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

MAR 13 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Metalaxyl Product Chemistry and Residue Chemistry Reregistration Standard Updates.

FROM: Edward Zager, Chief
Chemistry Branch - Reregistration Support (CBRS)
Health Effects Division (H7509C)

TO: Lois Rossi, Chief
Reregistration Branch
Special Review & Reregistration Division (H7508C)

and

Reto Engler, Ph.D., Chief
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

Attached are updates to the Product and Residue Chemistry Chapters of the Metalaxyl Reregistration Standard prepared by Acurex Corporation under supervision of CBRS. They have undergone secondary review in the Branch and have been revised to reflect Agency policies.

These documents provide an assessment of the status of data submitted in support of reregistration of Metalaxyl as of 01/15/91. Revised data requirement tables are included.

Please note that Confidential Business Information accompanies the Product Chemistry Update as Appendices A, B, C, D and E.

If you need additional input please advise.

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Attachment 1: Metalaxyl Product Chemistry Reregistration Standard Update

Attachment 2: Confidential Appendices A, B, C, D and E to Product Chemistry Reregistration Standard Update.

Attachment 3: Metalaxyl Residue Chemistry Reregistration Standard Update

cc (with attachments 1, 2 & 3): W. Smith, Metalaxyl Registration Standard file, Metalaxyl Subject File, C. Furlow (PIB/FOD), J. Burrell (FOD), Acurex

cc (with attachments 1 & 3): Circ. (7)
cc (without attachments): W. Boodee, RF

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METALAXYL

TASK 3

Reregistration Standard Update. Product Chemistry

January 31, 1991

Contract No. 68-DO-0142

Submitted to:

U.S. Environmental Protection Agency
Arlington, VA 22202

Submitted by:

Acurex Corporation
Environmental Systems Division
4915 Prospectus Drive
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ACUREX Corporation
Environmental Systems Division

METALAXYL

REREGISTRATION STANDARD UPDATE

PRODUCT CHEMISTRY

TASK 3

INTRODUCTION

A Product Search Listing conducted on 12/17/90 identifies a single registered manufacturing-use product of metalaxyl, the 90% technical (T), EPA Reg. No. 100-601, registered by Ciba-Geigy Corporation.

The Metalaxyl Guidance Document dated September, 1988 requires additional generic and product-specific product chemistry data for the technical product. Ciba-Geigy Corp. has submitted data (1989; MRIDs 41055201 and 41055202) in response to the Guidance Document. These data are reviewed below for their adequacy in fulfilling data requirements for the Ciba-Geigy 90% T (EPA Reg. No. 100-601).

Corresponding to each of the Topical Discussions listed below are the Guideline Reference Numbers from "Pesticide Assessment Guidelines - Subdivision D - Product Chemistry", referred to in Title 40 of the Code of Federal Regulations (40 CFR), Part 158, "Data Requirements for Registration", Subpart C, "Product Chemistry Data Requirements". These regulations and guidelines explain the minimum data that the Agency needs to adequately assess the product chemistry of metalaxyl.

	Guidelines Reference No. from 40 CFR §158.155-190
Product Composition and Manufacture	61-(1-3)
Analysis and Certification of Product Ingredients	62-(1-3)
Physical and Chemical Characteristics	63-(2-20)

SUMMARY

The following Product Chemistry data are required for the Ciba-Geigy 90% T (EPA Reg. No. 100-601):

- Data pertaining to product composition, a discussion of formation of impurities, preliminary analysis, certified limits, and enforcement analytical methods.

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PRODUCT IDENTITY AND COMPOSITION

61-1. Product Identity and Disclosure of Ingredients

The Metalaxyl Guidance Document dated September, 1988 does not require additional generic or product-specific data concerning product composition. Ciba-Geigy Corp. has submitted a confidential Statement of Formula for the technical product (MRID 41055201), which is summarized in Confidential Appendix A. The data reviewed in Confidential Appendix A do not fully satisfy requirements of 40 CFR §158.155 (Guideline Reference No. 61-1) regarding product composition for the 90% T because the nominal concentrations of the active ingredient and impurities were not reported.

61-2. Starting Materials and Manufacturing Process

The Metalaxyl Guidance Document dated September, 1988 requires additional generic and product-specific data concerning the starting materials and manufacturing process. Ciba-Geigy Corp. has submitted pertinent information (MRID 41055201), which is summarized in Confidential Appendix B. The data reviewed in Confidential Appendix B do not fully satisfy requirements of 40 CFR §158.160-162 (Guideline Reference No. 61-2) regarding this topic because no information was provided on either the duration of each step of the process or a description of measures taken to ensure the quality of the final product, including procedures involving the equipment used for blending product components and for filling and packaging. A description of the manufacturing process conditions favoring production of nitrosamines is also required.

61-3. Discussion of the Formation of Impurities

The Metalaxyl Guidance Document dated September, 1988 specified generic and product-specific data requirements for metalaxyl regarding detailed discussion of formation of impurities, including nitrosamines. In response to the Guidance Document and for registration of the 90% T (EPA Reg. No. 100-601), Ciba Geigy submitted information (1989; MRID 41055201 and 41055202) which is presented in Confidential Appendix C. Information discussed in Confidential Appendix C regarding formation of impurities in the 90% T does not satisfy the requirements of 40 CFR §158.167 (Guideline Reference No. 61-3) because a discussion was not presented regarding post-production contamination. In addition, the possible formation of nitrosamines from metalaxyl and its impurities must be discussed.

62-1. Preliminary Analysis

The Metalaxyl Guidance Document dated September, 1988 specified that five or more representative samples be analyzed for the amount of active ingredient and each impurity for

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which a certified limit is required. Complete validation data were requested for each analytical method used. In response to the Guidance Document for registration of the 90% T (EPA Reg. No. 100-601), Ciba Geigy submitted preliminary analysis data (1989; MRID 41055202) which is summarized and presented in Confidential Appendix D. These data satisfy the requirements of 40 CFR §158.170 (Guideline Reference No. 62-1) regarding preliminary analysis for the Ciba-Geigy Corp. 90% T (EPA Reg. No. 100-601). No additional data are required.

62-2. Certified Limits

The Metalaxyl Guidance Document dated September, 1988 specified generic and product specific data requirements regarding certification of ingredient limits. In response to the Guidance Document, Ciba Geigy submitted data (1989; MRID 41055201 and 41055202) which are reviewed in Confidential Appendix A. These data do not satisfy the requirements of 40 CFR §158.175 (Guideline Reference No. 62-2) regarding certified limits for the Ciba-Geigy Corp. 90% T (EPA Reg. No. 100-601).

The nominal concentration of 90% T, which appears on the product labels is inappropriate based on the reported lower limit for the active ingredient. The registrant should revise their label claim to be consistent with the nominal concentration reported on EPA form 8570-4 (Rev. 2-85). The presence of nitrosamines in metalaxyl production was subjected to an appropriate analysis scheme. However, the intervals specified in the Guidance Document were not followed or clearly stated by the registrant. Additional information is required: nitrosamines must be identified in six samples of each product (two of each shortly after production and six months after production). Certifications should be submitted on EPA Form 8570-4 (Rev. 2-85).

62-3. Enforcement Analytical Methods

The Metalaxyl Guidance Document specified that analytical methods be provided to determine the active ingredient and each toxicologically significant impurity (including nitrosamines) for which a certified limit is required. Enforcement analytical methods were submitted by Ciba Geigy (1989; MRID 41055202). Ciba Geigy submitted GLC methods employing flame ionization detection for the determination of metalaxyl and certain impurities in the 90% T (EPA Reg. No. 100-601). A general analytical method was used for quantitative evaluations by gas chromatography. Method AW-77/2 for metalaxyl prescribes an OV-101 packed GC column and stearic acid methyl ester as an internal standard. A second method for the specified impurities of metalaxyl was also provided. Metalaxyl percent by weight values were 96.83 ± 0.09 for ten injections of the same 90% T. A linearity check of the response for metalaxyl gave the correlation coefficient r = 0.99995. The coefficient of variations for impurities present at or above the 0.1% level were less than 4%. Chromatograms and chromatographic conditions were submitted for the methods as well as

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confirmatory IR Spectra of metalaxyl were provided. Method IA-28 was employed for the analysis of water by Karl Fischer titration. Nitrosamine content was evaluated by Method ASGSR-89-18, an NO_x analyzer method. Method validation data are presented in Confidential Appendix E.

Method AW-77/2 for detection of metalaxyl in the 90% T (EPA Reg. No. 100-601) satisfies the requirements of 40 CFR §158.180 (Guideline Reference No. 62-3) regarding enforcement analytical methods as does Method AK-77/3 for the impurities in metalaxyl. Method IA-28 for water and Method ASGSR-89-18 for nitrosamine content satisfied the 40 CFR §158.180 requirements as well. Validation data were not submitted for two impurities, each found at levels ≤ 0.1%, where no reference substance was used.

PHYSICAL AND CHEMICAL CHARACTERISTICS

The Metalaxyl Guidance Document dated September 1988 found the existing data adequate to satisfy the requirements for physical and chemical characteristics, 40 CFR §158.190 (Guideline Reference Nos. 63-2 through 63-21).

Product Chemistry Citations (used):

41055201 Lail, L. (1989) Technical Metalaxyl: Product Chemistry: Study No. PC-88-005. Unpublished study prepared by Ciba-Geigy Corp. 51 p.

41055202 Lail, L. (1989) Technical Metalaxyl: Product Chemistry: Study No. PC-88-005. Unpublished study prepared by Ciba Geigy Corp. 120 p.

TABLE A. GENERIC DATA REQUIREMENTS FOR THE METALAXYL TECHNICAL GRADE OF THE ACTIVE INGREDIENT.[1]

Data Requirement		Test Substance	Does EPA have data to satisfy this requirement?	Bibliographic Citation	Must additional data be submitted under FIFRA Sec. 3(c)(2)(B)?
40 CFR §158.155-190 Product Chemistry					
Product Composition					
61-2.	Beginning Materials and Production Process	TGAI	Partially	41055201 41055202	Yes[2]
61-3.	Formation of Impurities	TGAI	Partially	41055201 41055202	Yes[3]
Analysis and Certification of Product Ingredients					
62-1.	Preliminary Analysis of Product Samples	TGAI	Yes	41055201 41055202	No
Physical and Chemical Characteristics[4]					
63-2.	Color	TGAI	Yes		No
63-3.	Physical State	TGAI	Yes		No
63-4.	Odor	TGAI	Yes		No
63-5.	Melting Point	TGAI	Yes		No
63-6.	Boiling Point	TGAI	N/A[5]		No
63-7.	Density, Bulk Density, or Specific Gravity	TGAI	Yes		No
63-8.	Solubility	TGAI or PAI	Yes		No
63-9.	Vapor Pressure	PAI	Yes		No
63-10.	Dissociation Constant	TGAI or PAI	Yes		No

(Continued, footnotes follow)

TABLE A. (Continued).

Data Requirement	Test Substance	Does EPA have data to satisfy this requirement?	Bibliographic Citation	Must additional data be submitted under FIFRA Sec. 3(c)(2)(B)?
63-11. Octanol/Water Partitioning Coefficient	TGAI	Yes		No
63-12. pH	TGAI	Yes		No
63-13. Stability	TGAI	Yes		No
Other Requirements:				
64-1. Submittal of Samples	N/A	N/A		No

1. Data requirements pertain to the Ciba-Geigy 90% T (EPA Reg. No. 100-601). Additional data requirements are listed in the following Table B, "Product Specific Data Requirements for Metalaxyl Manufacturing-Use Products".

2. Complete information must be provided on the duration of each step of the manufacturing process, a description of the measures taken to ensure the quality of the final product, including procedures involving the equipment used for blending product components and for filling and packaging. A description of the manufacturing process conditions favoring production of nitrosamines must be provided..

3. A discussion must be presented regarding post-production contamination and must also address the possible formation of nitrosamines from metalaxyl and its impurities.

4. There are additional data requirements listed in Table B pertaining to physicochemical characteristics of those technical products which are also manufacturing use products.

5. Not required; a solid at room temperature.

TABLE B. PRODUCT SPECIFIC DATA REQUIREMENTS FOR THE METALAXYL MANUFACTURING-USE PRODUCTS.[1]

Data Requirement		Test Substance	Does EPA have data to satisfy this requirement?	Bibliographic Citation	Must additional data be submitted under FIFRA Sec. 3(c)(2)(B)?
40 CFR §158.155-190 Product Chemistry					
Product Composition					
61-1.	Product Identity and Disclosure of Ingredients	MP	Partially	41055201 41055202	Yes[2]
61-2.	Beginning Materials and Production Process	MP	Partially	41055201 41055202	Yes[3]
61-3.	Formation of Impurities	MP	Partially	41055201 41055202	Yes[4]
Analysis and Certification of Product Ingredients					
62-1.	Preliminary Analysis of Product Samples	MP	Yes	41055201 41055202	No
62-2.	Certification of Ingredient Limits	MP	Partially	41055201 41055202	Yes[5]
62-3.	Analytical Methods to Verify Certified Limits	MP	Yes	41055201 41055202	No
Physical and Chemical Characteristics					
63-2.	Color	MP	Yes		No
63-3.	Physical State	MP	Yes		No
63-4.	Odor	MP	Yes		No
63-5.	Melting Point	MP	Yes		No

(Continued, footnotes follow)

TABLE B. (Continued).

Data Requirement		Test Substance	Does EPA have data to satisfy this requirement?	Bibliographic Citation	Must additional data be submitted under FIFRA Sec. 3(c)(2)(B)?
63-6.	Boiling Point	MP	N/A[6]		No
63-7.	Density, Bulk Density, or Specific Gravity	MP	Yes		No
63-8.	Solubility	MP	Yes		No
63-9.	Vapor Pressure	MP	Yes		No
63-10.	Dissociation Constant	MP	Yes		No
63-11.	Octanol/Water Partitioning Coefficient	MP	Yes		No
63-12.	pH	MP	Yes		No
63-13.	Stability	MP	Yes		No
Other Requirements:					
64-1.	Submittal of Samples	N/A	N/A		No

(Continued, footnotes follow)

TABLE B. (Continued).

1. Data requirements pertain to the Metalaxyl 90% T (EPA Reg. No. 100-601). Additional data requirements are listed in the preceding Table A, "Generic Data Requirements for the Metalaxyl Technical Grade of the Active Ingredient".

2. Nominal Concentration and upper certified limit of the active ingredient must be provided. Certifications should be provided on EPA Form 8570 (Rev. 2-85).

3. Complete information must be provided on the duration of each step of the manufacturing process, a description of the measures taken to ensure the quality of the final product, including procedures involving the equipment used for blending product components and for filling and packaging. A description of the manufacturing process conditions favoring production of nitrosamines must be provided..

4. A discussion must be presented regarding post-production contamination and must also address the possible formation of nitrosamines from metalaxyl and its impurities.

5. A complete discussion must be provided regarding the possibility of degradation of the ingredients, altering the declared strength of the product for its stated life. All nitrosamines must be identified in six samples of each product; two samples of each analyzed shortly after production and six months after production. Certifications should be submitted on EPA Form 8570 (Rev. 2-85).

6. Not required; a solid at room temperature.

METALAXYL

REGISTRATION STANDARD UPDATE

PRODUCT CHEMISTRY

TASK 3

(Final Report)

CONFIDENTIAL APPENDICES

Appendix A:2 Page(s)
Appendix B:1 Page(s)
Appendix C:2 Page(s)
Appendix D:2 Page(s)
Appendix E:3 Page(s)

Confidential Appendices to the Scientific Review of the Registration Standard Update Report for the pesticide metalaxyl by the Dietary Exposure Branch [Confidential FIFRA Trade Secret/CBI].

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METALAXYL PRODUCT CHEMISTRY REVIEW

Page _____ is not included in this copy.

Pages ~~16~~ 14 through ~~25~~ 23 are not included in this copy.

The material not included contains the following type of information:

_✓_ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_✓_ Description of the product manufacturing process.

_✓_ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

_____ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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METALAXYL

TASK 3

Reregistration Standard Update. Residue Chemistry

January 31, 1991

Contract No. 68-DO-0142

Submitted to:

U.S. Environmental Protection Agency
Arlington, VA 22202

Submitted by:

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METALAXYL

REREGISTRATION STANDARD UPDATE

RESIDUE CHEMISTRY

Task - 3

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TABLE A. GENERIC RESIDUE CHEMISTRY DATA REQUIREMENTS FOR METALAXYL 39

METALAXYL

REREGISTRATION STANDARD UPDATE

RESIDUE CHEMISTRY

Task - 3

INTRODUCTION

Metalaxyl is a fungicide registered for use on alfalfa, almonds, apples, avocados, barley, beets, bentgrass, blackeyed peas, buckwheat, brassica leafy vegetables, citrus fruits, clover, corn, cotton, cowpeas, cucurbit vegetables, dill, fescue, forage grasses, hops, legume vegetables, lettuce, millet, oats, onions, okra, peanuts, peas, popcorn, pineapples, potatoes, raspberries, rice, sorghum, spinach, stone fruits, sugar beets, tobacco, trefoil, tomatoes, walnuts, and wheat.

The Metalaxyl Final Reregistration Standard and Tolerance Reassessment (FRSTR) Guidance Document dated 9/88 required data on animal metabolism, storage stability, residues in or on corn and peanut commodities, and processing studies on potatoes, sugar beets, corn, rice, sorghum, cottonseed, pineapples, and sunflower seeds. In addition, it was specified that a tolerance is needed for residues in or on soybean forage and hay and that a feed additive tolerance was required for dried hops.

In response to these requirements, Ciba-Geigy Corporation has submitted data concerning the nature of the residue in goats (1990; MRID 41664503) and hens (1990; MRID 41664504) and data from field trials with corn (1990; MRIDs 41689701 and 41689702). In addition, data submitted with a tolerance petition in 1986 are available pertaining to residues in the processed commodities of sugar beets. Data submitted on sample storage intervals (1990; MRID 41449001) have been reviewed by the Agency (R. Perfetti, DEB No. 7193). Data pertaining to the recovery of residues using FDA multiresidue protocols (1987 MRID 40503101 and 1989 MRID 41055203) have been forwarded to FDA for evaluation and inclusion in PAM Vol. I.

Metalaxyl was the subject of a Reregistration Standard and Guidance Document issued 12/81 and a Final Registration Standard and Tolerance Reassessment (FRSTR), Residue Chemistry Chapter dated 6/22/87 and Guidance Document dated 9/88. Since the issuance of the 1988 Guidance Document, tolerances have been established for residues in or on almonds, almond hulls, stone fruits, and strawberries, and a food additive tolerance has been established for residues of metalaxyl and metabolites in dried hops.

SUMMARY

The following data requirements remain outstanding:

- o Additional information is needed from the goat and hen metabolism studies.
- o An explanation of high control values obtained from analysis of corn forage and fodder using method AG-395 is required.
- o Additional data regarding storage stability and sample storage intervals is required.
- o The Guidance Document requirements for data on peanuts and the processed commodities of potatoes, cereal grains, cottonseed, and sunflower seed have not been addressed and remain outstanding.

QUALITATIVE NATURE OF THE RESIDUE IN PLANTS

Conclusions:

The qualitative nature of the residue in plants is adequately understood. The data reviewed for the Metalaxyl FRSTR and Guidance Document of 9/88 indicate that metalaxyl and its metabolites containing the 2,6-dimethylaniline moiety and N-(2-hydroxy methyl-6-methyl)-N-(methoxyacetyl)-alanine methyl ester are the residues of concern. No additional data are required.

Discussion of the data:

N/A.

QUALITATIVE NATURE OF THE RESIDUE IN ANIMALS

Conclusions:

The qualitative nature of the residue in animals is not adequately understood. The Metalaxyl Guidance Document dated 9/88 specified the need for metabolism studies on ruminants and poultry. In response to these requirements, Ciba-Geigy Corp. submitted data pertaining to the metabolism of metalaxyl in goats and poultry. Additional data is required from these studies. The deficiencies in the submitted data and the additional information required are summarized in the paragraphs that follow.

The data on lactating goats submitted by Ciba-Geigy Corporation (1990; MRID 41664503) are not adequate to delineate metabolism of metalaxyl in ruminants because the residues in milk and tissues were inadequately characterized. In milk, 40% (0.036 ppm) of the total radioactive residue is found as an unidentified metabolite "A". The registrant states that under mild base conditions the unidentified metabolite may be hydrolyzed to a known metabolite (CGA-107955). Additional data must be presented regarding the identity of this major component of the milk residue.

The following additional data are required:

- The registrant must submit additional information from the study described in MRID 41664503. Specifically, metabolite "A" isolated from milk of goat 2 needs to be characterized further.

The major portion of radioactivity analyzed in liver and kidney was found in metabolites CGA-107955 (13.5% and 31.5%, respectively), CGA-94689-isomer B (4.5% and 22.5%, respectively), and CGA-94689-isomer A (3.5% and 11.7%, respectively). The major portion of analyzed radioactivity in muscle was in metabolites CGA-107955 (18.4% and 17.4, respectively), CGA-62826 (10.9% and 9.1%, respectively), and CGA-67869 ((8.7% and 6.4%, respectively). The three major metabolites found in perirenal fat (goat 1) included two identified metabolites CGA-107955 (29.6%) and CGA-67869 (13.3%), and an unidentified metabolite "J" (11.6%). The three major metabolites found in perirenal fat (goat 2), extracted using an alternative scheme, were CGA-107955 (11.4%), CGA-94689 (5.8%) and CGA-67869 (4.8%). Milk samples from goat 1 and 2 were also extracted by two different extraction schemes. Similarly to perirenal fat (goat 1), milk samples from goat 1 contained the major portion of analyzed radioactivity as metabolites, CGA-107955 (7.3%) and CGA-67869 (4.8%), and the unidentified metabolite "J" (5.6%). Milk samples from goat 2 contained the greater portion of the analyzed radioactivity as metabolite "A" (40%), which is unidentified, and metabolites CGA-107955 (4.6%) and CGA-67869 (4.3%).

Metalaxyl in goats may be hydrolyzed to the ester alcohol and the acid alcohol which may then be N-dealkylated. Alternatively, oxidation can lead to either benzylic alcohol or phenolic compounds.

The data submitted regarding the metabolism of metalaxyl in hens (1990; MRIDs 41664504 and 41664506) are inadequate to fulfill data requirements for this topic because only 20% of the residue in liver, 30% in egg yolk, 9.3% in egg white, 2.4% in muscle, and none in skin/fat were identified. Components from protease digestion accounting for 32.9% of the total residue in egg whites were not resolved chromatographically and need further analysis. In addition, 45.7% of the egg white residue that was aqueous soluble should be identified. In egg yolk, radioactive components in the aqueous fraction (17.2%), the hexane fraction (21.8%), and insoluble residues (14.3% of the total) need to be characterized. Aqueous-soluble residues extracted after enzyme hydrolysis of whole liver should be characterized

along with the organosoluble residues (before hydrolysis) accounting for 17% of the total residue and the unextractable residues (ca. 20%). Unresolved residues in the organic fraction of muscle accounting for up to 60% of the residue and inidentified aqueous residues accounting for up to 34% need to be identified further. The following additional data are required:

- The registrant must submit additional information from the poultry study described in MRID 41664504. The unextracted solid residue from the liver should be subjected to acid hydrolysis. The final aqueous fraction collected after collagenase treatment of liver requires further separation and identification of the radioactive components. Additional chromatographic techniques should be employed to resolve CGA-79353 and to identify the co-eluting unknown in extracts from liver, breast, and thigh muscles. The unknowns in the skin and attached fat, the breast, and thigh muscles should be characterized further. The unresolved metabolites in the breast muscle should be resolved and quantified.

The metabolism of metalaxyl, in the hen, seems to follow two major pathways. In the first pathway, sequential demethylation of the ether and the ester groups gives first the alcohol CGA-67869 and then the hydroxy acid CGA-107955. Oxidation of the alcohol moiety of CGA-107955 results in the formation of the diacid CGA-78532, which in turn cleaves to give CGA-68124. Alternatively, oxidation of the benzylic carbon of metalaxyl produces the benzylic alcohol isomers CGA-94689 (two isomers). The benzoic acid CGA-108905 also forms. The benzoic acid undergoes demethylation to give the diacid CGA-108906. The registrant also suggested a minor pathway which consists of the hydroxylation of the phenyl ring resulting in the formation of CGA-100255; GC/MS indicated the presence of this metabolites in excreta. In the second pathway, CGA-107955 undergoes lipophilic, amino acid, and glucuronic acid conjugation. The latter two routes of conjugation also occur with CGA-78532, CGA-68124, CGA-10896, and CGA-94689. In addition CGA-94689 also forms lipoprotein conjugates in the egg yolk.

The Metalaxyl Guidance Document dated 9/88 specifically requests that representative samples from this test be analyzed by current enforcement methods to ascertain the validity of the method. No data were provided to meet this requirement; therefore, this test remains outstanding.

The molecular structures and chemical names of metalaxyl and known and suspected metabolites are given in Table 1.

References (used):

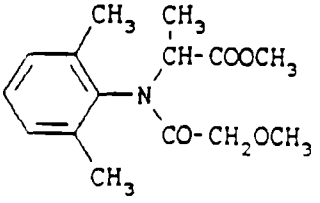
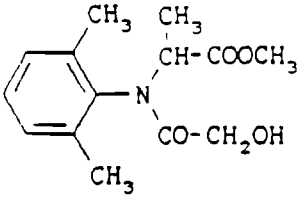
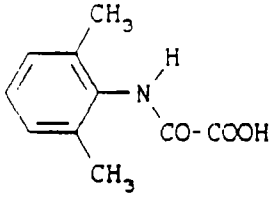
MRIDs: 41664503. 41664504. 41664506.

Discussion of the data:

Goats. Two lactating goats (Alpines) were dosed orally with uniformly [U- ^{14}C]phenyl labeled metalaxyl at a dose rate of 150 mg/day for 4 consecutive days (1990; MRID 41665403). This dose represents a feeding level of 76.9 ppm which is 4x the maximum theoretical dietary exposure based on tolerances for peanut vines and hay. The specific activity of the test substance was 37.7 μCi/mol with a radiochemical purity of >97%. During the dosing period, urine and feces were collected daily. Milk samples were taken once in the morning and once in the afternoon. The afternoon and the following morning milk from each goat were combined as a daily milk sample. Approximately 6 hours after the last dose, the test animals were weighed, sacrificed, and blood and tissue samples were collected. Approximately 76% of the administered radioactivity was eliminated in urine (66.6%) and feces (9.3%). The tissues contained approximately 1% of the total dose. The residues in milk were highest at the fourth day.

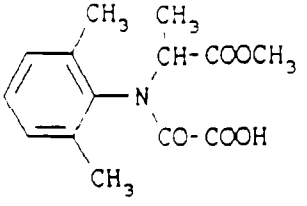
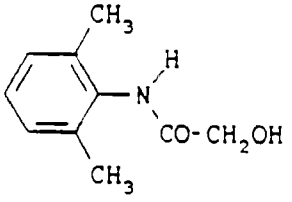
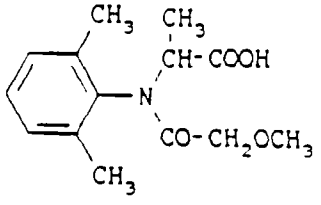
Samples were shipped from the Ciba-Geigy Vero Beach, Florida facility, where the biological phase of the studies was done, to the registrant's Greensboro, NC facility, where the analytical phase was performed. Whole blood was collected in heparin treated tubes and stored/shipped refrigerated. Tissue samples were taken and stored and shipped frozen. Intervals and conditions of sample storage once samples arrived at the Greensboro facility were not specified.

Table 1. Chemical names and molecular structures of metalaxyl and known and putative metabolites identified in animal tissues and used as standards in metabolism studies.

Company Code Chemical Name	Structure	Substrate	MRID
CGA-48988 (metalaxyl) N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester		**Poultry:** Liver Egg White Egg Yolk Breast Muscle Excreta Gizzard	 41664504 41664504 41664504 41664504 41664504 41664504
CGA-67869 N-(2,6-dimethylphenyl)-N-(hydroxyacetyl)-alanine methyl ester		**Ruminant:** Urine Tenderloin Milk Liver Kidney Leg Muscle Perirenal Fat **Poultry:** Gizzard Excreta Kidney Liver	 41664503 41664503 41664503 41664503 41664503 41664503 41664503 41664504 41664504 41664504 41664504
CGA-68124 [2,6-dimethylphenyl)-amino] oxoacetic acid		**Poultry:** Excreta[a] Heart[a] Gizzard[a] Liver[a] Breast Muscle[a] Thigh Muscle[a] Skin/Attached Fat[a] Egg White[a] Peritoneal fat[a] Kidney[a]	 41664504 41664504 41664504 41664504 41664504 41664504 41664504 41664504 41664504 41664504

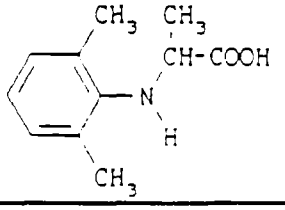
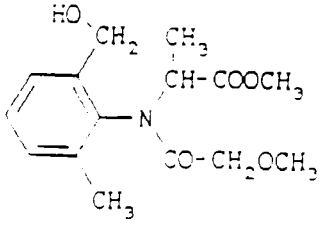
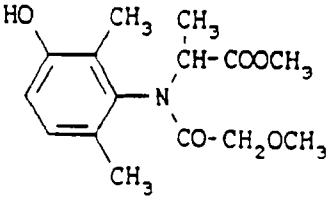
[a]Unresolved residues considered putative.
(continued)

Table 1. Metalaxyl and metabolites (continued)

Company Code Chemical Name	Structure	Substrate	MRID
CGA-79353 N-(carboxy-carbonyl)-N-(2,6-dimethylphenyl) alanine methyl ester		**Poultry:**	
		Excreta[a]	41664504
		Heart[a]	41664504
		Gizzard[a]	41664504
		Liver[a]	41664504
		Breast Muscle[a]	41664504
		Thigh Muscle[a]	41664504
		Skin/Attached fat[a]	41664504
		Egg White[a]	41664504
		Peritoneal fat[a]	41664504
		Kidney[a]	41664504
CGA-37734 N-(2,6-di-methylphenyl)-2-hydroxyacetamide		**Ruminant:**	
		Tenderloin[a]	41664503
		Milk[a]	41664503
		Liver[a]	41664503
		Urine[a]	41664503
		Kidney	41664503
		Leg Muscle	41664503
		Perirenal Fat	41664503
CGA-62826 N-(2,6-dimethyl-phenyl)-N-(meth-oxyacetyl)-alanine		**Ruminant:**	
		Tenderloin	41664503
		Milk	41664503
		Liver	41664503
		Urine	41664503
		Kidney	41664503
		Leg Muscle	41664503
		Perirenal Fat	41664503

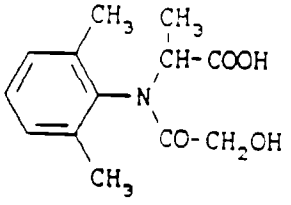
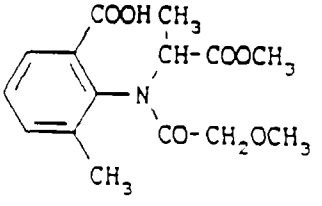
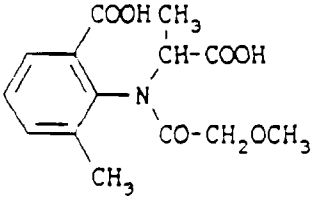
[a]Unresolved residues considered putative.
(continued)

Table 1. Metalaxyl and metabolites (continued)

Company Code Chemical Name	Structure	Substrate	MRID
CGA-67867 N-(2,6-di-methylphenyl) alanine		**Ruminant:** Urine	 41664503
CGA-94689 N-[(2-hydroxy-methyl)-6-meth-ylphenyl]-N-(meth-oxy acetyl)-alanine methyl ester		**Ruminant:** Milk Liver Kidney Leg Muscle Perirenal Fat Urine Tenderloin **Poultry:** Liver Egg White Egg Yolk Breast Muscle Thigh Muscle Excreta Gizzard Kidney Heart	 41664503 41664503 41664503 41664503 41664503 41664503 41664503 41664504 41664504 41664504 41664504 41664504 41664504 41664504 41664504 41664504
CGA-100255 N-(3-hydroxy-2,6-dimethylphenyl)-N-(methoxyacetyl) alanine methyl ester		**Ruminant:** Milk[a] Liver[a] Tenderloin[a] Urine[a] Kidney Leg Muscle Perirenal Fat	 41664503 41664503 41664503 41664503 41664503 41664503 41664503

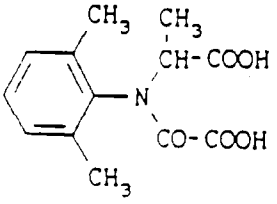
[a]Unresolved residues considered putative.
(continued)

Table 1. Metalaxyl and metabolites (concluded)

Company Code Chemical Name	Structure	Substrate	MRID
CGA-107955 N-(2,6-dimethylphenyl)-N-(hydroxyacetyl)-alanine		**Ruminant:** Milk Liver Urine Tenderloin Kidney Leg Muscle Perirenal Fat **Poultry:** Excreta Kidney Peritoneal Fat Liver Gizzard Heart	 41664503 41664503 41664503 41664503 41664503 41664503 41664503 41664504 41664504 41664504 41664504 41664504 41664504
CGA-108905 2-[(methoxyacetyl)(2-methoxy-1-methyl-2-oxoethyl) amino]-3-methylbenzoic acid		**Poultry:** Excreta Gizzard Kidney	 41664504 41664504 41664504
CGA-108906 2-[1-carboxyethyl) (methoxy acetyl) amino]-3-methyl benzoic acid		**Poultry:** Excreta[a] Heart[a] Gizzard[a] Liver[a] Breast Muscle[a] Thigh Muscle[a] Skin/Attached fat[a] Egg White[a] Peritoneal fat[a] Kidney[a]	 41664504 41664504 41664504 41664504 41664504 41664504 41664504 41664504 41664504 41664504

[a]Unresolved residues considered putative.
(continued)

Table 1. Metalaxyl and metabolites (concluded)

Company Code Chemical Name	Structure	Substrate MRID
CGA-78532 N-(carboxy-carbonyl)-N-(2,6-dimethyl-phenyl) alanine		**Poultry:** Excreta[a] 41664504 Heart[a] 41664504 Gizzard[a] 41664504 Liver[a] 41664504 Breast Muscle[a] 41664504 Thigh Muscle[a] 41664504 Skin/Attached Fat[a] 41664504 Egg White[a] 41664504 Peritoneal fat[a] 41664504 Kidney[a] 41664504

[a]Unresolved residues considered putative.

Total radioactive residues (TRR)

Aliquots of urine and milk, as well as soluble fractions from tissues, were radioassayed directly by liquid scintillation spectrometry (LSS). Blood, feces, homogenized tissues and subsamples of extracted materials were combusted and radioassayed by liquid scintillation spectrometry (LSS). The validated limit of detection for the radioassay was not reported. Recovery of residues in extracts ranged from 90% to 100% for all samples except for tenderloin (71%). The TRR expressed as ppm (µg metalaxyl equivalents/g) in milk and tissue samples are shown in Table 2.

Table 2. Total radioactive residue (TRR) found in milk and tissue of lactating goats administered [^{14}C] metalaxyl

Matrix	TRR (ppm)[a]	
	Goat 1	Goat 2
Milk	0.043[b]	0.089[c]
Liver	1.915	1.369
Kidney	2.296	1.062
Tenderloin	0.122	0.065
Leg Muscle	0.138	0.074
Peritoneal Fat	0.400	0.102

[a] ppm in metalaxyl equivalents
[b] Day 3
[c] Day 2

Extraction of residues

Seventy-four percent of the radioactive material in milk and 88% to 97% of the radioactive material in muscle, kidney and fat was solubilized with the initial organic solvent extraction.

A sample of milk (goat 2, day 2) was extracted, under unspecified conditions, with acetonitrile (ACN) to yield a precipitate and an ACN supernatant. The precipitate was

washed with ACN, treated with protease and then extracted with ethyl acetate (EtOAc) resulting in EtOAc and aqueous fractions and a non-extracted fraction. The EtOAc fraction was analyzed by thin-layer chromatography (TLC). The ACN supernatant was evaporated below 40 C to remove the ACN and then incubated with glucuronidase. After incubation, the sample was extracted with dichloromethane (DCM) followed by EtOAc. The resulting DCM and EtOAc fractions were analyzed by TLC. The aqueous fraction was treated with protease and extracted with EtOAc, but not analyzed further.

A milk sample from goat 1 (day 3) was extracted using a second scheme. The sample was separated by centrifugation into three fractions, fat, casein, and whey. The whey fraction was lyophilized and dissolved in methanol:acetonitrile (1:1, v/v) resulting in a whey supernatant and a precipitate. After protease treatment, the precipitate was extracted with EtOAc. The resulting EtOAc, aqueous, and precipitate fractions were not analyzed further. The whey supernatant was concentrated and incubated with glucuronidase and then partitioned with DCM followed by EtOAc. The resulting organic fractions were analyzed by TLC.

Liver, kidney, perirenal fat, and leg muscle from goat 1 and liver, kidney, leg muscle and tenderloin from goat 2 were extracted three to five times with methanol:water (80:20, v/v). Except for liver, the resulting insoluble residues were not further analyzed. The resulting supernatants were concentrated and then incubated with glucuronidase. After incubation, the samples were extracted sequentially with DCM and EtOAc and the organic fractions analyzed with TLC. The unextracted residues from liver were treated with protease and then extracted with EtOAc, resulting in aqueous and organic fractions which were analyzed by TLC. Perirenal fat (goat 2) was extracted with acetonitrile:hexane (4:1, v/v) resulting in an ACN fraction, a hexane fraction and a precipitate. The ACN fraction was concentrated, incubated with glucuronidase and then extracted with DCM and EtOAc. The resulting organic fractions were analyzed by TLC.

Levels of radioactivity in liquid fractions were assayed by LSS and radioactivity in solids was assayed by combustion of the samples after air-drying. The distribution of ^{14}C-activity in milk and tissue fractions is summarized in Tables 3 and 4.

Table 3. Distribution of radioactivity in extracts of milk from lactating goat (goat 2) administered [^{14}C] metalaxyl in diet.

Fraction	%[a]	ppm[b]
Acetonitrile Supernatant (74.4%)[c]		
ethyl acetate	10.3	0.009
dichloromethane	37.8	0.034
aqueous[d]		
ethyl acetate	3.7	0.020
aqueous	22.7	0.003
Precipitate (25.6%)[e]		
acetonitrile wash	3.6	0.003
ethyl acetate	17.7	0.016
aqueous	1.8	0.002
non-extracted	2.5	0.002
Total	100.1	0.089

a All percent values assigned to the fractions are based upon the total radioactive residue in the milk and are normalized to 100%

b ppm in metalaxyl equivalents

c Hydrolyzed with glucuronidase prior to extraction with dichloromethane followed by ethyl acetate

d Hydrolyzed with protease and extracted with ethyl acetate

e Washed with acetonitrile, treated with protease and extracted with ethyl acetate

Table 4. Distribution of radioactivity in extracts of tissue from lactating goats (goat 2) administered [^{14}C] metalaxyl in the diet

Fraction	Liver		Kidney		Muscle[b]		Fat[c]	
	%[a]	ppm	%	ppm	%	ppm	%	ppm
MeOH/water extract[d]	(25.4)	(0.347)	(93.2)	(0.990)	(88.5)	(0.065)	(96.8)	(0.387)
ethyl acetate	2.8	0.038	24.3	0.258	41.8	0.031	30.6	0.122
dichloromethane	16.8	0.230	54.6	0.580	26.3	0.019	51.5	0.206
aqueous	5.8	0.079	14.3	0.152	20.4	0.015	14.7	0.059
Nonextracted[e]	(74.6)	(1.02)	6.8	0.072	11.5	0.009	3.2	0.013
ethyl acetate	14.4	0.197						
non-extracted	6.1	0.084						
aqueous	54.1	0.741						
Total	100.0	1.369	100.0	1.062	100.0	0.074	100.0	0.40

[a] All percent values assigned to the tissue fractions are based upon the total radioactive residue in the tissue and are normalized to 100%.
[b] Data for leg muscle presented. Results for tenderloin are similar
[c] Data obtained from goat 1.
[d] Hydrolyzed with glucuronidase prior to extraction with dichloromethane followed by ethyl acetate.
[e] Hydrolyzed with protease prior to extraction with ethyl acetate.

Hydrolysis of residues

Native goat urine and aqueous soluble residues of tissues and milk extracts were treated with ß-glucuronidase at Ph 4.6 (sodium acetate buffer) for 12-24 hours at 37 °C in a shaking water bath.

Nonextractable residues from liver and milk were treated overnight with protease at Ph 7.0 (Tris-HCl buffer) at 37 °C in a shaking water bath.

The percentages of the total radioactive residues (TRR) accounted for by radioactivity solubilized by enzyme hydrolysis are presented in Tables 3 and 4.

Characterization of residues

The organosoluble fractions from urine, tissues and milk were co-chromatographed with standards on normal phase two-dimensional (2D)-TLC using silica gel plates. For re-chromatography, the radioactive areas were scraped from the plates and eluted from the silica gel with methanol. The solvent systems were chloroform:methanol:formic acid:water (83:13:1:1, v/v/v/v) followed by ethyl acetate:ethanol:acetic acid (90:9.5:0.5, v/v/v). The radioactive components in urine and milk which co-chromatographed with the standards on 2D-TLC were then further co-chromatographed with the corresponding standard on one-dimensional (1D)-reverse-phase TLC using pre-coated C-18 F-254 (Analtech) plates. The solvent system used to analyze urine was sodium acetate (0.01M, pH 4.7):methanol:acetonitrile (4:1:1, v/v/v). For the analysis of milk, sodium acetate (0.01M, pH 4.7):methanol:acetonitrile (2:1:1, v/v/v) was employed. The protease-released aqueous soluble fraction from liver was analyzed by 2D-TLC. The first dimension was developed with a solvent mixture consisting of n-butanol:acetic acid:water (4:1:1, v/v/v) followed with dimethoxyethane:dichoromethane:methanol (1:1:1, v/v/v). Radioactive compounds were detected by autoradiography and a TLC scanner. The compounds were identified by reference to migration distance of standards and quantified by direct integration from the scanner or LSS of the spots recovered from the plates. The chemical names, codes, and molecular structures of the standards used are depicted in Table 1.

The results of residue analyses are summarized in Tables 5 and 6. The molecular structures of metalaxyl and its identified and putative metabolites are illustrated in Table 1.

Metalaxyl, the parent compound, was not detected in any of the commodities. In milk samples from goat 1, 15.9% of the radioactivity was identified. Of the TRR in milk samples from goat 2, 24.1% of the radioactivity was identified including combined, unresolved E and F. The unidentified metabolite "A" accounted for 40% (0.036 ppm) of the TRR in milk from goat 2.

Of the TRR in liver (goat 2), 30% was accounted for by identified metabolites, including combined, unresolved E and F. The aqueous fraction, from the protease digestion of initially insoluble residues, accounted for the major portion of the residues (54.1%) and may contain polar conjugates although none was identified.

The total percent of radioactive residues accounted for by identified metabolites in kidney (goat 2) was 73.2%. The aqueous fraction (14.3%, 0.152 ppm) was not characterized.

In leg muscle and tenderloin, 63.8% and 59.4%, respectively, of the radioactivity was accounted for by identified metabolites. In the leg muscle, 20.4% (0.015 ppm) and in the tenderloin, 25.4% (0.017 ppm) of the radioactivity was accounted for in the aqueous fractions but not characterized.

In perirenal fat, 70.6% of the radioactivity in samples from goat 1 and 28.6% of the radioactivity in samples from goat 2 was accounted for by identified metabolites. In goat 1, 11.6% (0.046 ppm) of the radioactivity was accounted for as the unidentified metabolite "J".

Mass spectral analyses were performed on purified subsamples of urine. The structures of metabolites CGA-67869 and CGA-107955 were confirmed.

In summary, the metabolism of metalaxyl in goats has only been partially characterized. In milk (goat 2), 40% (0.036 ppm) of the total radioactive residue was found as the unidentified metabolite "A". The registrant states that under mild base conditions the unidentified metabolite may be hydrolyzed to a known metabolite (CGA-107955), although no data were provided to support this claim. Additional data are required.

Table 5. Quantitative distribution of metabolites in milk, kidney and liver of goat 2 after oral administration of [^{14}C]metalaxyl.

Metabolite Fraction	Metabolite ID	Milk		Liver		Kidney	
		%	ppm	%	ppm	%	ppm
A	Unknown	40	0.36	--		--	
B	CGA-67869	4.3	0.004	1.8	0.025	3.9	0.036
C	CGA-94689 (Isomer A)	2.3	0.002	3.5	0.048	11.7	0.124
D	CGA-94689 (Isomer B)	3.8	0.003	4.5	0.062	22.5	0.239
E	CGA-100255	8.7[a]	0.008[a]	5.1[a]	0.070[a]	2.7	0.029
F	CGA-37734	--		--		0.7	0.007
G	CGA-62826	0.4	<0.001	1.6	0.022	0.7	0.007
H	CGA-107955	4.6	0.004	13.5	0.185	31.5	0.335
I	Unknown	0.4	<0.001	0.2	0.003	1.0	0.011
J	Unknown	1.9[b]	0.002	3.7[b]	0.051[b]	4.1	0.044
K	Unknown	--		--		1.0	0.011
Total Analyzed		66.4	0.059	33.9	0.464	79.3	0.842
Identified		24.1	0.021	30.0	0.411	73.7	0.777
Total Residue			0.089		1.369		1.062

[a] Includes both E and F
[b] Includes both J and K

Table 6. Quantitative distribution of metabolites in leg muscle and peritoneal fat from goats administered [^{14}C]betalaxyl

Metabolite Fraction	Metabolite ID	Perineal Fat (Goat 1) %	ppm	Leg Muscle (Goat 2) %	ppm
B	CGA-67869	8.7	0.006	13.3	0.053
C	CGA-94689 (Isomer A)	3.9	0.003	6.2	0.025
D	CGA-94689 (Isomer B)	8.2	0.006	8.2	0.033
E	CGA-100255	5.4	0.004	3.4	0.014
F	CGA-37734	8.3	0.006	6.8	0.027
G	CGA-62826	10.9	0.008	3.1	0.012
H	CGA-107955	18.4	0.014	29.6	0.118
I	Unknown	n.d[a]		n.d	
J	Unknown	11.6	0.046	n.d	
M	Unknown	3.8	0.003	--	
Total Analyzed		67.6	0.050	82.2	0.328
Identified		63.8	0.047	70.6	0.282
Total Residue			0.074		0.400

[a] Not determined

Poultry. Ciba-Geigy (MRIDs 41664504 and 41664506) submitted data pertaining to the metabolism of metalaxyl in poultry. Five White leghorn laying hens were dosed daily for 4 days with uniformly ring-radiolabeled metalaxyl (specific activity 37.7 uCi/mg, radiochemical purity of >97%). Gelatin capsules containing 10 mg of [^{14}C]metalaxyl were administered orally to the test animals daily at dose rates equivalent to 100 ppm in the feed, representing 170x the maximum theoretical dietary exposure (based on tolerance for soybean and wheat grains). Excreta and eggs were collected each morning. Excreta samples were stored frozen. The eggs were separated into white and yolk and stored refrigerated. Approximately 6 hours after the last dose, the test animals were weighed, sacrificed, and blood and tissue samples were collected. Whole blood was collected in heparin treated tubes and stored/shipped refrigerated. The tissue samples were stored and shipped frozen. Excreta was shipped frozen, and egg whites and yolks were shipped refrigerated. No data were provided for the storage interval after arrival but prior to analysis.

Total radioactive residues (TRR)

Excreta samples and subsamples of tissues were homogenized with dry ice in a Wiley Mill. Blood, homogenized tissues, excreta, egg whites, egg yolks and subsamples of extracted materials were combusted with a Harvey Oxidizer. The collected $^{14}CO_2$ was measured by liquid scintillation counting using Oxosol C-14 cocktail. The limit of detection of the radioassay was not reported. The total radioactive residue for each regulated commodity is presented in Table 7. The excreta from the hens accounted for approximately 92% of the administered radioactivity.

Table 7. Distribution of radioactive residues in fractions of eggs and tissues from hens dosed with [^{14}C]metalaxyl at 100 ppm.

PPM Equivalents (% TRR)

Commodity (Fraction)	Total	Organo-soluble	Water-soluble	Post-extraction solid
Breast Muscle (Hexane) (dichloromethane) (ethyl acetate)	0.554	Total (61.1) 0.005 (0.9) 0.020 (3.6) 0.313 (56.5)	0.190 (34.2)	0.025 (4.5)
Thigh Muscle (Hexane) (dichloromethane) (ethyl acetate)	0.674	Total (56.5) 0.005 (0.7) 0.014 (2.1) 0.362 (53.7)	0.249 (36.9)	0.044 (6.5)
Liver[a] (dichloromethane) (ethyl acetate)	1.391	Total (38.2) 0.117 (8.4) 0.415 (29.8)	0.586 (42.1)	0.274 (19.7)
Skin/Fat (Hexane) (Acetonitrile)	0.318	Total (67.7) 0.012 (3.8) 0.203 (63.9)	0.062 (19.6)[b]	0.040 (12.6)
Egg Yolk (Hexane) (dichloromethane) (ethyl acetate)	0.206	Total (68.5) 0.045 (21.8)[a] 0.080 (39.0)[a] 0.016 (7.7)[a]	0.035 (17.2)[a,b]	0.029 (14.3)
Egg White (ethyl acetate)	0.179	Total (42.2) 0.076 (42.2)[a]	0.082 (45.7)[a]	0.022 (12.1)

[a] Total or portion obtained after enzyme or base hydrolysis
[b] Water fraction combined with methanol/water fraction or methanol fraction only

Extraction of Residues

Subsamples of excreta were homogenized with methanol:water (80:20, v/v). The mixture was centrifuged to separate the solid and extracts. The extracts were concentrated, dissolved in water and partitioned with DCM. The aqueous phase was acidified and partitioned with EtOAc. The organic and aqueous phases were analyzed by two-dimensional thin-layer chromatography (2D-TLC). The aqueous phase was also analyzed by reverse-phase (RP)-TLC.

Egg whites collected on day 4 were combined and lyophilized to dryness. The dried sample was homogenized with methanol and centrifuged to separate the solids from the extracts. The methanol extracts were analyzed by 2D-TLC. A subsample of the methanol extract was treated with protease as described below, and the aqueous phases were further analyzed by 2D-TLC and RP-TLC.

A subsample of day 2 and day 3 egg yolks was homogenized in turn, first with hexane, then acetonitrile, and then with methanol:water (80:20, v/v). The hexane extract was concentrated and then extracted with acetonitrile, leaving an oily residue that was redissolved in hexane. The acetonitrile extract was washed with hexane, and the hexane phases were combined and concentrated. The acetonitrile extracts were combined and concentrated. The methanol:water extract was concentrated and redissolved in methanol. A subsample of the acetonitrile extract was concentrated to dryness and dissolved in water. The sample was then treated with protease as described below and the DCM phase was analyzed by 2D-TLC.

A subsample of liver was homogenized with methanol:water (80:20, v/v). The mixture was centrifuged to separate the solid and extract phases. Each extract was concentrated to near dryness and the residue was dissolved in water and partitioned with DCM. The aqueous phase was acidified and then partitioned with EtOAc. All three phases were then analyzed by co-chromatography with excreta extracts.

Another subsample of the combined livers was treated with collagenase as described below, and the organosoluble residues were analyzed by 2D-TLC. The aqueous soluble residues resulting from the collagenase treatment were concentrated and treated with glucuronidase as described below. Then, the organic and aqueous fractions were analyzed by 2D-TLC.

Subsamples of muscle (breast and thigh) were homogenized with methanol:water(80:20, v/v). The mixture was centrifuged after each homogenization to separate the solid and the extract. The extract was concentrated to near dryness and the residue was dissolved in water and partitioned with hexane and then with DCM. The aqueous phase was then acidified and partitioned with ethyl acetate. The DCM, EtOAc, and aqueous fractions from the breast were analyzed by 2D-TLC. The water soluble residues were further analyzed by RP-TLC. The DCM, EtOAc, and water phases from the thigh were analyzed by 2D-TLC and RP-TLC.

A subsample of the combined skin and attached fat was homogenized, in turn with hexane, acetonitrile, methanol, then methanol:water. Each mixture was centrifuged to separate the solids and the extracts. The hexane extract was concentrated then extracted with acetonitrile. The unextractable oily residue was dissolved in hexane. The acetonitrile extracts were combined and washed with hexane. The hexane solubles were combined and concentrated. The acetonitrile, methanol, and methanol/water extracts were combined, concentrated and dissolved in acetonitrile. The acetonitrile solution was filtered, and the residual solids were dissolved in methanol and then filtered. The remaining undissolved material was dissolved in hexane. The acetonitrile and methanol extracts were analyzed by 2D-TLC; the acetonitrile solubles were further analyzed by RP-TLC.

A subsample of the peritoneal fat was homogenized sequentially with hexane, acetonitrile, methanol:water (80:20, v/v), and methanol:acetonitrile (ratio not stated). The mixture was centrifuged to separate the solids and extracts. The hexane extract was concentrated and extracted with acetonitrile. The unextractable oily residue was dissolved in a minimum amount of hexane. The acetonitrile extract was washed with hexane. The hexane solubles were combined and concentrated. The methanol:water and methanol:acetonitrile extracts were combined, concentrated, and dissolved in methanol. The acetonitrile and methanol-soluble extracts were analyzed by 2D-TLC. A subsample of the acetonitrile soluble metabolites was hydrolyzed under basic conditions, and the resulting EtOAc phase was analyzed by 2D-TLC and RP-TLC.

The distribution of radioactivity in the extracts of tissues and eggs is summarized in Table 7.

Hydrolysis of residues

Aqueous soluble residues from liver were incubated overnight with ß-glucuronidase (Type B-1, from bovine liver) in sodium acetate buffer (pH 4.6) at 37°C. A control aliquot omitting the enzyme was run concurrently. The hydrolysate was partitioned with EtOAc followed by DCM. The EtOAc and water layers were radioassayed. The aqueous and organic layers from the liver were analyzed by 2D-TLC.

Aqueous soluble residues from egg white and acetonitrile soluble metabolites from egg yolk were incubated with protease (Type XIV), from Streptomyces griseus, overnight at 37°C and pH 7.0. The aqueous solubles were extracted as described for glucuronidase. For egg yolk, the ACN sample was extracted into hexane and the hexane layer was then partitioned with ACN. The ACN layers were combined and then partitioned sequentially with DCM then EtOAC. The amount of radioactivity in each phase was measured by LSS. The DCM layer from the egg yolk was further analyzed by 2D-TLC. The EtOAc phase from egg white was analyzed by 2D-TLC and RP-TLC.

A subsample of the combined livers was treated with collagenase (Type IA) from Clostridium histolyticum overnight at 37 °C and pH 7.4. The sample was partitioned with DCM; the aqueous phase was then acidified and partitioned with EtOAc. The organic layers were analyzed by 2D-TLC. Duplicate aliquots of the three phases (DCM, EtOAc, and water) were measured for total ^{14}C-activity. Solids were air dried and triplicate subsamples were combusted to determine the unextractable radioactivity.

A subsample of the acetonitrile extract from the peritoneal fat, was concentrated then redissolved in methanol:water (90:10). The solution was cooled in an ice bath and one pellet of sodium hydroxide was added. After 2 hours at 5-10 °C the mixture was acidified to pH 2 with dilute formic acid and concentrated. The residue was dissolved in water and partitioned with ethyl acetate. Duplicate aliquots of the two phases (EtOAc and water) were analyzed for total ^{14}C-activity by LSS. The ethyl acetate layer was further analyzed by TLC.

Characterization of residues

Normal phase TLC analysis was performed on silica gel plates in saturated chambers. Extraction from the silica preparative TLC plates was accomplished by scraping the radioactive areas of interest into either methanol or a solution of methanol:acetone (1:1) and extracting the silica gel using sonication and centrifugation. The following solvent systems were used:

SS1:	chloroform/methanol/formic acid/water (83:13:1:1 v/v/v/v)
SS2:	ethyl acetate/ethanol/acetic acid (90:9.5:0.5 v/v/v)
SS4:	n-butanol/acetic acid/water (4:1:1 v/v/v)
SS8:	chloroform/acetonitrile (4:1 v/v)
SS11:	ethanol/ethyl acetate/hexane/formic acid (10:25:50:0.5 v/v/v/v)
SS12:	dimethoxy ethane/dichloromethane/methanol (1:1:1 v/v/v)

Various hen tissues and yolks were compared to excreta by 2D-TLC, using SS1 followed by SS2. The peritoneal fat ACN extract was also compared by 2D-TLC to the ACN extract of the skin and attached fat using SS4 followed by SS12. The hen excreta extracts were compared with the rat urine and the glucuronidase treated goat urine by 1D-TLC, using SS1.

Reverse-phase TLC was performed on C-18 plates either with or without a preconcentration zone. Some Analtech Reverse-Phase plates were also used for co-chromatography. The plates were developed in saturated chambers with one of the following solvent systems:

SS13:	acetonitrile/methanol/0.1 M acetate buffer (pH 4.6) (1:1:4 v/v/v)
SS14:	acetonitrile/methanol/0.1 M acetate buffer (pH 4.6) (1:1:2 v/v/v)

Flash column chromatography was performed on subsamples of the organosoluble excreta extracts. A glass column equipped with a solvent reservoir, was packed with flash chromatography grade silica gel and topped with of sand. The column was conditioned with of hexane. The sample, dissolved in a minimum amount of an appropriate solvent, was applied directly to the top of the column bed and eluted with a gradient of hexane (100%) followed by DCM (100%) and then by MeOH (100%). Aliquots of the collected fractions were radioassayed and the DCM fraction was concentrated and further analyzed by GC/MS.

Mass spectral data were obtained on a double focusing mass spectrometer with an integrated data system.

The organosoluble residues of excreta were analyzed and found to contain metalaxyl (3.5% of the TRR), CGA-107955 (8% TRR), CGA-108905 (2.3% TRR), and CGA-67869 (5.8% TRR). The identity of CGA-94689 was confirmed by GC/MS analysis of the DCM fraction. At least four unresolved metabolites and unknowns in these fractions accounted for a total of 12.2% of the TRR. Other unknowns in the organic layers totaled 2.7% of the TRR. The aqueous fraction consisted of six unknowns each representing 3.1-21.9% of the TRR which totaled 57%. The post extraction solid contained 3.1% of the TRR. No additional data were obtained from the enzyme hydrolysis of the aqueous layer. The registrant also claimed that CGA-100255 was a minor metabolite based on an indication from the GC/MS data, but no quantitative data were provided. Subsequent characterization of radioactive components in tissues was based on a comparison with results from these analyses of residues in excreta.

Co-chromatography of the methanol fraction from egg whites (pre-hydrolysis) with subsamples from organic excreta extracts indicated the presence of CGA-94689 and metalaxyl. Following protease hydrolysis of the initial methanol extract, the EtOAc fraction contained metalaxyl (4.9% of TRR) along with the two CGA-94689 isomers (1.4 and 3.0% of TRR). Unresolved metabolites (may have included CGA-108906, CGA-78532, CGA-68124, CGA-79353) and unknown components isolated from protease digestion accounted for a total of 32.9% of the TRR in egg whites. The aqueous fraction contained 45.7% of the TRR. These residues were insufficiently resolved by TLC; however, and no further analyses were performed. The unextracted residue represented 12.1% of the TRR and was not analyzed further.

Co-chromatography of the methanol fraction (before hydrolysis) from egg yolks with subsamples from organic excreta extracts indicated the presence of CGA-94689 and metalaxyl. Following protease hydrolysis, the DCM-soluble residues included CGA-94689 (22.2% of TRR), metalaxyl (7.9% of TRR), and two unknowns (1.8 and 7.1% of TRR). The aqueous (17.2%), hexane (21.8%), and non-extracted residues (14.3% of the TRR) were not characterized further.

Chromatographic analysis of the pre-hydrolysis organic and aqueous phases from liver confirmed the presence of metalaxyl and indicated the possible presence of CGA-108906, CGA-107955, CGA-67869, CGA-68124, and CGA-78532. The organosoluble fractions following collagenase treatment contained metalaxyl (1.3% of TRR), CGA-94689 (both isomers total 1.0% of TRR), CGA-67869 (0.7% of TRR), and CGA-107955 (17.1% of TRR). The unresolved and unidentified components accounted for a total of 16.9% of the TRR in liver. The post-hydrolysis analysis of the radioactivity in the aqueous phase (42.1% of the TRR) showed no resolved areas. The post-extraction solid contained 19.7% of the TRR and was not analyzed further.

The DCM phase from the thigh muscle contained CGA-94689 (0.6% of TRR). The organic and aqueous phases contained components that were unresolved from one another at a total of 65.4% of the TRR (may contain CGA-108906, CGA-78532, CGA-68124, CGA-79353). The

aqueous and organic phases also contained five unknowns (each accounting for 0.6-14.8% of TRR) representing a total of 26.9% of the TRR. The post extraction solid (6.5% of TRR) and the hexane phase (0.7% of TRR) were not further analyzed.

The DCM phase from the breast muscle extractions was analyzed and found to contain metalaxyl (0.4% of TRR) and CGA-94689 (both isomers 0.4 and 1.6% of TRR). The remaining residue in the organic phases (58.5% of TRR) was unresolved and may have included CGA-108906, CGA-78532, CGA-68124, or CGA-79353. The aqueous phase consisted of two poorly resolved areas that represented 15.1 and 19.1% of the TRR. The unextracted fraction contained 4.5% of the TRR and no further analysis was attempted.

The acetonitrile solubles from the skin and attached fat were considered by the registrant to be lipophilic conjugates based on their hydrolysis results from the peritoneal fat. The acetonitrile layer contained six unknowns, one of which was also found in the methanol fraction and represented 41.4% of the TRR. The other five unknowns represented 2.8-7.2% of the TRR (total 25.9% of TRR). The methanol layer contained unresolved residues representing a total of 16.3% of the TRR (may have contained CGA-78532, CGA-68124, CGA-79353, CGA-108906). Neither the hexane layer (3.84% of TRR) nor the post extraction solid (12.6% of TRR) were analyzed further.

The ACN fraction from the peritoneal fat was reportedly identical to the ACN fraction from the skin and attached fat by co-chromatography. The registrant suggests that the low polarity of the unknown ACN solubles indicated that they could be lipophilic conjugates, with cholesterol being a likely endocon. The methanol phase prior to hydrolysis contained unresolved and unknown residues representing a total of 1.3% of the TRR. A subsample of the acetonitrile phase was hydrolysed under mild conditions and the EtOAc phase was found to contained six unknowns that the registrant claimed were converted to metabolite CGA-107955 (49.8% of TRR) and two other unknowns (total 24.5% of the TRR) from the unknown lipophilic conjugates. The hexane (7.7% of TRR), post extraction solid (3.6% of TRR), and the water layer (13.1% of TRR) were not further characterized.

The characterization of the residues in eggs and tissues from laying hens is summarized in Table 8. The total radioactive residues reported were normalized to 100%.

Only 20% of the residue in liver, 30% in egg yolk, 9.3% in egg white, 2.4% in muscle, and none in skin/fat were identified. Components from protease digestion accounting for 32.9% of the total residue in egg whites were not resolved chromatographically and need further analysis. In addition, 45.7% of the egg white residue that was aqueous soluble should be identified. In egg yolk, radioactive components in the aqueous fraction (17.2%), the hexane fraction (21.8%), and insoluble residues (14.3% of the total) need to be characterized. Aqueous-soluble residues extracted after enzyme hydrolysis of whole liver should be characterized along with the organosoluble residues (before hydrolysis) accounting for 17% of the total residue and the unextractable residues (ca. 20%).

Table 8. Characterization of residues from eggs and tissues from hens.

		PPM Equivalents (% TRR)					
	Metabolite	Liver	Egg White	Egg Yolk	Breast	Thigh	Skin/Fat
A	CGA-48988 (parent)	0.018 (1.3)	0.009 (4.9)	0.016 (7.9)	0.002 (0.4)	--	--
B	CGA-94689 (isomer B)	0.013[c] (1.0)	0.003 (1.4)	--	0.002 (0.4)	--	--
C	Unknown	0.010 (0.7)	--	--	--	--	--
D	CGA-94689 (isomer D)	--	0.005 (3.0)	0.046 (22.2)	0.009 (1.6)	0.004 (0.6)	--
E	Unknown	--	--	0.004 (1.8)	0.006 (1.1)	0.004 (0.6)	--
F	CGA-67869	0.009 (0.7)	--	--	--	--	--
H	Unknown	--	--	--	--	0.100 (14.8)	--
I	Unknown	--	--	--	--	0.022 (3.3)	--
GHI	Unresolved Unknowns	--	--	--	0.171 (30.9)	--	--
K	CGA-107955	0.237 (17.1)	--	--	--	--	--
LM[a]	Unresolved	0.167 (12.0)	--	--	0.099 (17.8)	0.146 (21.6)	0.028 (8.7)
N[a]	Unresolved	0.049 (3.5)	--	--	0.019 (3.5)	0.295 (43.8)	0.024 (7.6)
LMN[a]	Unresolved	--	0.044 (24.8)	--	--	--	--
O[b]	Unknown	--	--	--	--	--	0.132 (41.4)

(continued, footnotes to follow)

Table 8. Residues in eggs and hen tissues (continued)

PPM Equivalents (% TRR)

	Metabolite	Liver	Egg White	Egg Yolk	Breast	Thigh	Skin/Fat
U1	Unknown	0.007 (0.5)	0.011 (6.4)	--	0.024 (4.3)	0.035 (5.2)	0.021 (6.6)
U2	Unknown	0.006 (0.4)	0.003 (1.7)	--	0.084 (15.1)	0.020 (3.0)	--
U3	Unknown	0.007 (0.5)	--	0.015 (7.1)	0.106 (19.1)	--	--
P[b]	Unknown	--	--	--	--	--	0.015 (4.6)
Q[b]	Unknown	--	--	--	--	--	0.015 (4.7)
Q'[b]	Unknown	--	--	--	--	--	0.009 (2.8)
R[b]	Unknown	--	--	--	--	--	0.023 (7.2)

[a] N may consist of CGA-78532, CGA-68124, CGA-79353; L is CGA-108906, and M is an unknown

[b] Unknown lipophilic conjugate based on hydrolysis results from peritoneal fat

[c] Contains both isomers B and D

Up to 60% and 34% of the residue in muscle organic and aqueous fractions, respectively, remains unresolved and requires additional attempts at identification

RESIDUE ANALYTICAL METHODS

Conclusions:

The Metalaxyl Residue Chemistry Chapter (FRSTR) of 6/22/87 discusses the GLC methodology available for data collection and enforcement of metalaxyl tolerances. Methods AG-348 and AG-349 correspond to Methods I and II in the Pesticide Analytical Manual (PAM) Vol. II (Pesticide Reg. Sec. 180.408). Method AG-395 is an improved modification of AG-348, exhibiting increased sensitivity in the measurement of CGA-94689 and decreased time required for analysis. AG-395 has undergone a successful Agency validation trial and has been validated using plant samples bearing ^{14}C-residues; (refer to the discussion in the 1987 Residue Chemistry Chapter). This method has been used to generate residue data on corn commodities which are reviewed in this update (1990; MRIDs 41689701 and 41689702).

It should be noted that, although the limit of detection of method AG-395 is stated as 0.05 ppm, untreated samples of corn forage and fodder from these recent tests bore apparent residues of 0.11 and 0.22 ppm, respectively. The registrant is asked to explain these high control values.

The Metalaxyl Guidance Document also states that metalaxyl metabolites containing the 2,6-dimethylaniline moiety and N-[2-(hydroxymethyl)-6-methylphenyl]-N-(methoxy-acetyl)-alanine methyl ester (CGA-94689) in or on crop samples be subjected to analysis using FDA Multiresidue Protocols. In response to this requirement, Ciba-Geigy Corp. has submitted data pertaining to the determination of metalaxyl, CGA-62826, and CGA-37734 (1987; MRID 40503101) and metalaxyl metabolites CGA-94689 (A and B isomers) and CGA-100255 (1989; MRID 41055203) in or on crop substrates and animal tissues using FDA Multiresidue Protocols. These data have been forwarded to FDA for review and inclusion in PAM Vol. I.

The nature of the residue in animals is not adequately understood. If the additional analyses requested for the recently submitted poultry and ruminant metabolism studies reveal additional metabolites of concern, supplementary methodology may need to be developed.

References (used):

MRIDs: 40503101. 41055203. 41689701. 41689702.

Discussion of the data:

N/A.

STORAGE STABILITY DATA

Conclusions:

The Metalaxyl Guidance Document dated 9/88 requires data examining the stability of residues of concern in stored plant and animal commodities and information regarding sample storage intervals. In response to these requirements, Ciba-Geigy has submitted data pertaining to the storage intervals of commodity samples used to generate data submitted in support of established tolerances (1990; MRID 41449001). The registrant has reported the intervals and conditions of storage for 134 raw and processed animal and plant commodities from 52 studies submitted in support of established tolerances. These data have been reviewed by the Agency (DEB No. 7193; 1/91). The validity of residue data generated using these samples will be determined following review of forthcoming storage stability studies, which the registrant has indicated are in progress.

The following MRID numbers indicate references cited in the Guidance Document that are not represented by storage information in the Ciba-Geigy submission.

MRID No.	Commodity
00071616	potatoes
00071673	poultry
00097511	lettuce
00098428	onions
	cucumbers
	melons
00100753	liver and kidney
	(dairy cattle and goats)
00128102	beets, beet greens
	sorghum stalks, sorghum grain
	sunflower seed, stalks

The registrant needs to submit information pertaining to the storage intervals and conditions for the samples analyzed in these studies.

- Storage stability data in support of all required and previously submitted residue data reflecting the actual storage conditions and intervals for samples used to generate the residue data. If residue depletion occurs, data will be required which depict the decline in levels of metalaxyl residues of concern in commodities stored under the range of conditions and for the range of intervals specified. Crop samples bearing measurable weathered residues or fortified with metalaxyl residues of concern must be analyzed immediately after harvest or fortification and again after storage intervals that allow for reasonable unforeseen delays in sample analysis. In laboratory tests using fortified samples, the pure active ingredient and pure metabolites must be used. However, if field-weathered samples are used, the test substance must be a typical end-use product. For additional guidance on conducting storage stability studies, the registrant is referred to an August, 1987 Position Document on the Effects of Storage Validity of Pesticide Residue Data available from NTIS under order No. PB 88112362/AS.

- The sample storage intervals and conditions must be supplied for all residue data submitted in support of tolerances, whether previously submitted or required in this update. The Registrant should submit sample storage information for the following samples cited in the Guidance Document as providing data in support of tolerances: potatoes (MRID 00071616), poultry and eggs (MRID 00071673), lettuce (MRID 00097511), onions, cucumbers and melons (MRID 00098428), liver and kidney of dairy cattle and goats (MRID 00100753), and beets and beet greens, sorghum stalks and grain, and sunflower seeds and stalks (MRID 00128102).

References (used):

MRID: 41449001.

Discussion of the data:

N/A.

MAGNITUDE OF THE RESIDUE IN PLANTS

Root and Tuber Vegetables Group

Sugar beet roots

Tolerances:

A tolerance of 0.1 ppm has been established for the combined residues of metalaxyl and its metabolites containing the 2,6-dimethylaniline moiety and N-(2-hydroxy methyl-6-methyl)-N-(methoxyacetyl)-alanine methyl ester, each expressed as metalaxyl, in or on sugar beet roots (40 CFR §180.408[a]). A feed additive tolerance of 1 ppm has been established for these same residues in sugar beet molasses (40 CFR §186.4000[a]).

Use directions and limitations:

According to the Metalaxyl Residue Chemistry Chapter (FRSTR) dated 6/22/87, the 2.65 lb/gal FlC formulation is registered for treatment of sugar beet seed at 0.5 oz ai/100 lb of seed.

Conclusions:

The Metalaxyl Guidance Document dated 9/88 requires data depicting residues of concern in dehydrated pulp, molasses and sugar processed from sugar beets bearing measurable residues. Data are available to indicate that residues concentrate up to 7x in molasses, but that no concentration occurs in pulp or sugar. Although residues in raw beets in this study were below the stated limit of detection of the analytical method, the raw data reveal that detectable residues of 0.036 ppm were present in or on beets used for processing. Residues were nondetectable in pulp and sugar and 0.24-0.25 ppm in molasses. These data (1987;

MRID 40569301) were submitted in support of PP#8F3617/FAP#8H5554 and were discussed by F. Griffith (DEB Nos. 3908-3912; 11/28/88); this petition is currently under review by the Agency. These data satisfy the requirement for data on sugar beet processing. No additional data are required.

References (used):

MRID: 40569301.

Discussion of the data:

N/A.

Cereal Grains Group

Tolerances:

A tolerance of 0.1 ppm has been established for the combined residues of metalaxyl and its metabolites containing the 2,6-dimethylaniline moiety and N-(2-hydroxy methyl-6-methyl)-N-(methoxyacetyl)-alanine methyl ester, each expressed as metalaxyl, in or on grain crops (40 CFR §180.408[a]). Tolerances of 0.2 and 2 ppm have been established for inadvertent residues of metalaxyl in or on wheat grain and wheat forage, fodder, and straw (40 CFR §180.408[b].

Food and feed additive tolerances of 0.1 ppm have been established for the inadvertent residues of metalaxyl in wheat milling fractions (40 CFR §185.4000[b] and 40 CFR §186.4000[b]).

Use directions and limitations:

The 25% WP and 2.65 lb/gal FlC formulations are registered for seed treatment of barley, corn (field corn, sweet corn, and popcorn), oats, rice, rye, and wheat seed at 0.25-0.5 oz ai/100 lb of seed. These same formulations and also the 2 lb/gal EC formulation are registered for treatment of sorghum seed at 0.25-1 oz ai/100 lb of seed. These treatments may be made using conventional slurry or mist seed-treating equipment. These are the same use directions listed for cereal grains in the Metalaxyl (FRSTR) Residue Chemistry Chapter of 6/87.

Conclusions:

The Metalaxyl Guidance Document requires data pertaining to residues in or on corn forage, fodder, and grain, and data depicting residues in the processed commodities of corn, rice, and sorghum. In response, Ciba-Geigy Corporation (1990; MRIDs 41689701 and 41689702) submitted data concerning residues of metalaxyl in or on corn forage, fodder, and grain from field trials conducted in 16 states. The registrant also submitted in accordance with Section 6(a)(2) of FIFRA, a letter (received 10/24/90) reporting tolerance-exceeding residues in corn fodder.

The submitted data indicate that residues were 0.36 ppm in or on corn fodder from a test conducted in GA. We note also that untreated fodder samples from this same test bore high apparent residues of up to 0.22 ppm. The registrant needs to explain these high apparent control values.

The currently established tolerance of 0.1 ppm applies to all commodities of representative crops in the cereal grains group. However, current regulations specify that forage, fodder, and straw of cereal grains represent a separate crop grouping from grain. In addition, separate tolerances are established for inadvertent residues in or on wheat grain and wheat forage, fodder and straw. Therefore, separate tolerances need to be established for residues in or on cereal grains (except wheat) and forage, fodder, and straw of cereal grains (except wheat). While the currently established level of 0.1 ppm is adequate to cover grains, a higher level will need to be set for forage, fodder, and straw. When the registrant provides the information requested from the recently submitted studies, they should also propose a tolerance for residues in or on the forage, fodder, and straw of cereal grains (except wheat) based on the available data. The following is required:

- The registrant must provide an explanation for the high apparent residues in or on untreated samples from the tests described in MRID 41689701. In addition the registrant must propose a tolerance for the combined residues of metalaxyl and metabolites in or on forage, fodder, and straw of cereal grains (except wheat) based on the available data.

Note to SRRD: Concomitant with the establishment of a separate tolerance for residues of metalaxyl in or on forage, fodder, and straw of cereal grains (except wheat) at a level supported by the available residue data, the 40 CFR 180.408 entry : "grain, crops" should be amended to reflect the appropriate commodity definition, "cereal grains (except wheat)."

In the current submission, the registrant has described a field trial in which foliar application was made to corn in addition to seed treatment in order to assure sufficient residues in or on grain to conduct a processing study. These data have not been received and no data have been submitted concerning the processed commodities of rice or sorghum as specified in the Guidance document. The following requirement remains outstanding:

- Data depicting the potential for concentration of residues of metalaxyl and metabolites in the following processed commodities: (i) meal, grits, crude oil, and refined oil from dry milling and starch, flour, crude oil, and refined oil from wet milling of corn grain; (ii) rice hulls, bran, and polished rice; and (iii) flour and starch from sorghum grain. Processing must be conducted using grain bearing measurable weathered residues. If residues concentrate in any commodity, an appropriate food or feed additive tolerance must be established.

We note that the 1988 Guidance Document requires data depicting residues in grain dust. However, since the only registered use of metalaxyl on cereal grains is pre-emergence (seed treatment), residues on the surface of grain are not expected; data on grain dust are not required.

References (used):

MRIDs: 41689701. 41689702.

Discussion of the data:

Ciba-Geigy Corp. (1990; MRIDs 41689701 and 41689702) submitted data from 17 tests conducted in CA, GA, IL(2), IN, IA, KS, MN, MS, MO, NE, NY, NC, OH, OK, PA, and TX pertaining to residues of metalaxyl in or on corn grain, forage and fodder harvested 59-174 days following the planting of seed treated using the 25% WP formulation at 0.5 oz ai/100 lb of seed (1x the maximum registered rate). The residues are summarized in Table 9.

Table 9. Residues in or on corn commodities grown from seed treated with the 25% WP formulation at 1x.

Commodity	Posttreatment Interval (days)	Residues (ppm) Treated	Residues (ppm) Untreated
Forage	62-125	<0.05-0.08	<0.05-0.07
Silage-stage forage	81-174	<0.05-0.09	<0.05-0.11
Fodder	132-236	<0.05-0.36	<0.05-0.22
Grain	132-236	<0.05	<0.05

Treated fodder samples from tests conducted in GA (0.36 ppm) and IN (0.13 ppm) bore tolerance-exceeding residues. In addition, untreated samples of fodder from CA (0.13 ppm) and GA (0.22 ppm) and a silage-stage forage sample from IA (0.11 ppm) contained high apparent residues.

The data were collected using GLC method AG-395, which has a stated limit of detection of 0.05 ppm for all commodities, although apparent residues up to 0.22 ppm were detected in control samples. Recoveries from samples fortified with metalaxyl at 0.05-2 ppm were 62-113% from forage, 66-145% from silage-stage forage, 66-140% from fodder and 73-120% from grain. Samples were stored at -15 C for 5-18 months prior to analysis.

Geographic representation is adequate. The test states of CA(<1%), GA(<1%), IL(17%), IN(9%), IA(19%), KS(2%), MN(8%), MS(<1%), MO(3%), NE(11%), NY(1%), NC(1%), OH(5%), OK(<1%), PA(1%), and TX(2%) accounted for a total of ca. 80% of the 1987 U.S. field corn production (1987 Census of Agriculture, Vol. 1, Part 51). The available data indicate that residues exceed the established tolerance in or on fodder samples the GA and IN tests. Furthermore, apparent residues were greater than 0.1 ppm in or on the untreated samples of fodder from the same GA test as well as one from a CA test and one control sample of silage-stage forage from the IA test. The registrant needs to explain these high control values and determine an appropriate level for a revised tolerance for residues in or on forage, fodder, and straw of cereal grains (except wheat). In addition, the 40 CFR 180.408 entry for "grain crops" should be amended to specify the appropriate crop group, cereal grains (except wheat). Finally, the requirement for data on the processed commodities of corn, rice, and sorghum remains outstanding.

Miscellaneous Commodities

Hops

Tolerances:

A tolerance of 0.5 ppm has been established for the combined residues of metalaxyl and its metabolites containing the 2,6-dimethylaniline moiety and N-(2-hydroxy methyl-6-methyl)-N-(methoxyacetyl)-alanine methyl ester, each expressed as metalaxyl, in or on green hops (40 CFR §180.408[a]). A food additive tolerance of 20 ppm has been established for these same residues in dried hops (40 CFR §185.4000[d]). Feed additive tolerances of 2 and 20 ppm have been established for these same residues in dry hops and spent hops (40 CFR §186.4000 [a] and [d], respectively).

Use directions and limitations:

The 2 lb/gal EC formulation is registered for soil application to hops at 0.5 lb ai/A in 20 gal of water. A single application per season is permitted and may be made over the crowns after pruning, but not after training. Crop refuse may not be fed to livestock. No PHI has been established. This is the same use pattern described in the Residue Chemistry Chapter dated 6/87.

Conclusions:

The Guidance Document dated 9/88 notes a interim tolerances of 50 ppm for residues of metalaxyl in dried and spent hops (expiration date 10/28/88). Subsequently, a food additive tolerance of 20 ppm was established for residues in dried hops. This level is 10x the feed additive tolerance for the same commodity. We recommend that the feed and food additive tolerances of for dried hops be set at the same level. The available data indicate that 20 ppm would be appropriate. The following is required:

- The registrant must propose a revision of either the feed additive tolerance for dry hops or the food additive tolerance for dried hops so that the tolerances are set at the same level. Raising the feed additive tolerance to 20 ppm to be consistent with the established food additive tolerance would be appropriate.

Note to SRRD: When a feed additive tolerance of 20 ppm is established for residues in dried hops, the existing 2 ppm tolerance should be revoked.

References (used):

N/A.

Discussion of the data:

N/A.

MAGNITUDE OF THE RESIDUE IN ANIMALS

Tolerances:

The following tolerances have been established for the combined residues of metalaxyl and metabolites containing the 2,6-dimethylaniline moiety and N-(2-hydroxy methyl-6-methyl)-N-(methoxyacetyl)-alanine methyl ester, each expressed as metalaxyl (40 CFR §180.408[a]): (i) 0.02 ppm in milk; (ii) 0.05 ppm in eggs; (iii) 0.05 ppm in meat and meat by-products of cattle, goats, hogs, horses, and sheep and poultry; and (iv) 0.4 ppm in the fat, kidney, and liver of these same animals.

Conclusions:

The Metalaxyl Guidance Document dated 9/88 reserves judgement on the available data concerning animal commodities and the adequacy of the established tolerances due to the outstanding requirement for data on animal metabolism. In addition data requirements for livestock feed commodities are still outstanding. Following receipt and review of the additional data requested from the livestock metabolism studies and from crop field trials and processing studies, the need for additional animal feeding studies will be determined and the established tolerances for residues in animal commodities will be assessed.

References (used):

N/A.

Discussion of the data:

N/A.

MASTER RECORD IDENTIFICATION NUMBERS

The following citations were taken from a Pesticide Data Management System search conducted on 12/9/90.

References (used):

40503101 Williams, B.; Condra, C. (1987) Multiresidue Method Testing of Metalaxyl and Metabolites in Crops and Animal Tissues: Final Report No. 36320. Unpublished study prepared by Analytical Bio-Cheistry Laboratories. 188p.

40534803 Perez, R. (1983) Validation of Analytical Method AG-395 Using [Carbon 14! - Metalaxyl Treated Lettuce (Residue Analytical Method): Study No. ABR-83033. Unpublished study prepared by Ciba Geigy Corp. 8 p.

40569301 Gold, B.;Cheung, M. (1987) Metalaxyl--Sugar Beets: [Residue Cheistry Data!: Project ID: ABR-87077. Unpublished study prepared by Ciba-Geigy Corp., in cooperation with Enviro-Bio-Tech, Ltd. 130 p.

41055203 Hubbard, H. (1989) Determination of the Metalaxyl Metabolites CGA-100255 and CGA-94689 (A&B Isoers) by U.S. Food and Drug Adinistration Multiresidue Procedures: Proj. ID ABR-88156. Unpublished study prepared by Ciba-Geigy Corp. 78p.

41250101 Cheung, M. (1989) Metalaxyl: Response to EPA Review of Metalaxyl on Sugar Beets: Project ID ABR-89075. Unpublished study prepared by Ciba-Geigy Corp. 97 p.

41449001 Ross, J. (1990) Metalaxyl Saple Storage Interval Summary: Lab Project Nos. ABR-90016; 409925. Unpublished study prepared by Ciba-Geigy Corp., Residue Chemistry Dept. 265 p.

41664503 Emrani, J. (1990) Metalaxyl: Metabolism of [carbon 14! Metalaxyl in Goats: Lab Project Number: ABR/90078. Unpublished study prepared by Ciba-Geigy Corp., and others 205 p.

41664504 Kennedy, E. (1990) Metalaxyl: Metabolism of [phi-carbon 14! - Metalaxyl in Hens: Lab Project Number: ABR/90077. Unpublished study prepared by Ciba-Geigy Corp., and others. 129 p.

41664506 McDonald, J. (1990) Procedure for Incubating Animal Tissue Samples with the Enzyme Collagenase: Lab Project Number: SOP/NUMBER/7/-22. Unpublished study prepared by Ciba Geigy Corp. 7 p.

References (not used):

[The following references do not contain infrmation relevant to this Ryeregistration Standard Update.]

40827401 Kuehne, R. (1988) Technical Metalaxyl: Additional Analysis of Metalazyl on Geran-treated Hops: Project ID. 2191-3/87. Unpublished study prepared by Ciba-Geigy Ltd. 16 p.

40909401 Cheung, M. (1988) Summary of Residue in Hops Follwing Applications of Ridomil 2E and Metalaxyl 48.8 WP: Lab. Proj. ID ABR-88152. Unpublished study prepared by Ciba-Geigy Corp. 47 p.

41664505 Ross, J. (1990) Preparation of Diazomethane: Lab Project Number: AG/345. Unpublished study prepared by Ciba-Geigy Corp., and others. 9 p.

41664507 Bell, D.; Freeman, B. (1971) Physiology and Biochemistry of the Domestic Fowl. P. 273-275. New York: Academic Press.

TABLE A. GENERIC DATA REQUIREMENTS FOR METALAXYL RESIDUE CHEMISTRY.

Data Requirement		Test Substance	Does EPA have data to satisfy this requirement?	Bibliographic Citation	Must additional data be submitted under FIFRA Sec. 3(c)(2)(B)?
40 CFR §158.240 Residue Chemistry					
171-2.	Chemical Identity[1]				
171-3.	Directions for Use[2]		(See Index)		
171-4.	Nature of the Residue (Metabolism) - Plants	PAIRA	Yes	N/A	No
171-4.	Nature of the Residue (Metabolism) - Livestock	PAIRA & plant metabolites	Partially	41663503 41664504 41664506	Yes[3,4]
171-4.	Residue Analytical Methods	TGAI & metabolites	Partially	40503101 41055203 41689701 41689702	Reserved[5]
171-4.	Storage Stability	TEP & metabolites	Partially	41449001	Yes[6,7]
171-4.	Magnitude of Residue in Plants				
	Root and Tuber Vegetables				
	- Beets	TEP	Yes	N/A	No
	- Potatoes	TEP	Yes	N/A	No
	(processed commodities)	TEP	Partially	N/A	Yes[8]

(Continued, footnotes follow)

TABLE A. (Continued).

Data Requirement	Test Substance	Does EPA have data to satisfy this requirement?	Bibliographic Citation	Must additional data be submitted under FIFRA Sec. 3(c)(2)(B)?
- Sugar beets	TEP	Yes	N/A	No
(processed commodities)	TEP	Yes	40569301	No[9]
Leaves of Root and Tuber Vegetables				
- Beet greens	TEP	Yes	N/A	No
- Sugar beet tops	TEP	Yes	N/A	No
Bulb Vegetables				
- Onions (bulb & green)	TEP	Yes	N/A	No
Leafy Vegetables (except Brassica)				
- Lettuce	TEP	Yes	N/A	No
- Spinach	TEP	Yes	N/A	No
Brassica Leafy Vegetables				
- Broccoli	TEP	Yes	N/A	No
- Cabbage	TEP	Yes	N/A	No
- Cauliflower	TEP	Yes	N/A	No

(Continued, footnotes follow)

TABLE A. (Continued).

Data Requirement	Test Substance	Does EPA have data to satisfy this requirement?	Bibliographic Citation	Must additional data be submitted under FIFRA Sec. 3(c)(2)(B)?
Legume Vegetables				
- Soybeans[10]	TEP	Yes	N/A	No
(processed commodities)	TEP	Yes	N/A	No
Foliage of Legume Vegetables	TEP	Yes	N/A	No
Fruiting Vegetables (Except Cucurbits)	TEP	Yes	N/A	No
Cucurbit Vegetables	TEP	Yes	N/A	No
Citrus Fruits	TEP	Yes	N/A	No
Pome Fruits				
- Apples	TEP	Yes	N/A	No
(processed commodities)	TEP	Yes	N/A	No
Stone Fruits	TEP	Yes	N/A	No

(Continued, footnotes follow)

TABLE A. (Continued).

Data Requirement	Test Substance	Does EPA have data to satisfy this requirement?	Bibliographic Citation	Must additional data be submitted under FIFRA Sec. 3(c)(2)(B)?
Small Fruits & Berries				
- Raspberries	TEP	Yes	N/A	No
- Strawberries	TEP	Yes	N/A	No
Tree Nuts				
- Almonds	TEP	Yes	N/A	No
- Walnuts	TEP	Yes	N/A	No
Cereal Grains				
	TEP	Partially	41689701 41689702	Yes[11]
(processed commodities)	TEP	No	N/A	Yes[12]
- Wheat	TEP	Yes	N/A	No
(processed commodities)	TEP	Yes	N/A	No
Forage, Fodder, and Straw of Cereal Grains				
- Wheat forage, fodder, & straw	TEP	Yes	N/A	No

(Continued, footnotes follow)

TABLE A. (Continued).

Data Requirement		Test Substance	Does EPA have data to satisfy this requirement?	Bibliographic Citation	Must additional data be submitted under FIFRA Sec. 3(c)(2)(B)?
	Miscellaneous Commodities				
	- Avocados	TEP	Yes	N/A	No
	- Cottonseed	TEP	Yes	N/A	No
	(processed commodities)	TEP	Partially	N/A	Yes[13]
	- Hops	TEP	Partially	N/A	Yes[14]
	- Peanuts	TEP	Partially	N/A	Yes[15]
	- Pineapples	TEP	Yes	N/A	No
	(processed commodities)	TEP	Partially	N/A	Yes[16]
	- Sunflower seed	TEP	Yes	N/A	No
	(processed commodities)	TEP	No	N/A	Yes[17]
	- Tobacco	TEP	No	N/A	No
171-4.	Magnitude of residue in Meat/Milk/ Poultry/Eggs	TGAI or plant metabolites	Partially	N/A	Reserved[18]

[1]The same chemical identity data are required as under 40 CFR §158.150-190, with emphasis on impurities that could constitute residue problems. Refer to Product Chemistry Data Requirements tables.

(Continued, footnotes follow)

TABLE A. (Continued).

[2] As stated in the Guidance Document, the label directions for dry bulb onions and fruiting vegetables.

[3]The registrant must submit additional information from the study described in MRID 41664503, specifically, metabolite "A" isolated from milk of goat 2 needs to be characterized further.

[4]The registrant must submit additional information from the study described in MRID 41664504. The unextracted solid residue from the liver should be subjected to acid hydrolysis. The final aqueous fraction collected after collagenase treatment of liver requires further separation and identification of the radioactive components. Additional chromatographic techniques should be employed to resolve CGA-79353 and to identify the co-eluting unknown in extracts from liver, breast, and thigh muscles. The unknowns in the skin and attached fat, the breast, and thigh muscles should be characterized further. The unresolved metabolites in the breast should be resolved and quantified.

[5]The qualitative nature of the residue in plants and animals has not been adequately described, therefore, the adequacy of the available methods cannot be ascertained. If the requested data on plant and animal metabolism indicate the presence of additional metabolites of toxicological concern, data depicting additional analytical methods will be required. If radiolabeled validation of existing analytical methodology for plants and animals (refer to "Qualitative Nature of the Residue in Plants" and "Qualitative Nature of the Residue in Animals" for additional details) indicates that a major portion of the total radioactive residue is not recovered and identified by the available methods, radiolabeled validation of new proposed methodology will be required.

[6]Storage stability data in support of all required and previously submitted residue data reflecting the actual storage conditions and intervals for samples used to generate the residue data. If residue depletion occurs, data will be required which depict the decline in levels of metalaxyl residues of concern in commodities stored under the range of conditions and for the range of intervals specified. Crop samples bearing measurable weathered residues or fortified with metalaxyl residues of concern must be analyzed immediately after harvest or fortification and again after storage intervals that allow for reasonable unforeseen delays in sample analysis. In laboratory tests using fortified samples, the pure active ingredient and pure metabolites must be used. However, if field-weathered samples are used, the test substance must be a typical end-use product. For additional guidance on conducting storage stability studies, the registrant is referred to an August, 1987 Position Document on the Effects of Storage Validity of Pesticide Residue Data available from NTIS under order No. PB 88112362/AS.

[7]The sample storage intervals and conditions must be supplied for all residue data submitted in support of tolerances, whether previously submitted or required in this update. The Registrant should submit sample storage information for the following samples cited in the Guidance Document as providing data in support of tolerances: potatoes (MRID 00071616), poultry and eggs (MRID 00071673), lettuce (MRID 00097511), onions, cucumbers and melons (MRID 00098428), liver and kidney of dairy cattle and goats (MRID 00100753), and beets and beet greens, sorghum stalks and grain, and sunflower seeds and stalks (MRID 00128102).

TABLE A. (Continued).

[8]No data have been submitted in response to the Guidance Document. Data are required depicting the potential for concentration of residues of metalaxyl in chips, granules, and wet and dry peel processed from potatoes bearing measurable, weathered residues. If residues concentrate in any commodity, an appropriate feed additive tolerance will be required.

[9]Data reviewed in conjunction with PP#8F3617/FAP#8H5554 are adequate to fulfill this requirement for a processing study with sugar beets. No additional data are required.

[10]The Guidance Document specifies that a tolerance must be proposed for residues in or on soybean forage, hay, and straw and recommends a level of 8 ppm. This requirement remains outstanding.

[11]The registrant must provide an explanation for the high apparent residues in or on untreated samples from the tests described in MRID 41689701. In addition the registrant must propose a tolerance for the combined residues of metalaxyl and metabolites in or on forage, fodder, and straw of cereal grains (except wheat) based on the available data. Concomitant with the establishment of a separate tolerance for residues of metalaxyl in or on forage, fodder, and straw of cereal grains (except wheat) at a level supported by the available residue data, the 40 CFR 180.408 entry : "grain, crops" should be amended to reflect the appropriate commodity definition, "cereal grains (except wheat)."

[12]The registrant has not responded to the requirements specified in the Guidance Document. Data are required depicting the potential for concentration of residues of metalaxyl and metabolites in the following processed commodities: (i) meal, grits, crude oil, and refined oil from dry milling and starch, flour, crude oil, and refined oil from wet milling of corn grain; (ii) rice hulls, bran, and polished rice; and (iii) flour and starch from sorghum grain. Processing must be conducted using grain bearing measurable weathered residues. If residues concentrate in any commodity, an appropriate food or feed additive tolerance must be established.

[13]No data have been submitted in response to the Guidance Document. Data are required depicting the potential for concentration of residues in hulls, meal, crude oil, refined oil, and soapstock processed from cottonseed bearing measurable weathered residues. If residues concentrate in any commodity, an appropriate food or feed additive tolerance must be proposed.

[14]The registrant must propose a revision of either the feed additive tolerance for dry hops or the food additive tolerance for dried hops so that the tolerances are set at the same level. Raising the feed additive tolerance to 20 ppm to be consistent with the established food additive tolerance would be appropriate.

[15]No data were submitted in response to the Guidance Document. Data are required depicting residues of metalaxyl and metabolites in or on nutmeats, hulls, hay, and vines of peanuts harvested following soil application made at planting using a G formulation at 0.25 lb ai/A and an at-pegging application at 1 lb ai/13,000

TABLE A. (Continued).

linear feet of row. The tests must be conducted in AL (5%) or GA(49%) and NC(10%) or VA(6%), since these states accounted for ca. 80% of the U.S. peanut production.

[16]No data were submitted in response to the Guidance Document. Data are required depicting the concentration of residues in juice and bran processed from pineapples bearing measurable weathered residues. If residues concentrate in either commodity, an appropriate food or feed additive tolerance will be needed.

[17]No data from a sunflower processing study were submitted in response to the Guidance Document. Data are required depicting the potential for concentration of residues in meal, hulls, crude oil, and refined oil processed from sunflower seed bearing measurable weathered residues. If residues concentrate in any commodity, an appropriate food or feed additive tolerance must be proposed.

[18]The Metalaxyl Guidance Document dated 9/88 reserves judgement on the available data concerning animal commodities and the adequacy of the established tolerances due to the outstanding requirement for data on animal metabolism. In addition data requirements for livestock feed commodities are still outstanding. Following receipt and review of the additional data requested from the livestock metabolism studies and from crop field trials and processing studies, the need for additional animal feeding studies will be determined and the established tolerances for residues in animal commodities will be assessed.

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