as presented. ILV data were generated for milk, liver, and eggs spiked with imidacloprid and its guanidine and 6-CNA metabolites as a mixture. The fortification levels were 0.02 ppm and 0.1 ppm in milk and eggs, and 0.1 ppm and 0.5 ppm. The petitioner claims the LOQ for this method is 0.02 ppm. The petitioner made duplicate injections into the GC/MS from the same solution. The animal tissues method was validated with a single set of recovery data. Total imidacloprid recoveries from milk ranged from 70.6% to 84.1% and from eggs ranged from 63.3% to 84.4%. Total imidacloprid residues from poultry liver ranged from88.9% to 103%.

The petitioner has not presented acceptable ILV data for the proposed imidacloprid animal tissues enforcement method. ILV data are required for imidacloprid, per se, and its olefin, hydroxy, urea, WAK3583, nitrosimino, and 6-CNA in ruminant liver, kidney, fat, muscle, and milk at the proposed tolerances and 2-5 times the proposed tolerances. ILV data are also required for imidacloprid, per se, and its olefin, hydroxy, dihydroxy, DIJ 10739, WAK 4126, 6-CNA, and WAK 4230 in eggs, poultry liver, and muscle tissues at the proposed tolerances and at 2-5 times the proposed tolerances. The petitioner is reminded that the TMV for tissues and milk cannot be completed without these additional ILV data. Based on the new recovery ILV data the milk and tissues TMV may be modified.

The petitioner presented the results of a study to determine the efficiency of the Bayer method 00191 to recover aged radiolabeled residues of ^{14}C-imidacloprid from caprine tissues. Reserve samples of caprine muscle, fat, liver, and milk were extracted with methanol/water as described in method 00191 followed by filtration through a Buchner funnel with fast filter paper. The retained solids were washed and dried. The radioactivity was determined in both the solids and in the extracts. From caprine milk, muscle and fat 94% to 98% of the imidacloprid radioresidues were in the extracts and only 2-6% of the residue was not extracted. 77% of the imidacloprid radiolabeled residues were extracted from the caprine liver.

The petitioner provided recovery data for the ^{14}C-imidacloprid equivalents through the clean-up and oxidation steps of Bayer method 00191. In summary, samples were partitioned with hexane and cleaned up through the XAD-4 resin column followed by basification with NaOH and oxidation by $KMnO_4$ to form 6-CNA. The ^{14}C-CNA residues were determined by TLC using silica 60 F254 plates and two different polarity mobile phases. 86% to 91% of the ^{14}C-imidacloprid equivalents were recovered from the extracts of caprine muscle, fat, and milk. From 70% to 76% of the ^{14}C-imidacloprid equivalents were recovered from the caprine liver extract. With a majority of the residue detected in the liver CBTS has some concerns over the significantly lower recoveries of imidacloprid equivalents reported. Results of the TLC analysis showed that > 95% of the residue on the TLC plates was 6-CNA.

CBTS concludes the petitioner has provided acceptable ^{14}C-imidacloprid recovery data from aged ^{14}C-imidacloprid caprine tissues to show the Bayer method 00191 can gather the magnitude of the residue data from poultry and ruminant feeding studies, and to enforce the proposed tolerances.

Response to Petitioner's Letter of February 5, 1993

In a letter dated February 5, 1993, and signed by J.S. Thornton, Miles Agricultural Division responded to CBTS concerns that the recovery data presented did not constitute an ILV for the enforcement method and that the ILV did not validate the method at the level of the proposed tolerances.

CBTS has concerns noted above on the ILV data that were generated in Germany that the petitioner needs to resolve. CBTS reiterates the ILV needs to be conducted on the proposed enforcement method, not the method used to gather the residue data. The method that was used in Germany to gather the residue data can not be used as an enforcement method with its use of controlled matrices and variable recoveries for the guanidine imidacloprid. Since the Agency did not have the ILV data in hand to review we could not determine if it in fact met our requirements. Thus, the petitioner was encouraged to submit his data for review before conducting additional tests.

The petitioner's ILV data have been reviewed above. The petitioner needs to resolve the deficiencies noted. In response to the five points in the petitioner's letter we agree that the petitioner understands the purpose of an ILV. After our review of the methods we feel that the there were 2 significant differences in the German method and the USA method. On review of the March 19, 1992, memorandum by W.D. Wassel, we point out that the Agency comments are still to be considered guidance and are tentative, not formal agreements. We also reiterate item 13 of this memorandum in that the petitioner should include the method quantitation limit and the <u>proposed tolerance</u> as fortification levels. Our review of item 5 in the petitioner's letter indicates that he understands that ILV data are to be generated at the proposed tolerance and at 2-5X the proposed tolerance. With the deficiencies noted, our response to the petitioner's February 5, 1993, letter is that he has not generated adequate ILV data as specified in PR Notice 88-5.

cc:R.F., Circu.,Reviewer(FDG),PP#4F4169,Imidacloprid Sub.File.
H-7509C:CBTS:Reviewer(FDG):CM#2:Rm804Q:305-5826:fdg5/10/93:edit:fdg:6/10/93.
RDI:SecHd:RSQuick:6/11/93:BrSrSci:RALoranger:6/16/93.