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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#8F3592/FAP#8H5550 - Avermectin B_1 (Abamectin) on Citrus - Evaluation of Analytical Method and Residue Data - Accession Nos. 404430-01 through 404430-10 - RCB Nos. 3142 and 3143

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and

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Merck, Sharp and Dohme has proposed the following permanent tolerances for avermectin (AVM), including its delta-8,9-isomer:

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*RCB is deferring to TB as to whether the 24-hydroxymethyl AVM should be included in the tolerance expression (see Ruminant Metabolism section of this review).

Commodities	Tolerance
Citrus whole fruit (RAC)	0.005 ppm
Cattle - meat and meat byproducts	0.005 ppm
- milk	0.001 ppm
Dried citrus pulp	0.03 ppm
Citrus oil	0.10 ppm

No permanent tolerances for residues of AVM, an insecticide produced by a strain of Streptomyces avermitilis have yet been established. However, a permanent tolerance request of 0.005 ppm for residues of AVM B_1 and its delta-8,9-isomer on cottonseed is currently pending (PP#7F3500, memorandum of C. Deyrup, July 29, 1987).

PP#4F3065, which proposed permanent tolerances on range and pasture grass of 0.001 ppm, was rejected because of deficiencies cited in the plant and animal metabolism studies and because of problems associated with the analytical methodology (PP#4F3065, memorandum of V.F. Boyd, September 13, 1984).

In conjunction with PP#7G3468/FAP#7H5518 and 50658-EUP-1 (memorandum of L. Cheng February 11, 1987) the following temporary tolerances were established and are in effect for the following commodities:

Cottonseed	0.005 ppm
Citrus fruit	0.005 ppm
Cattle fat, meat, and meat byproducts	0.010 ppm
Milk	0.001 ppm
Citrus oil and pulp	0.10 ppm

Conclusions

1. The formulation to be used on citrus is AGRIMEC 0.15 EC. All inerts in this formulation have been cleared under 40 CFR 180.1001.

2a. In a revised Section B/label under Remark a/ "Apply in 500-2000 gallons" should be changed to "Apply in 500-1000 gallons" to keep the total dosage applied to 20 fl oz AGRIMEC per acre per application or 0.025 lb ai/A/application.

2b. In a revised Section B/label, the petitioner will need to specify the treatment intervals and timing of applications during the growing season.

3a. RCB concludes that the residues of concern on citrus consist of AVM B_1a and its delta-8,9 isomer provided that TB expresses no concern regarding the presence of unidentified polar degradates which may comprise up to 70 percent of the total terminal residue. TB's opinion in turn regarding the toxicological significance of these polar degradates is predicated upon their favorable review and evaluation of the teratology tests and the Ames test conducted on these polar degradates when submitted by the petitioner

3b. If TB's evaluation of the teratology tests and Ames tests described in 2a above is unfavorable,wherein the identities of the polar degradates are needed, then RCB must conclude that the nature of the residue in plants is not adequately understood. The petitioner must then characterize the subject polar degradates as comprising a significant portion of the total toxic residue. Furthermore, the tolerance expression would probably need to be revised to include these polar degradates. Accordingly, this would require validated enforcement analytical methodology and additional residue data on citrus generated utilizing this methodology.

3c. If TB considerations permit, RCB concludes that metabolism studies using AVM B_1b are not needed; studies using AVM B_1a adequately reflect the metabolism of the technical product, which may contain up to 20% AVM B_1b.

3d. RCB defers to TB on the need for regulating the 24-hydroxymethyl AVM metabolite (free and conjugated). The 24-hydroxymethyl AVM metabolite accounts for 40 percent of the total radioactive residue (TRR) in kidney, 16.5 percent of the TRR in liver, 6 percent of the TRR in fat, and 11 percent of the TRR in milk.

3e. RCB concludes that the nature of the residue in ruminants is not adequately understood. The petitioner will need to conduct a goat metabolism study using ^{14}C-AVM; a higher dosage rate may be needed to identify the terminal residues.

4a. At this time RCB cannot determine whether the methodology (Merck Method No. 1009R01) which was used to generate all citrus RAC data and determine AVM B_{1a} (and its delta-8,9 isomer) provided adequate residue data. Another Merck Method (No. 1009 Revision No.2) recently (ACS/COB September 30, 1987) passed a successful EPA method validation. RCB will require bridging data between the two residue methodologies. To accomplish this bridging data, RCB recommends that selected RAC samples containing finite residues (i.e., > 5 ppb) be reanalyzed simultaneously by both methods and the results reported and compared. Simultaneous reanalysis would alleviate any concerns RCB would have concerning storage stability of existing RAC samples.

As an example, RCB would suggest reanalysis of Sample Nos. 70402359, -60, -63, -64; 70403492, -93; 70402343, -44, -47, -48, -49, -50.

4b. At this time RCB cannot conclude that Merck Method No. 1004 Revision No. 1 is adequate to enforce the proposed <u>food</u> and <u>animal feed</u> additive tolerances. It seems that the petitioner needs to revise this method as per Method No. 1009 Revision No. 2, that is, to standardize the testing of aluminum oxide in the method. The petitioner must also adequately address RCB's questions regarding high background or baseline levels on orange and tangelo dried peel control and sample chromatograms and submit specific processed commodity (dried citrus peel and oil) recovery data reflecting any revisions (such as standardizing the aluminum oxide) made in the procedure.

4c. With regard to determining AVM residues in animal commodities, RCB cannot determine

whether the proposed enforcement method (Merck Method No. 32A) is adequate for enforcement purposes until this method has passed a successful EPA method validation. A favorable conclusion regarding the adequacy of the proposed animal commodity enforcement methodology is also contingent upon the results of the requested ^{14}C-AVM goat metabolism study and TB's opinion regarding the need to regulate the 24-hydroxymethyl AVM metabolite (free and conjugated). If additional residues are determined to be of toxicological concern (such as 24-hydroxymethyl AVM), then appropriate analytical methodology for determining that compound will be needed.

4d. Ivermectin (22,23-dihydro AVM) is registered for use on large animals at a rate of 0.2 mg/kg body weight. The petitioner has said that the analytical methodology differentiates between ivermectin and AVM (C. Deyrup telecon with R. Dybas, Merck, July 2, 1987). RCB reiterates its need for chromatograms of representative animal commodities containing AVM and ivermectin to validate this claim.

4e. Residue Chemistry Data Requirements in 40 CFR 158.125(b)(15) require that regulated pesticide residues be subjected to one or more of the multiresidue procedures published in an Addendum to Pesticide Assessment Guidelines Subdivision O - Residue Chemistry Data Requirements for Analytical Methods in 40 CFR 158.125, Multiresidue Protocols.

To our knowledge, such testing has not been conducted on AVM. Therefore, the following data will be required: Residues of AVM in/on crop samples must be subjected to analysis by multiresidue protocols. Protocols for Method I, II, III, and IV are available from National Technical Information Service under Order No. PB 86 203734/AS.

5a. In the absence of the yet to be submitted storage stability data for <u>both</u> AVM B_{1a} and its delta-8,9 isomer on orange, lemon, and grapefruit, RCB cannot arrive at a final conclusion regarding the integrity of submitted citrus RAC samples.

5b. At this time, RCB can draw no conclusions on the adequacy of the residue data until the petitioner submits the requested bridging or validation data for Method No. 1009 Revision No. 2 and offers adequate documentation and justification as to why the residue values on oranges obtained from the two California locations (Santa Paula and Tulare counties) should not be considered. In addition, if TB expresses concern regarding unidentified polar degradates of AVM which may comprise up to 70 percent of the total terminal residue in citrus, then the petitioner must revise the tolerance expression to include identified polar degradates, develop validated enforcement analytical methodology to determine these degradates, and generate additional citrus residue data utilizing this methodology.

6a. Processing data previously submitted in conjunction with PP#5G3287/FAP#5H5474 showed no concentration of AVM B_{1a} residues in finished pulp, juice, and molasses fractions of oranges, tangerines, and grapefruit. Therefore, no FATs will be needed for these commodities.

6b. RCB, at this time, is unable to comment on the adequacy of the proposed food/feed additive tolerance in citrus oil and pulp until RCB's remaining questions regarding the nature of the residue in plants, adequacy of the submitted residue data, validation of Merck Method No. 1004 Revision 1 and submission of relevant sample storage stability data by the petitioner have all been addressed.

7a. The residue data from the cattle feeding study did not include analyses for the 24-hydroxymethyl metabolite or its conjugate. This metabolite has been found to comprise 40 percent of the TRR in goat kidney. If TB should conclude that 24-hydroxymethyl AVM and its conjugate are of toxicological concern, feeding studies reflecting analyses for these metabolites will also be required.

7b. RCB, at this time, is unable to comment on the adequacy of the proposed permanent tolerances on cattle meat, meat byproducts, and milk until the nature of the residue in ruminants

is adequately understood, the proposed enforcement methodology for animal commodities has has passed a successful EPA method validation and the need to include 24-hydroxymethyl AVM in the tolerance expression for animal commodities has been determined.

8. Neither Codex, Canada, nor Mexico have established tolerances for residues of AVM on citrus. There will be no compatibility problem if the proposed tolerance on citrus is established.

Recommendations

RCB recommends against the proposed tolerances for residues of AVM B_1a and its delta-8,9 isomer on citrus, dried citrus pulp, citrus oil and the meat, meat byproducts, and milk of cattle because of the reasons given under Conclusions 2a, 2b, 3a, 3b, 3d, 3e, 4a, 4b, 4c, 4d, 4e, 5a, 5b, 6b, 7a and 7b.

TB should be informed of RCB's deference to them regarding 24-hydroxymethyl AVM (free and conjugated) in ruminants (Conclusion 3d) and the need for investigating the metabolism of AVM B_1b (Conclusion 3c).

Note to PM: RCB suggests that the petitioner be given a copy of the complete review.

Detailed Considerations

Manufacturing Process

AVM B_1 is produced by a fermentation process using a strain of Streptomyces avermitilis. The technical product is extracted from the broth and purified by recrystallization. A more detailed description of the manufacturing process was given in RCB's May 1, 1986 review of EPA 618-OL (memorandum of L. Cheng). The technical product is a mixture of AVM B_1a and B_1b; the structures of these AVMs, which differ by the presence of an additional methylene group, are depicted in Attachment 2. The ratio of AMV B_1a to B_1b is >80:20. The technical material is about 95 percent AVM B_1 and contains about 0.5 percent of other AVMs of elucidated structures. The technical also contains about 1 percent of unidentified impurities related to the AVMs. TB is not concerned with the AMV-related impurities (PP#5G3287/FAP #5H5474, memorandum of W. Dykstra, March 3, 1986).

Formulation

The formulation to be used on citrus is AGRIMEC 0.15 EC Miticide/Insecticide which contains 2 percent AVM. One gallon of the emulsifiable concentrate (EC) contains 0.15 lb AVM B_1 as the active ingredient. AGRIMEC 0.15 EC is the same formulation as MK-936 0.15 EC for which all inert ingredients were cleared for use under §180.1001 (PP#6G3320, AVM on cottonseed, memorandum of A. Smith June 23, 1986).

The label describes abamectin as:

> Avermectin B_1, [A mixture of avermectins containing $\geq$ 80% avermectin B_1a (5-O-demethyl avermectin A_{1a}) and $\leq$ 20% avermectin B_1b (5-O-demethyl-25-di (1-methylpropyl)-25-(1-methylethyl) avermectin A_1a)]

Proposed Use

Citrus

AGRIMEC 0.15 EC is an emulsifiable concentrate which will control citrus rust mite, broad mite, and twospotted spider mite when applied according to the directions for use. Apply using conventional dilute or concentrate ground sprayers calibrated to deliver sufficient water for thorough coverage. Gallonage of spray will vary with size and number of trees per acre and density of foliage. In any case, thorough coverage is essential for good mite control. Applications should be made with a minimum of 0.20% oil in the spray mixture or not less than 1.0 gallon of oil per acre.

		For Dilute[a/] Sprays	For Concentrate[b/] Sprays
Crop	Pests	fl oz/100 gal	fl oz/acre
Citrus	Citrus rust mite Broad mite	1 - 2	5 - 20
	Twospotted spider mite	2	10 - 20
Citrus Spray Oil		Minimum 0.2%	Minimum 1 gal/acre

a/ Apply in 500-2000 gallons full cover dilute spray depending on tree height and planting density. Do not exceed 20 fl oz AGRIMEC per acre per application.

TREE HEIGHT	Less Than 10'	10' to 12'	14' to 16'	18' +
fl oz AGRIMEC/acre	5 - 10	7.5 - 15	10 - 20	15 -20

b/ For concentrate sprays - adjust the dosage to apply an amount per acre equal to that used in full cover dilute spray. Use the 5 fl oz/acre rate only on trees less than 10 feet in height.

Remarks:
- Do not apply within 7 days of harvest.
- Do not apply more than 60 fl oz per acre in any 12-month period.
- Always apply in combination with spray oil as directed.

Remark a/ "Apply in 500-2000 gallons" should be changed to "Apply in 500-1000 gallons" in a revised label/Section B. This will keep the total dosage applied to 20 fl oz product/acre/application.

The submitted label also does not specify the treatment interval or the timing of applications. Application protocols submitted with each of the residue trials conducted in this petition specify first application will be made postbloom in April/May followed by applications in Summer and Fall (September/October) with spray intervals of ca. 60 days. Actual spray intervals ranged from 34 to 86 days and 17 to 92 days between the first and second and second and third applications, respectively. Sixty-seven percent of all spray intervals were 66 days or less.

In a revised Section B/label under Remark a/ "Apply in 500-2000 gallons" should be changed to "Apply in 500-1000 gallons" to keep the total dosage applied to 20 fl oz AGRIMEC per acre per application or 0.025 lb ai/A/application. The petitioner also needs a revised Section B/label which specifies the approximate treatment intervals and the timing of applications during the growing season. The timing of applications and intervals selected by the petitioner should correlate with or support the submitted residue data, otherwise additional citrus residue data will be needed to support the use pattern selected.

Nature of the Residue in Plants

No additional plant metabolism studies were submitted in the current petition.

Metabolism studies of AVM in cotton (PP#5G3220, PP#7F3500) and citrus (PP#5G3287) have been submitted. A metabolism study of AVM on pasture grass was submitted with PP#4F3065 but was

found to be deficient because bait granules of AVM were added to the grass through a glass funnel so that contact with the grass was minimal. The petitioner contends that the metabolic profiles obtained from the metabolism studies on cotton, citrus, and the photodegradate profile obtained from water photolysis are all similar. The studies on citrus metabolism were discussed extensively in the review of PP#5G3287 (memorandum of L. Cheng, December 19, 1985). In that review RCB concluded that the degradation of AVM-B_1a is very complex and that the majority of the metabolites are highly polar in nature. Undegraded AVM-B_1a and its delta-8,9 isomer exist only as a small portion (2 to 13%) 2 weeks after application. For the purposes of that review RCB considered the parent and its delta-8,9 isomer as the residues of concern. For the permanent tolerance petition RCB considered AVM-B_1a and its delta-8,9 isomer as the residues of concern provided TB is not concerned with he "polar degradents" isolated from citrus and the "column wash" fractions from orange fruit.

In its memorandum of February 11, 1987 (PP#7F3468/FAP#7H5518, memorandum of L. Cheng), RCB addressed the question of using photolytically produced polar degradates for testing purposes instead of using residues produced on the fruit. RCB stated that the following three paragraphs are directed to the permanent tolerance petition.

"In our previous review (PP#5G3287) we stated that if more testing of the 'polar degradents' (the major component in the residue) should be required by TB, tests should be conducted with materials isolated from citrus rather than from in vitro (thin film) experiments. Merck responded that the use of the 'polar degradents' fraction isolated from in vitro was valid. The registrant argued that the polar degradate fraction is 'photolytically formed on the fruit surface' and not by plant metabolism, and that it would be difficult to generate on fruit sufficient quantities of the polar degradates for testing due to the low use rate. Furthermore, degradation on both citrus and glass surfaces had been shown to lead to the same products.

Merck's own data showed that degradation on citrus is only accelerated by light. Formation of the polar degradates still occurs in absence of light. While we agree with Merck's presentation that the HPLC activity profiles and the GPC characteristics of the citrus polar degradates and the thin film degradates are very similar, we have not concluded that the two polar degradates are identical. Any possible interaction between AVM B_1 and its degradation products with citrus peel would be absent on glass surface. Merck has characterized this fraction to be 'non-avermectin like' thus far, but the exact identity of the polar degradates remains unknown. Merck can either fully

identify the polar fraction or, if required by TB, conduct testing using the material isolated from the field.

Merck also responded to the other issue raised by RCB on the 8- and 12-week 'column wash' fractions of the orange peel. The registrant argued that repeated RP-HPLC treatments of the 'column wash' fractions led to similar profiles, and that NP-HPLC analysis also resulted in 'column wash' fractions, indicating these were not distinct nonpolar materials. Furthermore, particulate matter and oils (natural products) were obtained upon concentration of the 'column wash' fraction. Merck theorized that the amount of the natural products admixed with AVM B_1a residues were nonselectively bound to the HPLC column, resulting in 21 to 33 percent of the recovered activity as 'column wash' fractions in the 8- and 12-week orange peels. In view of the fact that less than 10 percent of the residues were isolated in the 'column wash' fraction in lemon and grapefruit throughout the entire 12-week study period, it appears that the 'column wash' fractions in the 8- and 12-week orange peels contained previously characterized components, and that these two 'column wash' fractions contained about 10 percent of the avermectin residues. The 'column wash' fractions in citrus would not pose any residue problem."

In its July 29, 1987 C. Dreyrup review of PP#7F3500 Avermectin B_1 on Cottonseed, RCB reiterated the above conclusion that investigations should be carried out on plant degradates rather than on residues generated from photolysis.

Two cotton metabolism studies were submitted with PP#6G3320. The data were resubmitted with PP#7F3500 along with some additional findings. In its review of these studies RCB commented that the metabolism of AVM by cotton has been shown to be extremely complex. Except for parent, the delta-8,9 isomer, and palmitic and linoleic acids, the petitioner has only been able to characterize the residues in terms of whether the residues are more or less polar than the parent. On this basis, it is difficult for RCB to conclude, as the petitioner has, that the metabolic pathways in citrus and cotton are the same. In both citrus and cotton, polar degradates comprise a major portion of the activity. Also, radioactive linoleic fatty esters were found in both citrus and cotton.

RCB suggested that the petitioner examine the polar cotton and citrus degradates with TLC using solvent systems aimed at moving polar residues. Since the polar degradates form a major portion of the terminal residues, if the petitioner could demonstrate that TLC chromatograms from corresponding extracts of the citrus and cotton studies are similar, and that chromatograms from NP-HPLC, GPC, and RP-HPLC with gradient elutions from the cotton and citrus study are all similar, RCB could then

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conclude that the metabolism of AVM by citrus and cotton is similar.

In PP#7F3500 RCB recommended that the polar residues, which form a major portion of the terminal residues in cotton, will need to be further investigated. For example, the petitioner should determine whether the macrocyclic ring is intact and whether the sugar moieties are still attached to the AVM nucleus. RCB furthermore concluded (TB considerations permitting) that metabolism studies using AVM B_1b are not needed; studies using AVM B_1a adequately reflect the metabolism of the technical product, which may contain up to 20% AVM B_1b.

Followup to RCB Reviews of PP#7G3468 (Citrus) and PP#7F3500 (Cotton)

Plant metabolism studies had demonstrated that up to 70 percent of the total radioactive residue in citrus consisted of unidentified polar degradates. Since these polar degradates do not result from animal metabolism of AVM, TB had requested that toxicological testing of the polar degradates be carried out. Merck asked for a meeting in order to discuss the material to be used in testing. RCB (PP#7G3468/FAP#7H5518, memorandum of L. Cheng, February 11, 1987; PP#7F3500, memorandum of C. Deyrup, July 29, 1987) had recommended that the toxicological testing be carried out on polar degradates isolated from citrus rather than on degradates arising from the photolysis of AVM coated on petri dishes.

At an August 21, 1987 conference with the petitioner, Merck predicted that it would not be possible to carry out toxicological testing with polar degradates isolated from fruit because co-extractants from the fruit would probably also be toxicologically active.

RCB responded that the petitioner had the option of identifying the polar residues. Merck argued that it would be impossible to identify the degradates, since they thought that there were probably over 100 components comprising the polar degradates. Merck said that since the polar degradates occur at the 7 to 12 ppb level in citrus, any component would have to be tremendously toxic to have an effect.

RCB countered that aside from describing these residues as "polar," the petitioner had submitted no further characterizations of the polar degradates. RCB suggested that the petitioner submit data demonstrating the complexity of the mixture of polar degradates. Until this meeting, RCB did not know whether the polar degradates consisted of 5 components or 100 components.

RCB could then ask TB if it were still concerned regarding the polar metabolites. RCB and TB need more information on the nature of the unidentified residues besides the fact that they are polar.

The petitioner agreed that this approach could be feasible, and the meeting ended.

RCB's position as a result of this meeting was summarized as follows:

1. RCB is not convinced that the polar degradates from fruit are the same as those from a thin film photolysis and believes that it may be impossible to establish that the residues from the two sources are sufficiently identical for testing purposes;

2. The petitioner should demonstrate the complexity of the nature of the polar degradates isolated from fruit; and

3. The petitioner should attempt to further characterize the nature of the polar degradates (e.g., ^{13}C-AVM with mass spectroscopy).

Subsequent to the August 21, 1987 conference, the petitioner submitted a protocol for the generation of AVM polar degradates to be subjected to toxicological testing (teratology testing and Ames test). Generating the required amount of material from citrus would involve treating and processing 240,000 fruit. The petitioner had proposed to treat fruit at a 30X rate to generate enough material for the teratology test and to photolytically generate the 1.5 g needed for the Ames test.

RCB suggested (see C. Deyrup October 20, 1987 review of Avermectin B_1 Proposed Test Protocol) that the petitioner submit chromatograms of the polar degradates from 1X and 30X radiolabeled studies so that RCB could determine whether the 30X experiment yields polar degradates which are qualitatively similar to those from the 1X study. RCB cautioned the petitioner that if the 30X and 1X studies yielded totally different profiles, the proposed 30X experiment may be irrelevant. RCB also suggested that the petitioner submit chromatograms of the polar degradates generated photolytically for comparison with the chromatograms from the fruit study.

In response to RCB's suggestion, the petitioner at a October 29, 1987 conference brought slides of the chromatograms of the polar degradates generated from a 1X treatment of oranges, a 10X

treatment of oranges, and from photolysis of a film of AVM in a petri dish.

The one major difference among the chromatograms was the relative contribution of the most polar degradates. Both the 1X and the photolytic studies contained a relatively large amount of these degradates; but this was not the case with the 10X study. About 10 percent of the AVM was left in the 1X study, about 8 percent of the AVM was left in the photolytic experiment, and about 35 percent of the AVM was left in the 10X study. The petitioner believes that the most polar degradates represent extensive degradation. Therefore, the 10X study exhibits the least conversion to these degradates because breakdown of AVM has not yet progressed to the extent observed in the other studies.

RCB responded that the petitioner's explanation was logical but it implied that there would be even less of these polar degradates in a 30X experiment. RCB asked the petitioner whether it would be possible to better characterize the more polar metabolites. The petitioner said that NMR and mass spectrometry could be useful in characterizing the degradates from the photolytic experiment, but the presence of the co-extractants would preclude the use of these techniques with any fruit-derived material. RCB pointed out that since the petitioner proposed to use material from 30X treated fruit for testing and since this material may be deficient in major residues present in 1X treated fruit, any evidence that this polar material is not structurally related to AVM would ease our concern on the significance of this residue. The petitioner revealed that FAB-MS of photolytically derived material has indicated that extensive oxidation has occurred.

The petitioner agreed that there could be even less of the more polar degradates in a 30X study, and pointed out that the photolytic polar degradates looked more representative of the 1X study than the 10X study did. Since the proposed 30X study might produce very little of the most polar degradates, RCB suggested that two teratology studies might provide better toxicological information. Photolytically-generated material would contain the more polar degradates, and the fruit-derived material would contain any possible residues resulting from the interaction of AVM and the fruit. TB said that two teratology studies would alleviate some of the concerns they had over the use of material derived solely through photolysis, or solely through the 30X treatment of fruit. The petitioner agreed to carry out two teratology studies.

Since the petitioner cannot characterize the fruit-derived material, RCB asked whether the petitioner could use TLC to demonstrate whether any of the more polar metabolites from the

fruit-derived material are actually present in the photolytically generated material. If the photolytically derived material is to serve as a surrogate source of the more polar degradates from fruit, any assurance that the two materials contain common components would be helpful. The petitioner said that TLC might be useful.

Based on the citrus metabolism data submitted to date, RCB concludes that the residues of concern on citrus consist of AVM $B_{1}a$ and its delta-8,9 isomer provided that TB expresses no concern regarding the identities of the polar degradates which may comprise up to 70 percent of the total terminal residue. TB's opinion in turn regarding the toxicological significance of these polar degradates is predicated upon their favorable review and evaluation of the teratology tests and the Ames test conducted on these polar degradates when submitted by the petitioner. If, on the other hand, TB's evaluation of the teratology test or Ames test is unfavorable, wherein the identities of the polar degradates are needed, then the petitioner must characterize the subject polar degradates as comprising a significant portion of the total toxic residue. Furthermore, the tolerance expression must be revised to include these polar degradates; validated enforcement analytical methodology developed; and additional residue data on citrus generated utilizing this methodology.

Nature of the Residue in Animals

No animal metabolism studies were submitted with this petition.

Rat metabolism data were submitted in conjunction with PP#4F3065 (Avermectin on Range and Pasture Grass, memorandum of F. Boyd, September 13, 1984), PP#5F3287 (Avermectin on Citrus, memorandum of L. Cheng, December 19, 1985) and PP#7G3468 (Avermectin on Citrus, memorandum of L. Cheng February 11, 1987).

The major metabolite identified in rats was 3" desmethyl AVM with a minor metabolite < 10 percent of the TRR identified as 24-hydroxymethyl AVM.

The delta-8,9 isomer is a photolysis product of AVM and has been found on oranges, cotton leaves, and celery leaves. It is not found in animals. Therefore, the petitioner investigated the metabolism of the delta-8,9 isomer by rats. Metabolites analogous to those detected in the AVM study were found, namely the corresponding 24-hydroxymethyl and 3" desmethyl isomers. The delta-8,9 isomer and its metabolites accounted for 92 to 98 percent of the extractable activity from rat tissues.

Goat metabolism studies were submitted with PP#5F3065 and PP#5G3287.

The major metabolite identified in goat tissues was 24-hydroxymethyl AVM also known as metabolite A in both the rat and goat studies. No metabolite B (3" desmethyl AVM), the major metabolite found in rats, was reported in any tissues. The petitioner cites studies which report that ivermectin (22,23-dihydro AVM), which is registered for use on animals, gives rise to the 3" desmethyl analog of AVM in swine.

In the absence of any additional animal metabolism studies in the current submission, RCB reiterates its previous (C. Deyrup memorandum of July 29, 1987 PP#7G3500 Avermectin B_1 on Cottonseed) summary comments and conclusion regarding animal metabolism studies:

"If TB considerations permit, RCB concludes that metabolism studies using AVM B_1b are not needed; studies using AVM B_1a adequately reflect the metabolism of the technical product, which may contain up to 20 percent AVM B_1b.

The petitioner will need to confirm the identity of the 24-hydroxymethyl metabolite through another analytical means besides co-chromatography with the standard with HPLC. Also, the conclusion that identical hydrolysis products arise from the 24-hydroxymethyl AVM standard and from the nonpolar conjugate should be tested by another analytical method (see RCB C. Deyrup October 30, 1987 memorandum of amendment to PP#7F3500).

RCB concluded that the goat metabolism study, which used ^3H-AVM was adequate to support a temporary tolerance on citrus and meat and milk (PP#7G3468, memorandum of L. Cheng, February 11, 1987).

However, RCB is not convinced that the tritium label is suitable for the establishment of permanent tolerances.

Although the tritium label appeared to be stable in the rat studies, RCB does not consider the use of tritium to be appropriate in ruminant studies for the following reasons:

1. The petitioner has demonstrated that the tritium is lost during metabolism in soil;

2. The metabolic pathways may be different in rats and ruminants; to RCB's knowledge, no 3" desmethyl AVM has yet been found in ruminants, although this is the major metabolite in rats;

3. The metabolism which resulted in the loss of the label and the formation of tritiated water in the soil study may have been microbial in nature, and a significant portion of ruminant digestion is also microbial in nature;

4. If tritium is lost through a redox reaction, RCB is not certain how much tritium would be released as water or how quickly the tritiated water would be released; and

5. Although ^{14}C volatiles are also lost in soil and plant metabolism studies, loss of tritium is of more concern because it is more likely that an unlabeled metabolite of toxicological concern could arise. The loss of volatile ^{14}C fragments would probably coincide with substantial AVM degradation.

RCB defers to TB on the need for regulating the 24-hydroxymethyl AVM metabolite (free and conjugated). The 24-hydroxymethyl AVM metabolite accounts for 40 percent of the TRR in kidney, 16.5 percent of the TRR in liver, 6 percent of the TRR in fat, and 11 percent of the TRR in milk. Extrapolating from the feeding studies to a 1X rate (based on the impact of cottonseed in the diet only), the 24-hydroxymethyl AVM level could be about 0.1 ppb in tissues and 0.003 ppb in milk. (If a tolerance on citrus should be established [PP#7G3468] residues of 24-hydroxymethyl AVM could range up to 5 ppb in tissue and 0.5 ppb in milk).

RCB concludes that the nature of the residue in ruminants is not adequately understood. The petitioner will need to conduct a goat metabolism study using ^{14}C-AVM; a higher dosage rate may be needed to identify the terminal residues."

Analytical Methodology

Citrus (RAC's)

Citrus residue data previously submitted to RCB (L. Cheng February 11, 1987 memorandum re: PP#7G3468/ FAP#7H5518) were analyzed using Merck Method No. 1004 "LC-Fluorescence Assay for Avermectin B_{1a} in and on Citrus Peel, Pulp, and Processed Fractions." This method was described in detail in the December 19, 1985 L. Cheng memorandum re: PP#5G3287/FAP#5H5474. The reported limit of detection was 1 to 2 ppb (2 ppb for lemon peel in RCB's judgment) with a limit of reliable measurement of 3 ppb stated in PP#7G3468. All controls were < 5 ppb. Recovery data were 60 to 110 percent at 1, 5, 10 and 20 ppb B_{1a} fortified in citrus peel and 61 to 110 percent at 1, 5, and 10 ppb B_{1a} fortified in citrus pulp.

The original Merck Method No. 1004 codetermined both AVM B_1a/B_1b and their delta-8,9-isomers as single components with the parent compound and its isomer having the same HPLC retention times. Due to variability in the derivatization reaction, the delta-8,9 isomer [which is about 30 to 40 percent of the AVM B_1a residue level in citrus (PHI = 7 days)] is only partially determined by this method. A new method (Merck Method No. 1009R01) is based on the formation of different fluorescent derivatives formed under stronger reaction conditions than the previous method (trifluoroacetic anhydride rather than acetic anhydride acylation). The new method measures quantitatively both AVM B_1a and the B_1a delta-8,9 isomer as a single component; recovery values from samples spiked with either AVM B_1a or the delta-8,9-isomer are essentially equivalent. This method also quantitates AVM B_1b, and by analogy the B_1b delta-8,9 isomer.

All of the citrus residue data (orange, tangelo, lemon, and grapefruit) submitted in the current petition were analyzed using Merck Method No. 1009R01 (whole fruit RAC) "HPLC-Fluorescence Determination for Avermectin B_1 and its delta-8,9 Z Isomer in Citrus Fruit" dated January 13, 1987.

In this method, residues of AVM B1 and its delta 8,9 Z isomer are extracted from citrus fruit homogenate by blending with methanol. The filtrate is extracted twice with isooctane and the isooctane discarded. Ten percent NaCl solution is added to the methanol extract and this mixture is extracted two times with 0.01% t-butanol in methylene chloride. The combined organic extracts are concentrated. Acidic alumina is used for column clean-up of the concentrate. The eluant is evaporated to dryness and a fluorescent derivative is formed by reaction with N,N dimethylformamide/trifluoroacetic anhydride/1-methylimidazole reagent (Reagent B-1) for 1 hour at 30 °C, followed by reaction with methanolic ammonium hydroxide (Reagent B-2) for 1/2 hour at 30 °C. The reaction mixture is dissolved in chloroform and passed through a silica gel column for separation of the derivatized residue from derivatization reagents. The eluant is taken to dryness and dissolved in methanol. The derivatized residue is determined by reversed-phase liquid chromatography with fluorescence detection. As derivatization of the B_1a delta-8,9 Z isomer results in a peak with the same retention time as the parent AVM B_1a, the derivatized residue quantitated represents the sum of AVM B_1a and its delta-8,9 Z isomer, as shown below:

avermectin B_1a + delta 8,9 Z isomer —(heat, Reagent B)→ avermectin B_1a → single fluorescent peak

Retention times for the fluorescent derivatives of AVM B_1a/AVM B_1a delta 8,9 Z isomer and AVM B_1b/AVM B_1b delta 8,9 Z isomer are approximately 10 minutes and 8.5 minutes, respectively. As AVM B_1b is at most 20 percent (usually less than 10%) of the active ingredient and residue levels are generally less than 5 ng/g, the peak representing AVM B_1b/AVM B_1b delta 8,9 Z isomer is identified but not quantitated when residues are between 2 and 5 ng/g. Residues of AVM B_1b/AVM B_1b delta 8,9 Z isomer above 5 ng/g are quantitated in the same manner as AVM B_1a/AVM B_1a delta 8,9 Z isomer, using the B_1a standard curve.

An analysis set is comprised of no less than 5 standards and no more than 12 samples. The standards are run before and after the samples to ensure the stability of the HPLC system, the standards and the samples. For each analysis set, the slope and intercept are determined from the linear regression of the standards' peak height vs. concentration in nanograms per milliliter. Occasionally it has been observed that the peak height for one standard is much lower than expected. Because it is known that this observation can be attributed to low derivatization reaction yield, a single errant standard may be discarded in determining the regression coefficients.

The concentration of AVM B_1a in a residue sample is determined as follows:

$$C=(PK\ HT-I)/S \qquad UNK=(CxFV)/SW$$

Where: C = concentration of AVM B_1a/AVM B_1a delta 8,9 Z isomer in ng/ml in the final volume used for HPLC analysis, PK HT = peak height of AVM B_1a/AVM B_1a delta 8,9 Z isomer fluoro derivative, I = intercept, S = slope, FV = final volume used for HPLC analysis, SW = sample weight, UNK = concentration of AVM B_1a/AVM B_1a delta 8,9 Z isomer in ng/g in the unknown residue sample.

The following recoveries were reported:

Matrix	Residue	Fortification Level ppb	% Recovery
Oranges	AVM B_1a	5 - 25	(72 - 99) $\bar{X}$=88
Oranges	AVM B_1a D8,9 isomer	5 - 25	(48 - 86) $\bar{X}$=75
Oranges	AVM B_1b	5	(94 - 100) $\bar{X}$=97
Grapefruit	AVM B_1a	5 - 25	(74 - 92) $\bar{X}$=79
Lemon	AVM B_1a	5 - 25	(74 - 76) $\bar{X}$=75
Lemon	AVM B_1a D8,9 isomer	5 - 23	(76 - 84) $\bar{X}$=80

For all citrus RACs analyzed, the limit of detection for both AVM B_1a and its delta-8,9 isomer was reported at 2 ppb, the reported limit of quantification 5 ppb and values obtained > 2 ppb but < 5 ppb reported as nonquantifiable. Numerous sample chromatograms submitted by the petitioner for all citrus RACs analyzed support those residue detection and quantification levels. All citrus control values for both AVM B_1a and its delta-8,9 isomer were reported at < 0.002 ppm. Submitted sample chromatograms also indicated excellent resolution between the AVM B_1a and its delta-8,9 isomer peaks and AVM B_1b and its delta-8,9-isomer peak.

Merck Method No. 1009R01 (dated January 13, 1987) in conjunction with PP#7G3468 (Avermectin B_1 on Citrus) was submitted to the EPA Beltsville laboratory for a method validation (see L. Cheng's memorandum dated April 13, 1987). However, as a result of this method validation request, Diane Rains of the Beltsville laboratory indicated that the revised method (No. 1009R01) emphasizes the use of a particular lot of acid washed aluminum oxide from J.T. Baker (Lot #619464) and that substitution of this column material from manufacturers other than J.T. Baker may give unacceptable results. The revised method further states that "if the specified lot number can no longer be obtained Merck should be notified before you begin using a different lot of alumina." Ms. Rains has indicated that such constraints placed on the use of aluminum oxide do not satisfy the requirements for an enforcement method.

The Beltsville chemist noted in her April 20, 1987 memorandum that if Merck provides a method for testing the aluminum oxide and provides a way to standardize it, this would not be a problem.

Merck subsequently submitted the requested information and procedures on how to validate new lots or additional manufactured sources of alumina other than J.T. Baker Lot No. 619464 specified in Method No. 1009R01. RCB recommended (see L. Cheng May 22, 1987 memorandum) that the submitted information be incorporated into Method No. 1009R01. This method incorporation was made and the redesignated method Merck Method No. 1009 Revision No. 2 dated July 31, 1987 was subjected to an EPA method validation on whole oranges (Avermectin B_1 at 0.0051 and 0.010 ppm) (see J. Wilner ACS/COB September 30, 1987 memorandum to E. Zager, RCB). In this memorandum, the Beltsville chemist concluded that the method appears suitable for enforcement action although it is a little long.

RCB notes that all of the citrus residue data submitted in the current petition were generated with Merck Method No. 1009R01 not with the revised Method No. 1009R02 which was favorably validated on oranges in ACS/COB's September 30, 1987 method validation. Therefore to validate some of the citrus RAC residue data provided in this petition RCB will require that the petitioner standardize his aluminum oxide as specified in Method No. 1009R02 to achieve adequate bridging data between the two residue methodologies. To accomplish this bridging data RCB recommends that selected RAC samples containing finite residues (i.e., > 5 ppb) be reanalyzed simultaneously by both methods and the results reported and compared. Simultaneous reanalysis would alleviate any concerns RCB would have concerning storage stability of existing RAC samples. As an example, RCB would suggest reanalysis of samples Nos. 70402359, -60, -63, -64; 70403492, -93; 70402343, -44, -47, -48, -49, -50.

Citrus Processed Fractions

(Dried Citrus Pulp and Citrus Oil)

All of the citrus fractionation samples (dried peel [pulp] and oil of oranges, tangelos and grapefruit) submitted in the current petition were analyzed using Merck Method No. 1004 Revision No. 1 "HPLC-Fluorescence Method for Determining Avermectin B_1 and Its Delta 8,9 Isomer in Citrus Processed Fractions" dated June 26, 1987.

This method for AVM B_1 and its delta 8,9 isomer is applicable to the analysis of citrus processed fractions, including dried peel, chopped peel, oil, and processing liquids. Residues of AVM are extracted from the fruit matrix by blending with methanol. Nonpolar coextractives are removed by washing with isooctane. The methanol solution is made aqueous with sodium chloride solution and the AVM residues are extracted into methylene chloride containing 0.01% t-butanol. The methylene chloride extracts are dried with

sodium sulfate, concentrated and then purified using acidic alumina column cleanup. The eluant is evaporated to dryness and the residue is derivatized by reaction with trifluoroacetic anhydride and 1-methylimidazole in dimethylformamide to form fluorescent derivatives. The derivatization is completed by reaction in methanolic ammonium hydroxide. The derivatized residue is purified and then determined by reverse phase HPLC with fluorescence detection.

Samples of processing liquids, such as rinses, are assayed by loading the sample onto a C-8 solid phase extraction column and eluting with acetonitrile. An aliquot of the acetonitrile eluant is derivatized by the same trifluoroacetic anhydride reaction to form the fluorescent derivatives which are assayed by HPLC with fluorescence detection.

Derivatization of AVM B_1a delta-8,9 isomer by trifluoracetic anhydride results in a peak with a retention time identical to that of the parent AVM B_1a derivative. The AVM B_1b derivative elutes before the AVM B_1a + delta-8,9 peak so two separate residues can be quantitated: AVM B_1b as one and AVM B_1a + AVM B_1a delta-8,9 isomer as the second.

Under the given chromatographic conditions, the fluorescent derivatives of AVM B_1a or AVM B_1a delta 8,9 elute at the same retention time, approximately 10 minutes, while the AVM B_1b derivative elutes at approximately 8 minutes.

An analysis set is comprised of no less than 5 standards and no more than 12 to 15 samples. The standards are run before and after the samples to ensure the stability of the HPLC system, the standards, and the samples. The peak heights of the standards or samples are measured at the retention time of the components of interest in cm or electronic units, depending on the data acquisition system. For each analysis set, the slope and intercept are determined from the linear regression of the standards' peak height versus concentration in nanograms per milliliter. A single errant standard may be discarded in determining the regression coefficients if there is reason to suspect the standard.

The concentration of AVM B_1a + AVM B_1a delta 8,9 isomer or AVM B_1b in a residue sample is determined as follows:

$$C=(PK\ Ht-I)/S \qquad Unk=(CxFV)/(SWxFrac)$$

where: C = concentration of B_1a + 8,9 or B_1b in ng/mL in the final volume used for HPLC analysis, Pk Ht = peak height of the components of interest, I = intercept of the regression line, S = slope of the regression line, Unk = the concentration of AVM B_1a + delta 8,9 or AVM B_1b in the unknown residue sample,

FV = final volume used for HPLC analysis, SW = sample weight used in assay, and Frac = the fraction of sample used for the derivatization reaction (usually 0.5).

This method (Method No. 1004 Revision 1) is similar to Merck's Method No. 1009R01 for whole citrus fruit which was discussed above. However, some additional sample preparation steps for dried citrus peel and oil have been added.

The following recoveries were reported:

Matrix	Residue	Fortification Level ppb	% Recovery
Washed Fruit	AVM B_1a	5 - 50	(78-101) $\bar{X}$ = 88
" "	$B_1aD8,9$ isomer	5	(57-75) $\bar{X}$ = 66
Dried Peel (Pulp)	AVM B_1a	10 - 75.2	(85-118) $\bar{X}$ = 101
" "	$B_1aD8,9$ isomer	9.6 - 72	(68-97) $\bar{X}$ = 86
Chopped Peel (Pulp)	AVM B_1a	10	(76-81) $\bar{X}$ = 78
" "	$B_1aD8,9$ isomer	9.4	(77-81) $\bar{X}$ = 79
Citrus Oil	AVM B_1a	50	(73-83) $\bar{X}$ = 74
" "	$B_1aD8,9$ isomer	47 - 49	(52-78) $\bar{X}$ = 63

The reported limit of reliable quantitation for the fruit fractions was 5 ng/g. The residue peak representing AVM B_1a + AVM B_1a delta 8,9 isomer was identified but not quantitated (reported as NQ) when present at levels between 2-5 ng/g for fruit fractions. For the processing liquids the detection and quantitation limits for both components were 0.1 ng/mL. The residue peak representing AVM B_1b was treated the same as the AVM B_1 + delta 8,9 isomer, but since AVM B_1b is at most 20 percent (usually less than 10%) of the active ingredient, the B_1b peak is seldom present. Residues at or below the detection limit for either component were reported as not detected (ND).

Numerous sample chromatograms submitted by the petitioner for all citrus fractions analyzed support these residue detection and quantification levels. With the exception of one tangelo prewash rinse control sample at 0.3 ppb, all control values were reported as ND or < 0.002 ppm. RCB notes, however, that chromatograms of a number of orange dried peel (e.g., sample Nos. 402DPC, 400DP and 401DP) and tangelo dried peel (e.g., sample Nos. 502DPC, 500DP and 501DP) samples including controls indicated background or baseline levels not only high but off scale in many instances making analytical measurements difficult if not impossible. The petitioner should explain the reasons

for those high background levels for orange and tangelo dried peel control and sample chromatograms and discuss what procedures were implemented or are proposed to reduce the background levels. As previously observed with Merck Method No. 1009R01, the submitted sample chromatograms for Merck Method No. 1004 Revision No. 1 also indicated excellent resolution between the AVM B_1a and its delta 8,9-isomer peak and AVM B_1b and its delta-8,9 isomer peak. Because generic rather than specific commodity recovery data only were provided (i.e., washed fruit, dried citrus peel [pulp], citrus oil) the petitioner should submit to RCB specific processed commodity recovery data on orange peel (pulp), grapefruit peel (pulp), tangelo peel (pulp), orange oil, grapefruit oil and tangelo oil.

The EPA Method Validation of Method No. 1009 Revision No. 2 indicates that Merck needs to revise Method No. 1004 as per Method No. 1009 Revision No. 2 to standardize the testing of the aluminum oxide used in the method. The petitioner must also adequately address the questions posed by RCB regarding high background or baseline levels on submitted chromatograms and submit specific processed commodity (dried peel and citrus oil) recovery data reflecting any revisions made in the original procedure (Method No. 1004 Revision No. 1).

Animal Commodities

Since feed items are associated with citrus, a method capable of determining the residues of concern in animal commodities is required. Methodology for the determination of AVM was submitted with PP#7G3468 and was submitted for an EPA method validation (PP#7G3468,Method Trial Request, memorandum of L. Cheng, February 19, 1987). Residues are extracted from tissue with isooctane. Derivatization is with acetic anhydride/DMF/1-methylimidazole. After clean-up with a Sep-Pak, the residues are determined by HPLC. Recoveries from tissues fortified at levels of 2.34-46.8 ppb ranged from 81 to 96 percent.

Ethanol and ammonium hydroxide are added to milk, and residues are extracted with ethyl acetate and isooctane. After concentration, the residues are partitioned between acetonitrile and hexane. The acetonitrile is evaporated, and the residues are derivatized with acetic anhydride/DMF/1-methylimidazole. Recoveries of AVM ranged from 65 to 110 percent from samples spiked at levels of 1 to 5 ppb.

Subsequent to the original EPA method validation request, the registrant has revised its proposed enforcement method for the analysis of abamectin residues in meat and milk. The revised method is Method #32A entitled "HPLC-Fluorescence Assay for Avermectin B_1a, B_1b and the Avermectin B_1a Delta-8,9-Isomer In Bovine Tissues and Milk." The major modifications are listed below (see L. Cheng November 5, 1987 memorandum Followup Method Trial request PP#7G3468):

- o Use of trifluoroacetic anhydride instead of acetic anhydride followed by methanolic ammonium hydroxide as the derivatizing agents (same as those used for citrus);

- o An extra clean-up step in the tissue analysis (liver, kidney, and fat but not muscle) using an aminopropyl column before derivatization;

- o Because of slight changes in the HPLC conditions (composition of mobile phase, temperature and pressure), retention time of the analytes is now about 17 minutes for tissues and 8 minutes for milk. Derivatives of abamectin and its delta-8,9-isomer have the same retention time.

The revised method does not address the concern about the differentiation between ivermectin and AVM B_1. Ivermectin is 22,23-dihydroavermectin B_1 and is a registered drug for use on large animals (see C. Deyrup's memorandum of July 29, 1987, PP#7F3500).

Since Beltsville laboratories have not yet tested the original version of the meat and milk method (memorandum of September 30, 1987 Jay Wilner), RCB requested that the revised Method No. 32A be tested for two chemicals on three commodities instead.

The results of the followup of the EPA Method Validation request are still pending.

At this time RCB cannot determine whether the proposed enforcement method (Merck Method No. 32A) is adequate for enforcement purposes until the method has passed a successful EPA Method Validation. A favorable conclusion regarding the adequacy of the proposed animal commodity enforcement methodology is also contingent upon the results of the requested ^{14}C-AVM goat metabolism study and TB's opinion regarding the need to regulate the 24-hydroxymethyl AVM metabolite (free and conjugated). If additional residues are determined to be of toxicological concern (such as 24-hydroxymethyl AVM) then appropriate analytical methodology may need to be developed.

Ivermectin (22,23-dihydro AVM) is registered for use on large animals at a rate of 0.2 mg/kg body weight. The petitioner has said that the analytical methodology differentiates between ivermectin and AVM (C. Deyrup telecon with R. Dybas, Merck, July 2, 1987). RCB reiterates its need for chromatograms of representative animal commodities containing AVM and ivermectin to validate this claim.

Residue Data

Storage Stability Studies

Experiments were begun in 1983 by the petitioner to examine the storage stability of AVM B_1 residues in citrus. Homogenized orange peel samples were fortified at 1, 10, and 25 ng/g B_1a; homogenized lemon and grapefruit peel samples were fortified at 5 and 25 ng/g. Samples were stored at below -10 °C until analysis by the AA derivatization described as the confirmatory method in Method 1009 Revision No. 1 January 13, 1987. Recoveries of AVM B_1a from grapefruit peel after 9 months and 4 years storage were 87 and 77 percent; orange peel after 13 months and 4 years storage were 73 and 67 percent and for lemon peel after 9 months and 4 years storage were 87 and 86 percent. The results were somewhat erratic and considered preliminary by Merck. Therefore, the petitioner has stated that additional stability studies on orange, lemon, and grapefruit whole fruit fortified with both AVM B_1a and the delta isomer have been started and the data will be submitted at a later time.

In the absence of this yet to be submitted storage stability data, RCB cannot arrive at any conclusions regarding the integrity of the orange, tangelo, grapefruit, and lemon residue samples submitted in the current petition which were stored frozen at -10 °C for 4 to 6; 3 1/2 to 5 1/2; 4 1/2 to 6; and 4 months, respectively, between field sampling and residue analysis.

Citrus Residue Data

The petitioner has submitted citrus residue data from 15 1986 trials conducted in the following major citrus growing states: Florida (8); California (4), Texas (2), and Arizona (1). Twelve residue studies were run on the RAC; in one of these studies samples were taken only on days 0 and 7 (the proposed PHI) while in the other three studies dissipation data were obtained at 0, 1, 3, and 7 days postapplication. Three of the residue studies served as fractionation studies which were run on orange, grapefruit, and tangelo, and samples were taken 3 days postapplication.

The formulation used in all trials was abamectin 0.15 EC, tank mixed with a narrow range crop oil and diluted with water. The maximum proposed use rate of 0.025 lb ai/A (1X) and an exaggerated rate of 0.05 lb ai/A (2X) was included in 13 of the 15 tests. In three tests (fractionation studies on orange, grapefruit, and tangelo), abamectin was applied at an exaggerated 4X rate of 0.1 lb ai/A. Narrow range crop oil was tank mixed at a rate of 0.25 percent or a minimum of 1 gallon per acre in all abamectin treatments. Spray volumes ranged from 100 to 1000

gallons of spray per acre with 500 gal/acre the most common volume used. Standard commercial spraying equipment was used in all tests with applications through airblast speed sprayers in 13 tests and oscillating handgun, vertical boom, and Kinkelder (concentrate sprayer) used in one test each. A minimum of three applications were made in all trials and four were made in one of the tests. First application was made postbloom (April/May), followed by applications in summer and fall (September/October) with spray intervals of about 60 days. Actual spray intervals ranged from 34 to 86 days and 17 to 92 days between the first and second and second and third applications, respectively. Sixty-seven percent of all spray intervals were 66 days or less.

RAC samples were picked and packed directly into plastic impregnated cloth sample bags and were transferred, unwashed into frozen storage within 1 to 3 hours after harvesting. The samples remained in frozen storage (at or below -10 °C) prior to and during shipment for analysis. Samples were all maintained at -10 °C in the laboratory until analysis.

1. Oranges

In 7 ((2) Florida; (1) Arizona; (3) California; and (1) Texas) residue trials at a 1X application rate and at the proposed PHI of 7 days, residues of AVM B_1a and its delta-8,9 isomer on whole orange samples ranged from < 0.002 to 0.0112 ppm and at 1.4X and 2X ranged from 0.0120 to 0.0147 ppm and < 0.002 to 0.0121 ppm, respectively. No residues of AVM B_1b (< 0.002 ppm) were detected in any trial. At all application rates residues of AVM B_1a delta 8,9-isomer were generally < 0.005 ppm.

2. Tangelos

In 2 (Florida) residue trials at a 1X application rate and at the proposed PHI of 7 days, residues of AVM B_1a, its delta 8,9-isomer and AVM B_1b were all reported at < 0.002 ppm.

3. Grapefruit

In 2 (Florida and Texas) residue trials at a 1X application rate and at the proposed PHI of 7 days, residues of AVM B_1a and its delta 8,9-isomer on whole grapefruit (pink and white) were all reported at < 0.002 ppm and at 2X ranged from < 0.002 ppm to > 0.002 but < 0.005 ppm. B_1b residues were all reported at < 0.002 ppm.

4. Lemons

In a (California) residue trial at both 1X and 2X application rates and at the proposed PHI of 7 days, residues of AVM B_1a and its delta 8,9-isomer on whole lemons were all reported at < 0.002 ppm.

RCB's Comments/Conclusions Re: Residue Data

Residues of AVM B_1a, and its delta 8,9-isomer were reported in whole oranges above the limit of quantification (0.005 ppm) in two of the three California field trials; Study No. 001-86-196R (Santa Paula) and Study No. 001-86-596R (Tulare County). In the former study at a 1X rate, residues ranged from 0.0078 to 0.0081 ppm and in the latter study at a 1X rate residues ranged from 0.0101 to 0.0112 ppm.

The petitioner contends that these two studies were improperly conducted based on the reports filed by the field biologists responsible for monitoring these field residue trials and results are being reported only as supplemental data. Regarding Study No. 001-86-196R the petitioner stated that one treated set at 0 day contained no residues and one set at 7 days contained unrealistically high residues. What the petitioner failed to mention was that both 1X treatment sets had higher residues at 0 days than at 7 days. Regarding Study No. 001-86-596R the petitioner contends that the 7-day residue sample reflected a 1.4X application rate (0.0120, 0.0147 ppm) which is true; however, another 7-day residue sample reflecting a 1X application rate (0.0101; 0.0112 ppm) had a 0-day counterpart which contained AVM B_1a and delta 8,9-isomer residues of < 0.005 ppm. The petitioner's arguments regarding the alleged anamolous residue data are not adequately supported and in addition it is very unlikely that aberrant or anamolous residue data would be obtained from two different locations within the same State both producing control values below the method limit of detection (< 0.002 ppm). Furthermore, to clarify this issue, RCB has recommended under the Analytical Methodology section of this petition that these subject samples be reanalyzed by Merck's revised Method No. 1009R02 to provide bridging data to this method which has undergone a successful EPA method validation by ACS/COB on September 30, 1987.

RCB can draw no conclusions at this time regarding the adequacy of the submitted citrus residue data to support the proposed tolerance until the petitioner submits the requested bridging or validation data for Method No. 1009R02 and offers adequate documentation and justification as to why the residue values on oranges obtained from the two California locations (Santa Paula and Tulare Counties) should not be considered. In

addition, if TB expresses concern regarding unidentified polar degradates of AVM which may comprise up to 70 percent of the total terminal residue in citrus then the petitioner must revise the tolerance expression to include identified polar degradates, develop validated enforcement analytical methodology to determine these degradates and generate additional citrus residue data utilizing this methodology.

Citrus Fractionation Study

Processing data previously submitted in PP#5G3287/FAP#5H5474 showed no concentration of AVM B_1a residues in finished pulp, juice, and molasses fractions of oranges, tangerines, and grapefruit. Concentration of AVM B_1a residues was observed in dried pulp, 3.6 for Hamlin oranges, 4 for tangerines, and about 1 for grapefruit; those respective concentration factors in oil were 2.7, 4.3, and 6. RCB had recommended in its L. Cheng December 19, 1985 review of that same petition that for a permanent tolerance petition, Merck needs to conduct a processing study on grapefruit starting with finite residues on the fruit.

In support of a petition for registration of Abamectin 0.15 EC for use as a miticide on citrus, field trials were run in Lake Alfred, Florida in the fall, 1986. Three applications at 4 times the recommended maximum use rate (or at 0.1 lb ai/acre) were made on grapefruit, Hamlin oranges and Orlando tangelos. Whole fruit samples were taken on the day of the last (third) application and 3 days after that application. Fruit harvested 3 days after application were processed at the pilot plant facility at the University of Florida, Lake Alfred, FL. Fractions were collected from the processing in addition to the field fruit (RAC), unwashed whole fruit and washed whole fruit. Whole fruit and fractionation samples were frozen immediately after collection and shipped frozen to the MSDRL facilities at Three Bridges NJ for analysis. Oil fraction samples were not frozen but were refrigerated and shipped separately. The exaggerated (4X) application rate and fruit sampling at 3 days rather than at the PHI of 7 days was employed to ensure whole fruit residues above 5 ng/g, the limit of reliable quantitation of the assay method.

RCB notes that the previously submitted processing study in PP#5G3207/FAP#5H5474 was conducted at a 1X application rate with samples collected at the recommended 7-day PHI.

1. Hamlin Orange. Residues of AVM B_1a and its delta 8,9-isomer on the whole fruit as a result of AVM B_1a treatments ranged from 9.5 to 10.2 and averaged 9.9 ppb. Residues on washed whole fruit were all reported at < 5 ppb. Residues in dried peel ranged from 41.4 to 46.8 ppb ($\bar{X}$ = 44.1 ppb) and in the oil fraction

ranged from 48.3 to 62.5 ppb ($\bar{X}$ = 54.1 ppb). RCB calculates the average concentration factors in orange dried peel and orange oil as 4.5 and 5.5, respectively. Comparable values reported in PP#5G3287/ FAP#5H5474 were 3.6 and 2.7.

2. Tangelo. Residues of AVM B_1a and its delta 8,9-isomer as a result of AVM B_1a treatments ranged from 16.9 to 17.1 and averaged 17.0 ppb. Residues on washed whole fruit ranged from 9.0 to 9.4 and averaged 9.2 ppb. Residues in dried peel ranged from 70.5 to 78.3 and averaged 73.7 ppb and in the oil fraction ranged from 114.5 to 120.6 and averaged 117.6 ppb. RCB calculates the average concentration factors in tangelo dried peel and tangelo oil as 4.3 and 6.9, respectively. Comparable values reported in PP#5G3287/ FAP#5H5474 for tangerines were 4.0 and 4.3, respectively.

3. Grapefruit. Residues of AVM B_1a and its delta 8,9-isomer as a result of AVM B_1a treatments ranged from 7.4 to 10.6 and averaged 9.0 ppb. Residues on washed whole grapefruit were all reported as < 5 ppb. Residues in dried peel ranged from 8.6 to 13.0 and averaged 10.9 ppb and in the oil fraction ranged from 83.9 to 89.0 and averaged 87.3 ppb. RCB calculates the average concentration factors in grapefruit dried peel and grapefruit oil as 1.2 and 9.7, respectively. Comparable (although estimated) values reported in PP#5G3287/FAP#5H5474 for grapefruit were < 1 and 2 to 6, respectively.

RCB's Comments/Conclusion Re: Fractionation Study

No sample storage stability data were submitted with the orange, tangelo, and grapefruit fractionation studies although citrus peel (dried pulp) and oil fractions were stored for periods of time approaching 3 months for the peel and 2 months for the oil samples. However, because of the relatively short time intervals involved, RCB will utilize the petitioner's forthcoming RAC storage stability data to validate the samples obtained from the citrus fractionation study.

RCB, at this time, is unable to comment on the adequacy of the proposed food/feed additive tolerance in citrus oil and pulp until RCB's remaining questions regarding the nature of the residue in plants, adequacy of the submitted residue data, validation of Merck Method No. 1004 Revision 1 and submission of relevant sample storage stability data by the petitioner have all been addressed.

Meat, Milk, Poultry, and Eggs

No animal feeding studies were submitted with the current petition. In conjunction with PP#7G3468, temporary tolerances for AVM B_1 and its delta 8,9-isomer were established in meat (0.01 ppm) and milk (0.001 ppm) arising from the use on citrus.

These temporary tolerances were based on the results of a 28-day feeding study (submitted with PP#7G3468) that was conducted on lactating cows fed AVM at levels of 10 ppb, 30 ppb, or 100 ppb in the diet. The following levels of AVM were detected.

Dose (ppb)	AVM Level (ppb)			
	Liver	Muscle	Fat	Kidney
10	3-4	1-2	2	1-2
30	5.0-7.6	2	4-6.0	2
100	18-20	2	9.8-14	4-5

AVM residues were not detected in the milk of cows fed at the 10 ppb level. At the 30 ppb feeding level, one milk sample (5-day) was reported to contain 1 ppb AVM. At the 100 ppb feeding level, AVM residues were first detected on Day 2 at a level of 1 ppb. The highest residue level was reported to be 4 ppb on Day 14. On Day 28, AVM residues in milk ranged from 1 to 1.1 ppb.

Recovery data were submitted with the feeding study. Recoveries of AVM from milk fortified at levels of 0.5 ppb to 4 ppb ranged from 65 to 124 percent. Recoveries of AVM from tissue fortified at levels of 5 to 20 ppb ranged from 82 to 95 percent. Recovery data provided with the proposed enforcement method (also submitted with PP#7G3468) reflected fortifications of tissue from 2.34 to 46.8 ppb. Recoveries at the lowest fortification level ranged from 81 to 85 percent.

Beef and dairy cattle may consume up to 33 percent dried citrus pulp in their diet. The dietary burden of AVM B_1 would be 33% x 0.1 ppm or 33 ppb. Results from the feeding study indicate that at the 30 ppb ingestion level, greater than 5 ppb of AVM B_1 residues would be transferred to liver and fat of cattle, but not in milk. Thus the proposed temporary tolerances of 0.005 ppm on meat and meat byproducts of cattle were not adequate. The registrant subsequently proposed, in an amendment, tolerances of 0.01 ppm on meat, meat byproducts, and fat of cattle which are currently in effect.

In a copending petition (PP#7F3500) proposing the establishment of a permanent tolerance of 0.005 ppm for residues of AVM B_1 and its delta 8,9-isomer on cottonseed, residues resulting from the proposed use on cottonseed and cottonseed hulls which

may comprise up to 40 percent of the diet of beef cattle and up to 25 percent of the diet of dairy cattle would impose a dietary burden of about 0.002 ppb for beef cattle and about 0.0012 ppb for dairy cattle.

The residue data from the cattle feeding study did not include analyses for the 24-hydroxymethyl metabolite or its conjugate. This metabolite has been found to comprise 40 percent of the TRR in goat kidney. If TB should conclude that 24-hydroxymethyl AVM and its conjugate are of toxicological concern, feeding studies reflecting analyses for these metabolites will also be required.

RCB, at this time, is unable to comment on the adequacy of the proposed permanent tolerances on cattle (meat and meat by-products) and milk until the nature of the residue in ruminants is adequately understood, the proposed enforcement methodology for animal commodities has passed a successful EPA method validation and the need to include 24-hydroxymethyl AVM in the tolerance expression for animal commodities has been determined.

Other Considerations

Neither Codex, Canada, nor Mexico have established tolerances for residues of AVM on citrus. There will be no compatibility problem if the proposed tolerance on citrus is established.

Attachment 1 - International Residue Limit Status
Attachment 2 - Avermectin B_1a and B_1b Structure

cc: (with Attachments 1 and 2): TOX,Circu.,R.F., PP#8F3592/
FAP#8H5550,Reviewer-M.F.Kovacs Jr.,PMSD/ISB(Eldredge)
RDI:JH Onley:4/5/88:RD Schmitt 4/5/88
TS-769:RCB:M.Kovacs:CM#2:Rm.810 x7689:Typist Kendrick:4/15/88
Edited by:mfk:4/20/88

ATTACHMENT 1

INTERNATIONAL RESIDUE LIMIT STATUS

J. Lee 3/18/88

CHEMICAL Avermectin

CODEX NO. _____

CODEX STATUS:

☑ No Codex Proposal Step 6 or above

Residue(if Step 8): _____

Crop(s)	Limit (mg/kg)

PROPOSED U.S. TOLERANCES:

Petition No. ~~PP# 8F3592/FAP# 8H5555~~

RCB Reviewer ~~M.F. Kovacs Jr.~~

Residue: ~~Avermectin + delta 8,9 isomer~~

Crop(s)	Limit (mg/kg)
Citrus	0.005
Cattle (meat + mbp)	0.005
Milk	0.001
Dried Citrus Pulp	0.03
Citrus oil	0.10

CANADIAN LIMITS:

☑ No Canadian limit

Residue: _____

Crop(s)	Limit (mg/kg)

MEXICAN LIMITS:

☑ No Mexican limit

Residue: _____

Crop(s)	Limit (mg/kg)

NOTES:

ATTACHMENT 2
PP#8F3592/FAP#8H5550

AVERMECTIN B_1
MK-936

a-Component R = C_2H_5 ≥ 80%
b-Component R = CH_3 ≤ 20%

* - location of tritium label
• - location of carbon-14 labels