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

FEB 13 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#8F3592/FAP#8H5550 - Citrus Derived Polar Degradates of Avermectin B_1a Used for Teratology Testing - Characterization of the Degradates - Amendment of November 8, 1988; MRID Nos. 409127-1 and 408833-1

DEB Nos.: 4735, 4736, and 4737

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and

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Health Effects Division (TS-769C)

THRU: Charles L. Trichilo, Ph.D., Chief
Dietary Exposure Branch
Health Effects Division (TS-769C)

Merck Sharp and Dohme submitted an amendment to Toxicology Branch (TB) including results of teratology testing of polar degradates from citrus. As a part of that amendment, data on the chemical characterization and production of the degradates were also presented. This review is an evaluation of those data.

This submission contains three reports:

Report 1 - Field study production of abamectin polar degradates in Clermont, FL.

Report 2 - Radiolabeled studies at Lake Alfred, FL Citrus Center to demonstrate similarity of degradates from 1X and 30X applications.

Report 3 - Isolation, processing, and identification of polar degradates from Report 1 Field Study.

The three reports will be evaluated in numerical order.

Recommendation

DEB recommends favorably for use of the citrus field produced polar degradates of avermectin B_1a as requested for toxicology testing. The terminal residues on citrus are chemically characterized in the same manner as the polar degradates previously described by DEB and previously requested to be toxicologically evaluated by TB.

Conclusions

1. Field studies using C^{14}-abamectin and nonlabeled abamectin were performed in Florida using Hamlin oranges.

2. The C^{14}-labeled chemical was used for:

   a. Identifying and characterizing the polar degradates;

   b. Devising adequate methods for removal and purification of polar degradates; and

   c. Confirming simulated commercial handling of citrus fruit with dissipation of applied abamectin.

3. HPLC radioprofiles of the citrus-derived polar degradates show their chemical characteristics to be representative of field-produced degradates, as previously characterized, and makes them suitable for toxicology testing.

Report 1 - Generation of the Polar Degradates of Abamectin on Oranges for Toxicity Testing

Abamectin is used at a low rate of application (0.02 to 0.025 lb ai/A). In order to obtain sufficient quantity of degradates for toxicity testing it was necessary to apply a 30X (0.75 lb ai/A) exaggerated rate of abamectin. A standard air blast sprayer was used to apply the abamectin in 500 gallons of water, to runoff. The formulation ingredients without abamectin were applied in like manner as a control. Sufficient Hamlin variety oranges were treated in this manner to allow harvesting of 10,000 fruit, each from the treated and control acreage. Details of pesticide application, climatic conditions, and harvesting are provided.

A small scale study using C^{14}-avermectin was performed in like manner to the above. The fruit in this study was analyzed to determine quantity of polar degradates in the rinsate from washed fruit and to follow the degradates through the processing procedure.

All fruit harvested from the field studies were stored immediately at 40 °F and were transported under refrigeration at 44 °F to the laboratory for analysis.

Report 2 - Generation and Isolation of the C^{14}-Polar Degradates of Abamectin from Citrus Fruits

In order to determine the quality/quantity of polar degradates and ascertain with C^{14}-labeling that the field protocol, as in Report 1, would produce degradates necessary for toxicity testing, five mature, 5-year-old orange trees in plastic pots were employed in this field/laboratory study. A sixth tree was maintained in an outdoor environment with all of its fruit receiving a 1X application of C^{14}-avamectin and all fruit simulating commercial wash and storage treatment.

The fruit of the five trees received C^{14}-abamectin at 1X (low specific activity), 30X (high specific activity), or blank formulation (without abamectin) as a paint application (applied to individual fruit by brush). In this manner it was possible to tag individual fruit on each tree so that various fruit on each tree received one of the four treatment solutions. Fruit were sampled at 0, 1, and 2 weeks following pesticide application. Samples were also obtained of simulated rainfall rinse, methanol rinses, and water rinses. These samples afforded an opportunity to follow the presence and dissipation of C^{14}-abamectin and its polar degradates.

Details of the protocol used in the Lake Alfred study and experimental circumstances noted during the course of the C^{14}-study are contained in the Appendix of Report 2.

Report 3 - Abamectin Degradation on Citrus Fruit and Preparation of Degradates for Teratology Studies

Field studies, as described in Reports 1 and 2, were performed with mature Hamlin oranges on the trees. In order to determine a rinse method for removal of B_1a residues, C^{14}-treated and nonlabeled B_1a-treated oranges were rinsed with methanol a single orange at a time. Two such methanol rinses showed removal of 80 to 90 percent C^{14}-B_1a-residues when assayed by HPLC. The peak patterns of the 1X and 30X treated oranges were found to be very similar. In such a manner, it was possible to devise a citrus rinsing apparatus capable of methanol rinsing 1200 to 1300 oranges as a batch process.

In a similar fashion, using the C^{14}-B_1a-treated oranges it was possible to determine loss of residues due to simulated normal rainfall and/or a simulated commercial washing of harvested fruit.

To determine the quantity of C^{14}-activity absorbed into the peel of the fruit it was necessary to prepare an acetone powder of the citrus peel and extract it with methanol.

Extraction and clean-up of the solvent fractions prior to C^{14} quantitation and HPLC qualitation are described in detail. In like manner the processing, extraction, and partial purification of the polar avermectin degradates for teratology testing are also described.

Using a Zorbax or IBM C18 column eluted with methanol/water, in increasing concentrations, it was possible to compare HPLC radioprofiles of the citrus-derived degradates. These profiles are presented in Figures 1, 2, and 3 (attached) taken directly from pages 90, 92, and 93, Laboratory Project Identification, PLM# -3, -4, Document No. 2 of the November 8, 1988 study submission.

Comparing each of the seven radioprofiles, qualitatively, as to peaks within the fractions and occurrence of same fractions, a distinct similarity is evident. These seven radioprofiles are of two methanol rinses of C^{14}-B_1a-treated fruit, 30X application and 14-day PHI (Figure 1); methanol rinses of 30X and 1X oranges harvested at 7 or 14 days, all four profiles (Figure 2); simulated commercial washwater rinse and methanol rinse, two profiles (Figure 3).

The radioprofiles in Figures 1, 2, and 3 also are patterned as the profiles previously presented for citrus (PP#8F3592, memorandum of M. Kovacs, April 25, 1988) and cotton leaves (PP#7F3500, memorandum of F. Boyd, August 5, 1988). These data show that the polar degradates derived from the methanol washes of approximately 10,000 oranges treated with abamectin at 30X application and harvested at 14 days PHI are adequately representative of the polar degradates of abamectin found on B_1a-treated oranges.

Attachments

cc: (With Atachments): Tox, Circu., R.F., PP#8F3592, Reviewer - V.F. Boyd, PMSD/ISB (Eldredge)
RDI: J.Onley, 1/23/89: R.D.Schmitt, 1/23/89
TS-769:DEB:F.Boyd:CM#2:RM810:X7379:
KENCO:1/25/89:Corrected by vg:2/2/89

ATTACHMENT TO MEMO OF F. Boyd, PP#8F3592/FAP#7H5550, 2/10/79

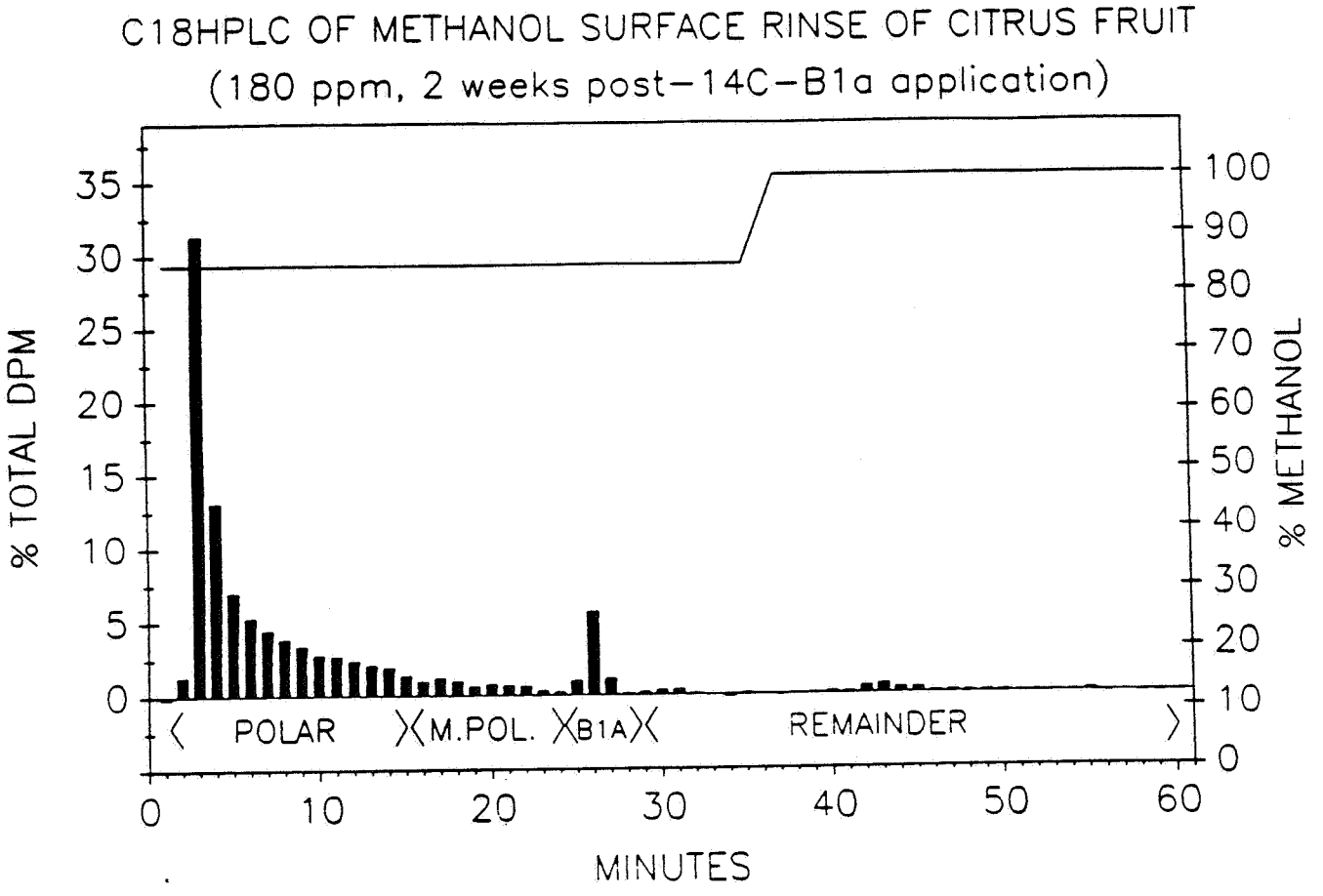

Fig. 1. C18HPLC radioprofile of residues in composite sample from 2 consecutive 15 ml methanol rinses of 5 ^{14}C-B1a-treated orange fruit (180 ppm, 13.0 uCi/mg B1a, 14 days PHI). Polar residues- t_R < 0.6 t_R of B1a; Moderately polar (M.POL.) residues- t_R between 0.6 and 0.95 of B1a. Right y axis indicates eluent composition.

ATTACHMENT TO MEMO OF F. Boyd, PP# 8F3592/FAP# TH5550, 2/10/89

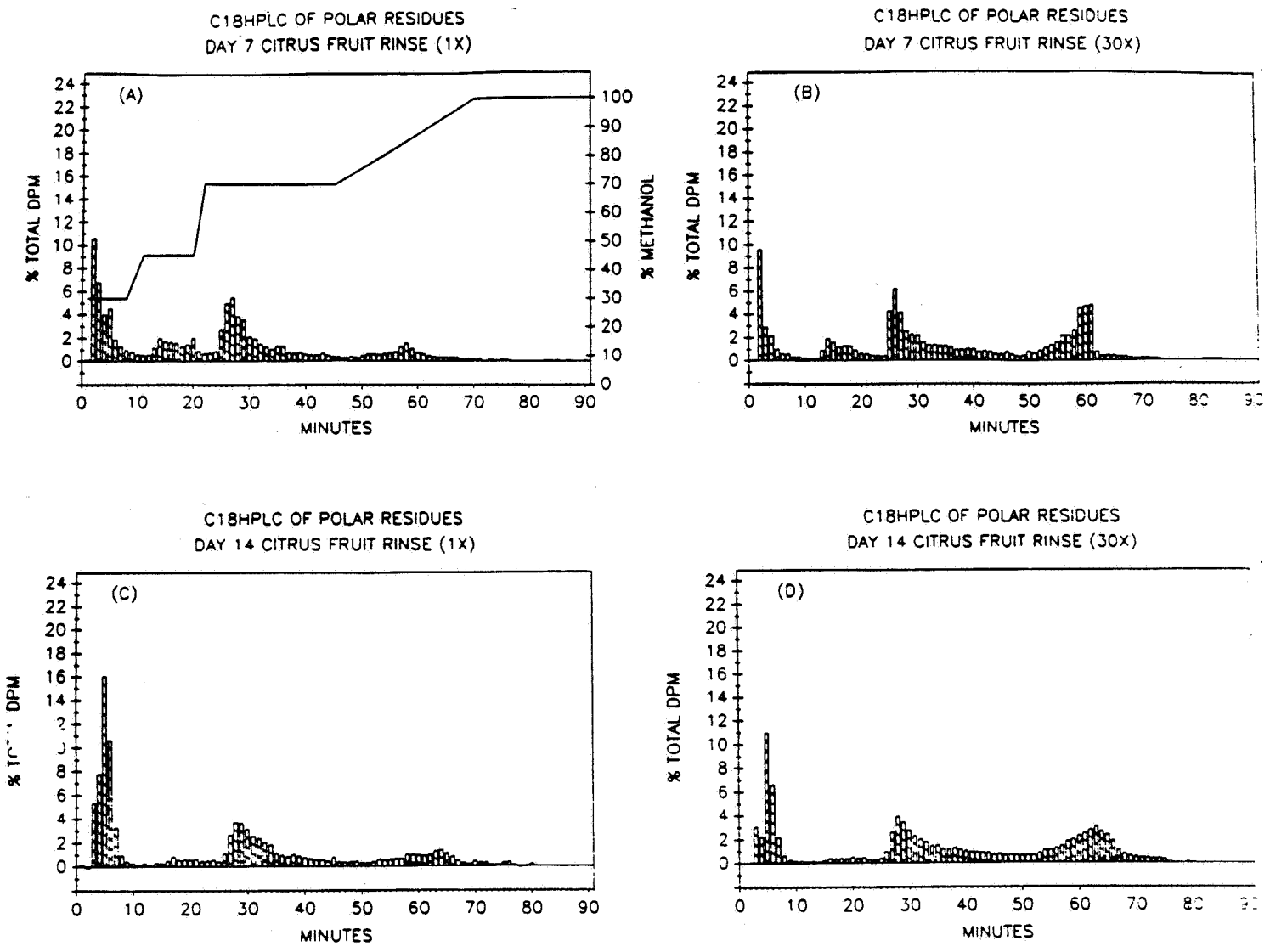

Fig. 2. C18HPLC radioprofiles after rechromatography of polar residues (as in Fig. 2) from composite samples of methanol rinses of ^{14}C-B1a-treated orange fruit (180 ppm (30X) or 6 ppm (1X), 13.0 uCi/mg B1a, 7-14 days PHI). Right Y axis in 4A indicates eluent composition for 4A-D.

ATTACHMENT TO MEMO OF F. BOYD, PP#8F3592/FAP#8H5550, 2/10/89

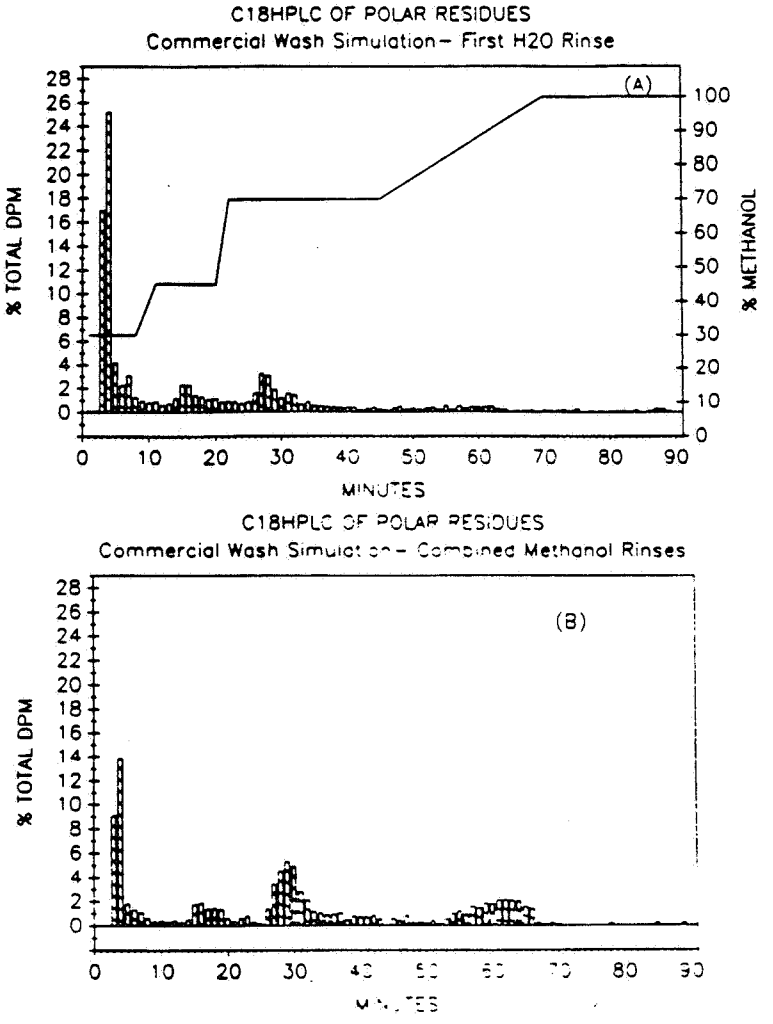

Fig. 3. Effect of simulated commercial orange washing procedure on polar residues from ^{14}C-Bla-treated orange fruit (6 ppm, 13.0 uCi/mg Bla, 7 days PHI). Oranges were brushed sequentially with water, detergent solution, and water; the fruit were then rinsed twice with methanol. A) C18HPLC radioprofile of polar residues in composite sample from first water rinse from 5 oranges. B) C18HPLC radioprofile of polar residues in composite sample of 2 methanol rinses of 5 oranges.