CASWELL FILE

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

007081

MAR 15 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Avermectin B_1 (Abamectin) - PP#8F3592/8H5550/7F3500 - Mouse Teratology Study with Citrus-Derived Polar Degradates of Abamectin

Caswell No.: 63AB
Project No.: 9-0456
Record No.: 235664, 235666, 235667
MRID No.: 409127-01

FROM: William Dykstra, Reviewer *William Dykstra 3/2/89*
Review Section I
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

TO: George T. LaRocca, PM 15
Insecticide-Rodenticide Branch
Registration Division (H7504C)

THRU: Edwin Budd, Section Head *Budd 3/3/89 JWA*
Review Section I
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

Requested Action

Review mouse teratology study performed with citrus-derived polar degradates of abamectin.

Conclusions and Recommendations

The mouse teratology study performed with citrus-derived polar degradates of abamectin was negative for maternal toxicity, teratogenicity, and developmental toxicity at 1.0 mg/kg/day (HDT). The study is acceptable as Core Minimum data.

1

Reviewed By: William Dykstra William Dykstra 3/2/89
Section I, Toxicology Branch - IRS (H7509C)
Secondary Reviewer: Edwin Budd
Section I, Toxicology Branch - IRS (H7509C)

007081

DATA EVALUATION REPORT

Study Type: 83-3 - Teratology, Mouse TOX Chem No.: 63AB

Accession No.: N/A MRID No.: 409127-01

Test Material: L-930,463 (Citrus-Derived Abamectin Polar Degradates). See Review for details.

Study No.: TT#88-713-0

Sponsor: Merck and Company, Inc.

Testing Facility: Merck Sharp and Dohme Research Laboratories

Title of Report: Oral Developmental Toxicity Study in Mice.

Authors: L.R. Gordon

Report Issued: November 1, 1988

Conclusions:

There were no compound-related effects with respect to maternal toxicity, teratogenicity, and developmental toxicity in CF_1 mice at dosages up to 1.0 mg/kg/day (HDT) with citrus-derived polar degradates administered during days 6 through 15 of gestation.

NOEL for maternal toxicity = 1.0 mg/kg/day (HDT)
NOEL for developmental toxicity = 1.0 mg/kg/day (HDT)

Classification: Core-Minimum

Special Review Criteria (40 CFR 154.7): N/A

Review:

- Oral Developmental Toxicity Study in Mice; L-930,463 (Citrus-Derived Abamectin Polar Degradates); Merck Sharp and Dohme Research Laboratories Project No. TT#88-713-0; November 1, 1988.

  Test Material - L-930,463; Lot No. L-930,463-000S001; Citrus-derived polar degradates of abamectin; a concentrate of the methanol washings from the surface of abamectin-treated citrus, containing approximately 5 ppt of polar MK-0936 (abamectin) degradates. A more detailed description of the test material and its preparation is presented in the memorandum from Dr. Louis Crouch to Dr. Richard Robertson (both of Merck Sharp & Dohme), dated September 28, 1988 (attached) and in the Dietary Exposure Branch (DEB) memorandum by V. Frank Boyd, dated February 13, 1989 (also attached).

  Animals - Crl:CF_1BR mice were obtained from Charles River Breeding Laboratories, Wilmington, MA. The mice were approximately 10 weeks of age and weighed 22.9 to 31.2 g at initiation of the study. Males and females were housed singly (except during mating) in plastic cages with Beta-Chip® bedding at a temperature from 20 to 27 °C and a 12-hour light/dark cycle.

  During mating, each female was housed with one untreated male of the same strain. Females were selected for study when daily examinations of the vagina revealed the presence of a copulatory plug. The day of finding the plug was designated Day 0 of gestation. The mice had free access to pellets of Purina Certified Rodent Chow #5002 and tap water.

Methods:

Ramdomized groups of 25 mated female mice were assigned to a dosing vehicle control group (0), a 100 mg/kg/day carrier control group, a 200 mg/kg/day carrier control group, and three polar degradate test groups at 0.25, 0.50, and 1.0 mg/kg/day of abamectin-related polar degradates. The highest dosage level of polar degradates to be tested in the study (1.0 mg/kg/day) was previously agreed to by Merck Sharp and Dohme and Toxicology Branch (TB). See TB memorandum by William Dykstra, dated September 21, 1987 (attached).

The carrier control material designated as L-930,462 (Lot No. L-930,462-000H001) contained concentrated organic washings from the surface of vehicle (containing no abamectin)-treated citrus. The dosing vehicle was 0.5% methylcellulose in deionized water.

The dose levels of 100 and 200 mg/kg/day carrier controls were selected since they are equivalent to the level of total L-930,462 residues contained in the dosing suspensions of L-930,463 at 0.50 and 1.0 mg/kg/day, respectively.

The dosing volume was 10 mL/kg based on most recent body weight.

Each pregnant female mouse of each respective group was dosed once daily by gavage from days 6 through 15 of gestation.

Females were observed daily for physical signs. Maternal body weight was recorded on days 0, 6, 8, 10, 12, 14, 16, and 17 of gestation. Food consumption was measured during the following intervals: Days 3 to 5, 6 to 8, 9 to 11, 12 to 14, and 15 to 17 of gestation.

All female mice were sacrificed on day 17 of gestation and the uteri were examined. Implants were counted and classified as live fetuses, dead fetuses, or resorptions. All fetuses were weighed and examined for external abnormalities. Every third fetus in each litter and all externallly malformed and dead fetuses were given a visceral examination by dissection. The heads of these fetuses (excluding dead fetuses) were fixed in Bouin's solution and later examined by free-hand coronal sections. All fetuses were fixed, cleared, and stained with alizarin red for skeletal examination.

All sacrificed adult females were necropsied and examined grossly for lesions.

Statistical analyses were performed with $p < 0.05$ being significant.

Results:

There were 24, 25, 23, 23, 23, and 25 pregnant females in the 0, 100, 200, 0.25, 0.50, and 1.0 mg/kg/day groups, respectively.

There were no compound-related toxic signs, deaths, or abortions during the study.

There were no compound-related effects in maternal body weight and food consumption. Although average maternal body weight gain was slighly increased (about 15%) in the 200 mg/kg/day carrier control group in comparison to the vehicle control between days 16 and 17 (3.4 g in vehicle control vs. 3.9 g in the 200 mg/kg/day/carrier control group), this finding was not considered compound-related since the 1.0 mg/kg/day polar degradate-treated group was not similarly increased (3.6 g in the 1.0 mg/kg/day polar degradate group).

Although average food consumption was slighly increased (4 to 19 percent above vehicle control) during treatment in the 200 mg/kg/day carrier control group and the 0.25 and 0.50 mg/kg/day polar degradate groups, these same groups also had slightly increased food consumption before treatment (before day 6) and therefore these findings were not considered compound-related. Additionally, the 1.0 mg/kg/day polar degradate group (HDT) did not show any consistent increases in food consumption and, therefore, the increases during treatment in the other groups were not dose-related.

There were no compound-related effects in mean implants per pregnant female (range from 12.2 to 13.4 for all groups), live fetuses per pregnant female (range from 11.5 to 12.3 in all groups), and percent dead fetuses plus resorptions per implant (range from 8.8 to 11.6 for all groups).

There were no compound-related effets in mean live fetal body weight. Live fetal body weights ranged from 0.92 to 0.96 g for all groups.

The number of fetuses externally examined ranged from 264 to 297 for all groups.

There were no compound-related external malformations.

Exencephaly occurred in 2 fetuses (2 litters) in the 100 mg/kg/day carrier control group, 1 fetus (1 litter) in the 0.25 mg/kg/day polar degradate group and in 3 fetuses (2 litters) of the 0.5 mg/kg/day polar degradate group. Male fetus #15 of dam 88-0228 of the 0.5 mg/kg/day polar degradate group which had exencephaly also had polydactyly. Since there were no fetuses with exencephaly from the 200 mg/kg/day carrier control group or the 1.0 mg/kg/day polar degradate group (HDT), the findings in the other groups were not dose-related and were not considered compound-related.

Cleft palate occurred in 1 fetus (this fetus, #2 from female 88-0134, also had micrognathia) from the vehicle control group, 2 fetuses (2 litters) from the 100 mg/kg/day carrier control group, 1 fetus (1 litter) from the 200 mg/kg/day carrier control group, 1 fetus (1 litter) from the 0.5 mg/kg/day polar degradate group, and 2 fetuses (2 litters) from the 1.0 mg/kg/day polar degradate group. Due to the presence of cleft palate in the vehicle control group and the comparably low incidence in other groups, the findings of cleft palate in the polar degradate mid- and high-dose groups were not considered compound-related.

Encephalomeningocele was observed in one vehicle control fetus and one fetus (which also had microphthalmia) from the 0.25 mg/kg/day polar degradate group. These external malformations were considered compound-related, since they only

occurred in the vehicle control group and only the 0.25 mg/kg/day polar degradate group, occurred in single incidences, and were not dose-related.

Two fetuses (2 litters) in the 200 mg/kg/day carrier control group had tail malformations. Since these findings were not observed in the 1.0 mg/kg/day polar degradate group, these were not considered compound-related.

There were no compound-related visceral abnormalities.

The vehicle control group did not have any visceral abnormalities. There was one fetus with visceral abnormalities (malformation or variation) in one litter of the 100 and 200 mg/kg/day carrier control groups and of the 0.25 and 0.50 mg/kg/day polar degradate groups. In the 1.0 mg/kg/day polar degradate group, there were two fetuses (2 litters) with visceral abnormalities.

The visceral abnormalities for all groups included ventricular septal defect (1 fetus), cerebral malformation (1 fetus), diffuse hemorrhagic kidney (2 fetuses), displaced testis (1 fetus) and one fetus with overriding aorta, pulmonary stenosis and cor triloculare combined (this fetus was in the 1.0 mg/kg/day polar degradate group).

Due to the isolated occurrence of these visceral abnormalities and the low incidences, they were not considered compound-related.

There were no compound-related skeletal abnormalities.

There were no sternebral malformations in the vehicle control group. Sternebral malformations were observed, however, in six fetuses (3 litters) of the 100 mg/kg/day carrier control group, nine fetuses (8 litters) of the 200 mg/kg/day carrier control group, two fetuses (2 litters) of the 0.25 mg/kg/day polar degradate group, two fetuses (2 litters) of the 0.50 mg/kg/day polar degradate group, and four fetuses (4 litters) of the 1.0 mg/kg/day polar degradate group.

The incidences of sternebral malformations in fetuses and litters in all the polar degradate groups and the 100 mg/kg/day carrier control group (but not the 200 mg/kg/day carrier control group) were within the range of historical controls from Merck as stated in the report.

The fetal incidence range in historical controls was 0 to 2.6 percent, in comparison to the incidence of fetuses affected in the study (excluding the 200 mg/kg/day carrier control group) which ranged from 0 to 2.0 percent. The litter incidence range in historical controls was 0 to 25 percent, whereas the incidence of litters affected in the study (excluding the 200 mg/kg/day carrier control group) ranged from 0 to 16.0 percent.

6

Additionally, the relatively high incidence of sternebral malformations seen in the high-dose carrier control group was not observed in the 1.0 mg/kg/day polar degradate group which received the same exposure to the high-dose carrier control substance.

Also, the occurrence of these malformations usually appeared as single fetuses and single litters with the exception of two litters which were in the carrier control groups.

In view of these circumstances, the occurrence of sternebral malformations was not considered compound-related.

All other skeletal findings occurred either singly or with comparable frequency between the vehicle control, carrier controls, and polar degradate treated groups.

These findings included atlas malformation, cervical vertebrae malformations, thoracic vertebrae malformations, missing vertebrae, extra vertebrae, hypoplastic rib, atlas variation, axis variation, lumbar count variation, cervical rib, lumbar rib, and sternebral variation.

The incidence of fetuses and litters with sites of incomplete ossification did not show any compound-related effects. The frequency of fetuses and litters with sites of incomplete ossification was comparable between vehicle control, carrier controls, and polar degradate treated groups.

There were no compound-related gross lesions observed at necropsy of the adult female mice.

Conclusion:

There were no compound-related effects with respect to maternal toxicity, teratogenicity, and developmental toxicity in CF_1 mice at dosages up to 1.0 mg/kg/day (HDT) with citrus-derived polar degradates administered during days 6 through 15 of gestation.

NOEL for maternal toxicity = 1.0 mg/kg/day (HDT)
NOEL for developmental toxicity = 1.0 mg/kg/day (HDT)

Classification:

Core Minimum (no adverse toxicological effects observed at highest dosage level tested).

Attachments

Avermectin toxicology review

Page 8 is not included in this copy.

Pages _____ through _____ are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product 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

X 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.

Dykstra

007081

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

FROM: V. Frank Boyd, Ph.D., Chemist
Tolerance Petition Section II
Dietary Exposure Branch
Health Effects Division (TS-769C)

TO: George T. LaRocca, PM 15
Insecticide-Rodenticide Branch
Registration Division (TS-767C)

and

Edwin Budd, Section Head
Toxicology Branch I - Insecticide, Rodenticide Support
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.

9

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.

11

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. Byrd, PP# 7F3572/FAP# 7H5553,
2/10/97

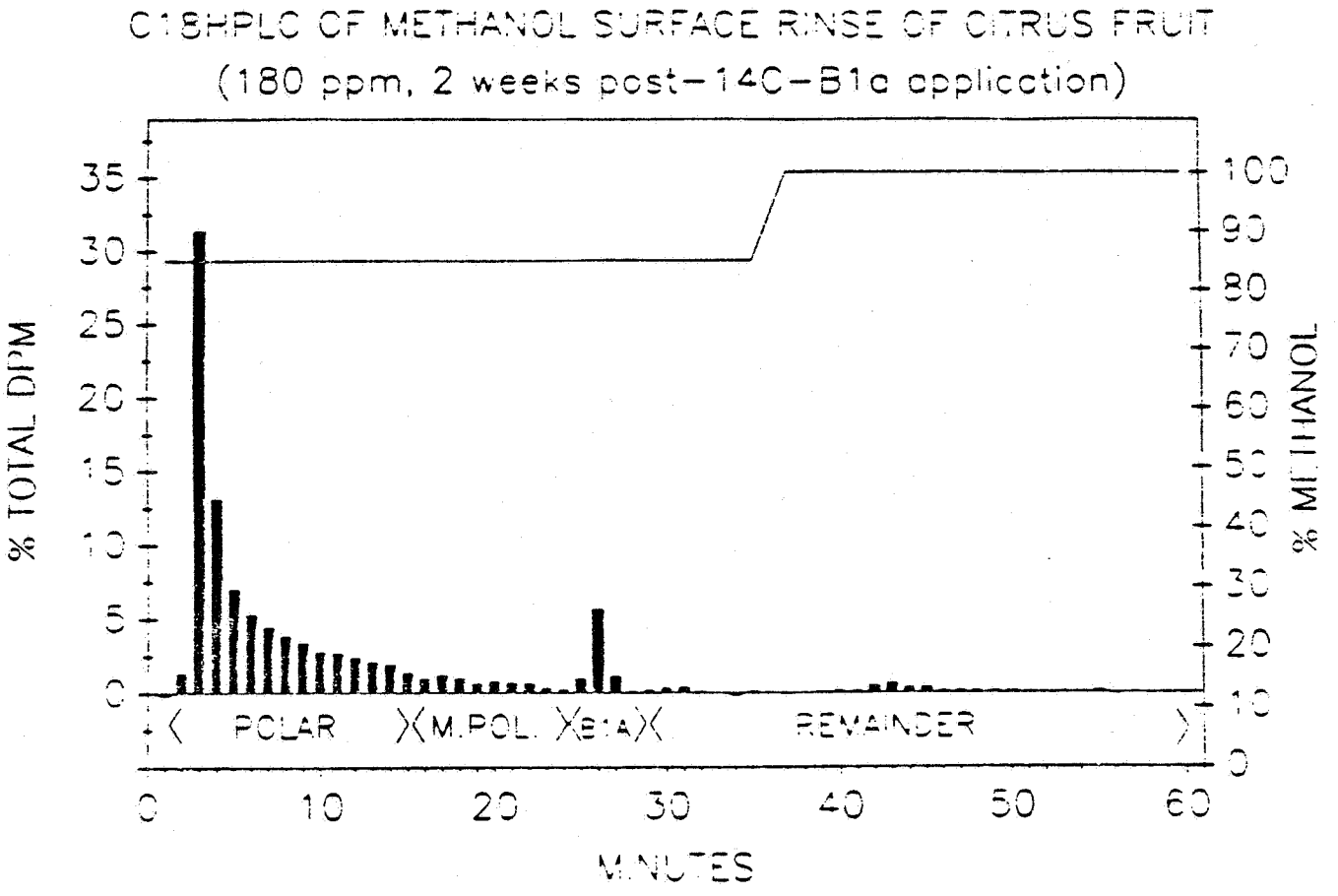

Fig. 1. C18HPLC radioprofile of residues in composite sample from 2 consecutive 15 ml methanol rinses of 5 ^{14}C-B1a-treated orange fruit (180 ppm, 13.0 uCi/mg B1a, 14 days PHI). Polar residues- t_R < 0.6 t_R of B1a; Moderately polar (M.POL.) residues- t_R between 0.6 and 0.95 of B1a. Right y axis indicates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

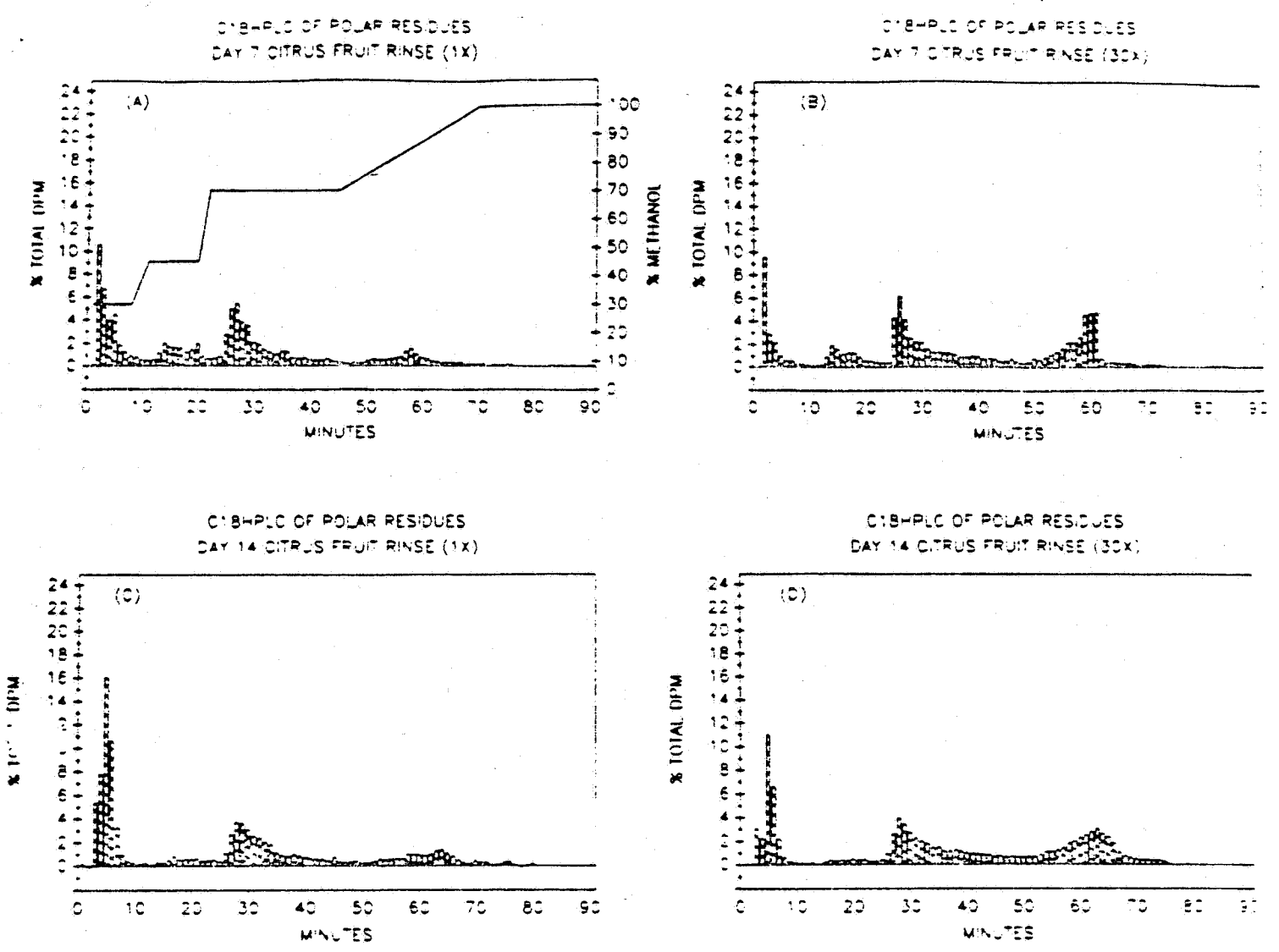

Fig. 2. C18HPLC radioprofiles after rechromatography of polar residues (as in Fig. 2) from composite samples of methanol rinses of ^{14}C-Bla-treated orange fruit (180 ppm (30X) or 6 ppm (1X), 13.0 uCi/mg Bla, 7-14 days PHI). Right Y axis in 4A indicates eluent composition for 4A-D.

007081

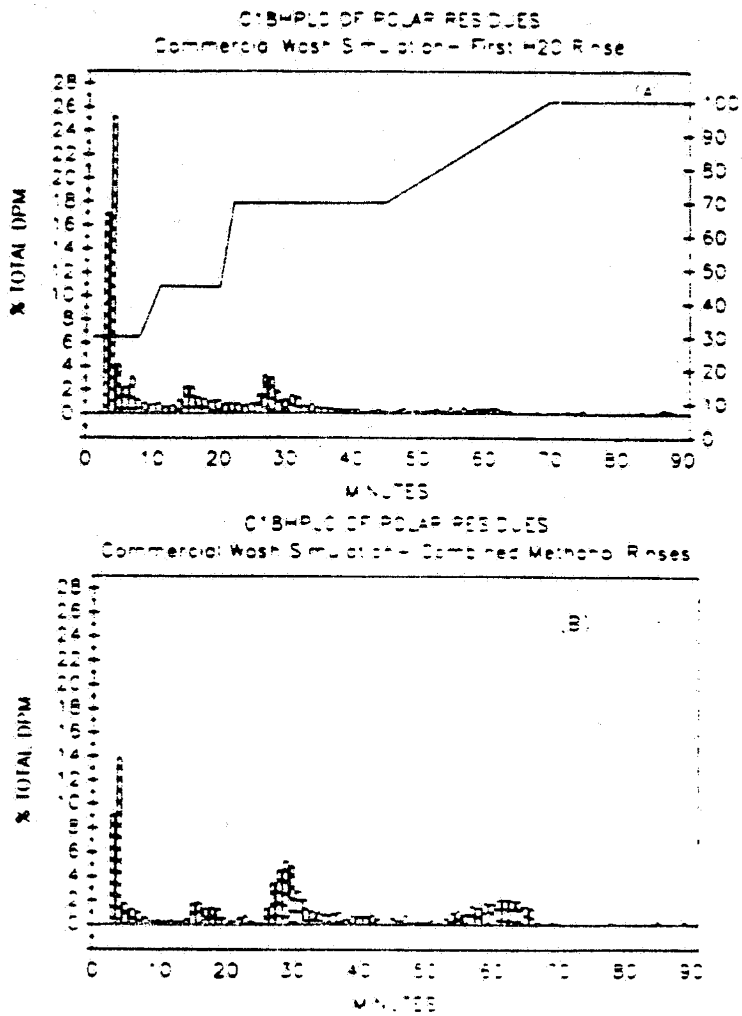

Fig. 3. Effect of simulated commercial orange washing procedure on polar residues from ^{14}C-B1a-treated orange fruit (6 ppm, 13.0 uCi/mg B1a, 7 days PHI). Oranges were brushed sequentially with water, detergent solution, and water; the fruit were then rinsed twice with methanol. A) C18HPLC radioprofile 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.

REVIEWER

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

007081

SEP 2 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Avermectin - Meeting With Registrant (Merck)

Caswell No.: 63AB

FROM: William Dykstra
Toxicology Branch
Hazard Evaluation Division (TS-769C)

William Dykstra 8/26/87

TO: George T. LaRocca, PM 15
Insecticide-Rodenticide Branch
Registration Division (TS-767C)

9/2/87

THRU: Edwin R. Budd, Section Head
Review Section II, Toxicology Branch
Hazard Evaluation Division (TS-769C)

8/27/87

On August 3, 1987, a meeting was held between representatives of Merck, Toxicology Branch (TB) (Dr. Farber, E. Budd, and W. Dykstra), and A. Heyward of PM Team 15 from Registration Division to discuss additional data requirements for permanent tolerances on citrus with Avermectin (Abamectin).

1. For the delta-8,9-isomer, the following data were requested:

   a. Ames assay (with and without activation); depending on the results of this study, additional mutagenicity data may be required. Before making a final decision on the need for additional mutagenicity testing, the results of the chronic feeding/oncogenicity studies on avermectin will also be considered. These studies are presently under review in TB.

17