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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Science Review of a New Analytical Method and a Residue Study for EthylBloc™ (EPA Reg. No. 071297-1) Containing 0.14% 1-Methylcyclopropene (Chemical No. 224459) in Support of a Tolerance Exemption Petition (Petition No. 0F06144); an Existing EUP (EPA Reg. No. 71297-EUP-1); and Agrofresh Inc./Rohm & Haas Response to the Agency Memorandum from R. S. Jones to D. Benmhend (Dated 02/15/2001). DP Barcode D273531; Case No. 063215; Submission No. S594166; MRID Unassigned.

FROM: Russell S. Jones, Ph.D., Biologist
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511C)

THRU: Freshteh Toghrol, Ph.D., Senior Scientist for F. Toghrol 5/3/01
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511C)

TO: Driss Benmhend, Regulatory Action Leader
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511C)

ACTION REQUESTED

In response to a request for additional data (see Memorandum from R. S. Jones to D. Benmhend, dated 2/15/2001), AgroFresh, Inc. [formerly BioTechnologies for Horticulture, Inc (BTH, Inc.; a subsidiary of Rohm and Haas Company)] has submitted a new radioisotope analytical method for the determination of C^{14}-1-methylcyclopropene (C^{14}-1-MCP) residues in apples (MRID Not Assigned). In addition, the registrant provided magnitude of the residue data for apples treated with C^{14}-1-MCP using the aforementioned radioisotope analytical method. This new analytical method and the accompanying magnitude of the residue data were submitted in support of: (i) a petition (Petition No. 0F06144) for an exemption from the requirements of tolerances for residues of 1-MCP on stored food commodities; (ii) a label amendment to add indoor use on post-harvested fruits and vegetables; and (iii) in support of an amendment to an existing "crop destruct" EUP (EPA Reg. No. 71297-EUP-1) for EthylBloc™ (EPA Reg. No. 071297-1). 1-MCP is the active ingredient in the end-use product, EthylBloc™ which contains 0.14% 1-MCP. EthylBloc™ is a powdered product that releases 1-MCP as a gas when mixed

with water or a buffering agent. The end-use product is currently registered for non-food use on floral and nursery crops.

In addition to the action request described above, the registrant submitted a response (see letter from S. Longacre to D. Benmhend, dated 3/1/2001) to a previous Science Review conducted in regard to items (i), (ii), and (iii) listed in the paragraph above (see Memorandum from R. S. Jones to D. Benmhend, dated 2/15/2001). The AgroFresh, Inc./Rohm & Haas response to the BPB Memorandum of 2/15/2001, followed by BPPD comments, will be included in the "Background" section of this review.

CONCLUSIONS AND RECOMMENDATIONS FOR THE NEW ANALYTICAL METHOD AND THE MAGNITUDE OF THE RESIDUE STUDY ON STORED POSTHARVEST APPLES

1. For purposes of the EUP, the registrant submitted an acceptable preliminary analytical method (Liquid Scintillation Counting, or LSC) for determining ^{14}C-1-MCP residues in treated apples. This method has a limit of quantitation (LOQ) of 1 ppb.

2. For purposes of the EUP, the registrant submitted acceptable residue data for apples using the new analytical method. The submitted data, when corrected for mean analytical method recoveries, demonstrate that residues of 1-MCP on apples will not exceed 8.6 ppb in/on apples at 24 hours following a 24-hr treatment with 1200 ppb (1.2 ppm; or 1.2x the maximum label application rate) of ^{14}C-1-MCP. The aforementioned residue value is equivalent to a calculated value of 7.2 ppb 1-MCP following a 24-hr treatment with 1000 ppb 1-MCP (1.0 ppm; or 1x the maximum label application rate).

3. Residues of ^{14}C-1-MCP (either corrected for LSC method recovery or uncorrected) were observed to be below the non-radiolabel method (GC/FID) limit of detection (10 ppb) within 24-hours following the end of exposure to 1-MCP gas at 1200 ppb (1.2x the maximum label use rate). The GC/FID analytical method is the method that would be used for enforcement purposes.

4a. Based on data reported by Sisler and Serek (1999. *Compounds controlling the ethylene receptor*. Bot. Bull. Acad. Sinica 40: 1-7), up to 50% of all ethylene receptors in plant tissue bound with 1-MCP molecules following treatment with 1-MCP gas may release 1-MCP within 30 days of exposure (see Discussion below).

4b. Using a worst-case scenario that treated apples will contain up to a calculated level of 7.2 ppb 1-MCP at 24 hours posttreatment [a 24-hour exposure to 1000 ppb 1-MCP gas (see Conclusion 2 above)], it is calculated that 1-MCP residues may decline to levels (approximately 0.9 ppb) slightly below the limit of detection of the radiolabel analytical method (1 ppb) within approximately 90 days posttreatment (see Conclusion 4a above).

4c. Under normal agricultural practices, apples may be stored for 6-12 months (Source: Michigan Apple Commission and Washington Apple Commission). Therefore, under normal agricultural practices, it is expected that when stored, postharvested apples are treated EthylBloc™ at the maximum label use rate (releasing 1000 ppb of 1-MCP), 1-MCP residues will be non-detectable in/on apples by any known analytical method, within six months of treatment (a probable minimum postharvest storage interval for apples). It is emphasized here that only *non-labeled 1-MCP* will be applied to stored apples under normal agricultural practices.

STUDY SUMMARY

Three experiments were reported wherein apples (cv. 'Red Delicious' and/or 'Gala') were treated with radiolabeled ^{14}C-1-methylcyclopropene (^{14}C-1-MCP) at a concentration of 1.2 ppm (1200 ppb or 1.2x the maximum label use rate) for 24 hours under simulated storage conditions at 0°C and/or at room temperature. After 24 hours of exposure to ^{14}C-1-MCP, the chambers were vented to remove ^{14}C-1-MCP. Treated apples were then stored for up to two weeks at 0°C and/or at room temperature with a slow air purge. During the storage period, apple samples were collected periodically and analyzed for ^{14}C-1-MCP via a Liquid Scintillation Counting (LSC) method developed by the registrant to determine ^{14}C-1-MCP residues; the analytical limit of quantitation (LOQ) was 1 ppb. Samples consisted of three apples each removed from the top, middle, and bottom of each chamber at each sampling interval. Total radioactive residues (TRR) were analyzed immediately after sampling. Mean TRR in apples for all experiments ranged 2.4-5.2 ppb. When corrected for the average method recovery determined at each sampling interval, the mean TRR values were equivalent to 2.8-6.1 ppb (µg TRR/kg apple). The highest individual corrected residue value (8.6 ppb) was in a 'Gala' apple sample collected from the middle of a treatment chamber at 24-hr posttreatment. Overall, there were no apparent differences between apples treated and stored at 0°C or at room temperature, although the data were insufficient to assess how temperature differences might affect 1-MCP residues in apples.

Analytical method recoveries ranged 84.8-90.1% from 17 apple samples fortified with 1 and 10 ppb ^{14}C-1-MCP. Mean analytical method recovery for all sample sets was 87.1% with a standard deviation of 7.5%. Using a nonradiolabel analytical method (GC/FID), analyses of headspace gas in the treatment chambers demonstrated that ^{14}C-1-MCP was stable for the exposure period of each experiment (at least 24 hours).

Classification: The submitted analytical method for the determination of 14C-1-MCP in apples and the magnitude of the residue study are acceptable for purposes of the EUP.

Study Deficiencies: Minor; do not affect study conclusions. No raw data or sample chromatograms presented for analytical methods. No data presented for sampling intervals longer than 2 weeks posttreatment.

DISCUSSION

The registrant presented data pertaining to an LSC method to determine radiolabeled ^{14}C-1-methylcyclopropene (^{14}C-1-MCP) in/on apples and then used this method to measure total radioactive residues (TRR) in apple tissue up to 2 weeks following exposure to ^{14}C-1-MCP gas; the limit of quantitation (LOQ) was reported to be 1 ppb. A GC/FID method for determining non-radiolabeled 1-MCP, similar to a previously submitted and Agency-accepted GC/FID method, which had a 10 ppb limit of detection (LOD)was also submitted. The LSC method did not directly measure 1-MCP or any potential metabolites, but only measured total radioactive residues (TRR). However, under conditions of the study, the only source of measurable radioactivity in the apple tissues would from ^{14}C-1-MCP. In the three experiments conducted by the registrant, apples in simulated storage conditions were treated with ^{14}C-1-MCP gas at a concentration of 1.2 ppm (1200 ppb or 1.2x the maximum label use rate) for 24 hours under simulated storage conditions at 0°C and/or at room temperature. After 24 hours of exposure to ^{14}C-1-MCP, the chambers were vented to remove ^{14}C-1-MCP. The registrant presented data that were uncorrected for analytical method recoveries. These data were corrected by the Agency reviewer and the corrected values were used for the risk assessment.

There were no apparent effects of temperature or the location of the chamber from which samples were collected (top, middle or bottom) across all three experiments (see Tables 1b, 2b, and 3b in the DER). Additionally, there were no changes in residue levels over the 24-hour to two week experimental period. Corrected mean TRR in apples for all experiments ranged 2.8-6.1 ppb or µg TRR/kg apple. The highest individual corrected residue value (8.6 ppb) was in a 'Gala' apple sample collected from the middle of a treatment chamber at 24-hr posttreatment (at 1.2x the maximum label use rate, or 1200 ppb ^{14}C-1-MCP). Thus, at 24-hours posttreatment, the highest observed radioactive residue value in apple tissue was 14% below the limit of detection (10 ppb) for non-labeled 1-MCP. This value can be further corrected to estimate what the residue value would have been if the active ingredient had been applied at the maximum label use rate (1x, or 1000 ppb) via the following calculation:

(8.6 ppb TRR/1000 ppb ^{14}C-1-MCP)/1200 ppb ^{14}C-1-MCP = 7.2 ppb TRR

Therefore, in this series of experiments, if ^{14}C-1-MCP were applied to apples at a 1x rate, the theoretical maximum TRR would be 7.2 ppb, or 28% below the limit of detection for non-labeled 1-MCP (10 ppb).

Except for the data in this study, there is no information available regarding the average length of time that a 1-MCP molecule remains bound to ethylene receptors in apples. However, in a series of studies using different ethylene inhibitors and plant tissues, Sisler and Serek [*Compounds controlling the ethylene receptor*. Bot. Bull. Acad. Sinica (1999) 40: 1-7] demonstrated that 50% of the receptors in banana tissue become free of 1-MCP molecules within 43,200 minutes (30 days) posttreatment. Based on these data, residues of 1-MCP in apple may theoretically decline to approximately 0.9 ppb (slightly below the 1 ppb LOQ of the radiolabel LSC analytical method) within approximately 90 days posttreatment (24-hr = 7.2 ppb; 30-days = 3.6 ppb; 60 days = 1.8 ppb; 90-days = 0.9 ppb) if the following assumptions are made:

(i) receptors in apple and banana tissues have similar 1-MCP release rates (i.e. an approximate 30-day receptor-binding half-life); and

(ii) a worst case scenario that apples will contain 7.2 ppb 1-MCP 24 hours following treatment with 1000 ppb 1-MCP gas (1x the maximum label use rate).

No data are currently available regarding whether 1-MCP will be metabolized in plants, although it is unlikely that any 1-MCP metabolites will have any toxic properties given the simple nature of the molecule. Products of 1-MCP metabolism (if any) would be present in extremely tiny amounts. Physical data show that amongst its class of 3-carbon cyclic molecules, 1-MCP is considered to be relatively stable. Hopf et al. 1985. [*Gas phase kinetics of pyrolysis of 1-methyl-1-cyclopropene*. J. Org. Chem. 36: 1320-1321. (cited by Sisler et al. 1996. *Comparison of cyclopropene, 1-methylcyclopropene, and 3,3-methylcyclopropene as ethylene antagonists in plants*. Plant Growth Regulation 18: 169-174)] stated that "1-MCP is stable in the gas phase at 10 Torr (1.3%) for several months at room temperature."

Based on the data presented in the studies conducted by the registrant, and on data/information obtained from the technical literature, it is likely that residues of non-labeled 1-MCP (applied at a 1x rate via the end-use product EthylBloc) will be non-detectable via enforcement analytical methods (GC/FID; non-radioactivity) by 24-hours posttreatment. Residues of radiolabeled ^{14}C-1-MCP will likely be non-detectable by 90-days posttreatment. It is emphasized here that only *non-labeled 1-MCP* will be applied to stored apples under normal agricultural practices.

BACKGROUND

EthylBloc™ (EPA Reg. No. 071297-1) is currently registered for non-food use on floral and nursery crops in enclosed, indoor areas. More recently, the registrant requested an experimental use permit (EUP; EPA Reg. No. 71297-EUP-1) to permit the commercial indoor testing of EthylBloc™ on postharvest stored apples (EPA File No. 71297-1). Under the EUP, the

registrant intended to use a maximum of 52.9 lbs of EthylBloc™ (equivalent to 0.074 lbs of 1-MCP) on 10.8 million lbs of postharvested apples. A maximum of six trials are currently in progress in three states [CA (2), PA (2), and WA (2)] and are being conducted in commercial apple storage facilities at each location. Apples were harvested from a total of 491 acres of apple trees. Treatments at each location were conducted in a room with a volume of approximately 2500 m^3 and containing approximately 1.8 million apples. Nominal concentrations of 1-MCP in each trial were 1000 ppb over a 24- to 48-hr treatment interval. Following treatment, the apples were to remain in storage under refrigeration and low oxygen concentration for up to 9 months. During the 9-month storage interval, samples are being collected for analysis of various physiological parameters. This EUP was deemed acceptable by BPPD (see Memorandum from R. S. Jones to D. Benmhend, dated 9/28/2000) provided it included a crop destruct requirement. The crop destruct requirement was included because there were no established tolerances or tolerance exemptions for residues of 1-MCP on apples or other food commodities. In the EUP review of 9/28/2000, BPPD stated that the EUP could be amended at a later date to a non-crop destruct, after a permanent exemption from the requirement of tolerances had been established for 1-MCP. A petition (PP# 0F06144) to establish a permanent exemption from the requirement of tolerances for residues of 1-MCP on stored food commodities was submitted to the Agency in April 2000 and is the subject of this review.

REVIEW AND COMMENT ON THE AGROFRESH, INC/ROHM & HAAS (AFRH) RESPONSE (DATED 3/1/2001) TO THE BPPD SCIENCE REVIEW (MEMORANDUM FROM R. S. JONES TO D. BENMHEND, DATED 2/15/2001).

The registrant submitted a response (see letter from S. Longacre to D. Benmhend, dated 3/1/2001) to a previous Science Review (see Memorandum from R. S. Jones to D. Benmhend, dated 2/15/2001) conducted in regard to items (i), (ii), and (iii) listed in the "Action Requested" section above. The Science Review of 2/15/2001 also addressed comments submitted by Valent BioSciences [(formerly Abbott BioSciences); see letter from M. Tichon to EPA/PIRIB (dated 7/20/2000) regarding the petition PP#0F06144] submitted in regard to AgroFresh, Inc.'s FQPA Notice of Filing for the tolerance exemption petition (published in the Federal Register on 6/21/2000). The AFRH response of 3/1/2001 is attached to this document for reference.

1. BPB Comment to AFRH Response to Conclusions/Recommendations Nos. 1 and 2:

   The reviewer's statement that the submitted data "*support the petition for an exemption from the requirements of a tolerance for 1-methylcyclopropene (1-MCP)*" should be interpreted that while the submitted data were acceptable to support the petition, they were not necessarily the only data that would be needed to adequately address FQPA concerns so as to *grant* the tolerance exemption and permit the EUP to be amended from "crop destruct" to "non-destruct." Other potential dietary and health concerns were detailed in the Science Review of 2/15/2001.

2. BPB Comment to AFRH Response to Conclusions/Recommendations Nos. 3:

Analytical methods and magnitude of the residue studies/data that can be used to address dietary exposure/health concerns are addressed in the Science Review (see Conclusions 1 through 4 a-c above).

3. BPB Comment to AFRH Response to Conclusions/Recommendations Nos. 4:

We concur with AFRH's statement that we meant "90-day subchronic study" instead of a "chronic (one year) study." The registrant states that it has conducted a female rat two-week inhalation study with 1-MCP in support of the tolerance exemption petition. The registrant further states that "no effects were observed at 100 ppm and that minimal spleen and/or blood effects were observed at 300 and 1000 ppm."

Since inhalation data will not inform decisions pertaining to *dietary* exposure, these data will not influence decisions regarding the tolerance exemption petition, label amendment, or the EUP amendment. However, these data will be required for registration of EthylBloc™ for use on stored food commodities.

4. BPB Comment to AFRH Response to Conclusions/Recommendations Nos. 5:

The registrant has developed a workplace exposure air monitoring method for CMP and expects to submit a final report to the Agency in June 2001. Since data pertaining to CMP in the workplace will not inform decisions pertaining to *dietary* exposure, these data will not influence decisions regarding the tolerance exemption petition, label amendment, or the EUP amendment. However, these data will be required for registration of EthylBloc™ for use on stored food commodities.

5. BPB Comment to AFRH Response to Conclusions/Recommendations Nos. 6:

The registrant is conducting three *in vivo* mutagenicity studies in closed systems and an *in vivo* mouse micronucleus study. The registrant stated that no mutagenic effects had been observed. Data pertaining to mutagenicity following exposure to gaseous 1-MCP will not inform decisions pertaining to *dietary* exposure. Therefore, these data will not influence decisions regarding the tolerance exemption petition, label amendment, or the EUP amendment. However, these data will be required for registration of EthylBloc™ for use on stored food commodities.

6. Comment to AFRH Response to Conclusions/Recommendations Nos. 7:

BPPD concurs with the AFRH statement that "no additional ecotoxicity or environmental fate studies with 1-MCP or EthylBloc product are required" provided the use of 1-MCP or EthylBloc product are restricted to indoor, enclosed post-harvest use. Decisions

pertaining to the granting of a conditional or permanent exemption from the requirement of a tolerance for 1-MCP on all stored food commodities, a label amendment to permit application of 1-MCP or EthylBloc product, an amendment to the EUP to change it from "crop destruct" to non-destruct" will be made by BPPD risk managers.

CONFIDENTIAL APPENDIX

The Following Section Contains Confidential Business Information (CBI)

DATA EVALUATION REPORT

Primary Reviewer: Russell S. Jones, Ph.D., BPPD
Secondary Reviewer: Freshteh Toghrol, Ph.D., BPPD

STUDY TYPE:	Product Chemistry: Analytical Methods (Subdivision M Guideline 151-16) and Magnitude of the Residue (Non-Guideline)
TOX. CHEM. No.:	None
CASE No.	063215
PC CODE:	224459
DP BARCODE:	D273531
SUBMISSION No.:	S594166
MRID Nos:	Unassigned
TEST MATERIAL:	EthylBloc™
STUDY Nos.:	Report Number TR34-01-30
SPONSOR:	AgroFresh, Inc./Rohm & Haas Co. (formerly BioTechnologies for Horticulture, Inc.) 727 Norristown Road, P. O. Box 904, Spring House, PA 19477-0904.
TESTING FACILITIES:	AgroFresh, Inc./Rohm & Haas Co. (formerly BioTechnologies for Horticulture, Inc.) 727 Norristown Road, P. O. Box 904, Spring House, PA 19477-0904.
TITLE OF REPORTS:	14C-1-Methylcyclopropene (1-MCP) Preliminary Results of 14C-Residues in Apples.
AUTHORS:	D. J. Verona
REPORT ISSUED:	April 5, 2001
QUALITY ASSURANCE:	The studies were conducted under Good Laboratory Practices (GLP) guidelines. A compliance statement was signed by the study author and the sponsor/submitter on 04/05/2001.

SUMMARY: Three experiments were reported wherein apples (cv. 'Red Delicious' and/or 'Gala') were treated with radiolabeled ^{14}C-1-methylcyclopropene (^{14}C-1-MCP) at a concentration of 1.2 ppm (1200 ppb or 1.2x the maximum label use rate) for 24 hours under simulated storage conditions at 0°C and/or at room temperature. After 24 hours of exposure to ^{14}C-1-MCP, the chambers were vented to remove ^{14}C-1-MCP. Treated apples were then stored for up to two weeks at 0°C and/or at room temperature with a slow air purge. During the storage period, apple samples were collected periodically and analyzed for ^{14}C-1-MCP via a Liquid Scintillation Counting (LSC) method; the analytical limit of quantitation was 1 ppb. Samples consisted of three apples each removed from the top, middle, and bottom of each chamber at each sampling interval. Total radioactive residues (TRR) were analyzed immediately after sampling. Mean TRR in apples for all experiments ranged 2.4-5.2 ppb; when corrected for the average method recovery determined at each sampling interval, the mean TRR values are equivalent to 2.8-6.1 ppb (µg TRR/kg apple). The highest individual corrected residue value (8.6 ppb) was in a 'Gala' apple sample collected from the bottom of a treatment chamber at 24 hr posttreatment.

Analytical method recoveries ranged 84.8-90.1% from 17 apple samples fortified with 1 and 10 ppb ^{14}C-1-MCP. Mean analytical method recovery for all sample sets was 87.1% with a standard deviation of 7.5%. Using a nonradiolabel analytical method (GC/FID), analyses of headspace gas in the treatment chambers demonstrated that ^{14}C-1-MCP was stable for the exposure period of each experiment for at least 24 hours.

CLASSIFICATION: Acceptable.

STUDY DEFICIENCIES: Minor; do not affect study conclusions. No raw data or sample chromatograms presented for analytical methods. No data presented for sampling intervals longer than 2 weeks posttreatment.

I. ANALYTICAL METHODS (151-16) and Magnitude of the Residue (Non-Guideline); MRID Unassigned

The registrant submitted a new analytical method for the determination of ^{14}C-1-MCP residues in apples following treatment with 1200 ppb (1.2 ppm) of ^{14}C-1-MCP.

Test Substance:

Active Ingredient:	1-Methylcyclopropene (1-MCP); CAS No. 3100-04-7
Other Ingredient:	α-cyclodextrin; CAS No. 10016-20-3
Lot Number	

(formulated test substance):	1034.0001
Specific activity complex:	3.03 mCi/g
% 1-MCP:	3.16%
Specific Activity of 1-MCP:	4.70 mCi/mM or 87.0 mCi/g
Appearance (formulated test substance):	white powder

Treatment Chambers:

Material:	1/8-inch aluminum with welded seams
Internal Dimensions:	75.4- x 35.3- x38.4-cm
Internal Volume:	99 L (measured); 102.6 L (calculated)
Air Circulation:	electric fan mounted on bottom of the shelf (see below) at the center of the chamber.
Temperature Control:	Via coolant solution circulated through chamber jacket and coils from a refrigerated circulator
Other Features:	Perforated aluminum shelf approximately 5.4 cm from bottom of chamber; Double wall on one side to from a coolant jacket; Refrigeration coils at bottom of chamber below shelf; ports on sides and top of chamber to permit 1-MCP entry, as well as sampling and venting; Thermometer or thermocouple to monitor temperature; Removable top sealed with Viton gasket and bolts.

Experimental: Apples obtained from controlled atmosphere (CA) storage were refrigerated at 0-3°C for an unspecified time prior to treatment. There were two treatment regimes: ambient temperature (with 'Gala' apples) and 0°C (with 'Red Delicious' and 'Gala'). Just prior to the ambient treatment, apples were removed from refrigerated storage and permitted to reach room temperature, then placed in a treatment chamber. In the low temperature treatment, apples were immediately removed from refrigeration and placed in a treatment chamber. Treatment chambers were made of aluminum and had a volume of 99 L. Regardless of temperature regime, each treatment chamber contained approximately 25 kg of apples.

Application of 1-MCP: A 1000 ppm stock of 1-MCP was prepared via generation from a complex of 1-MCP and α-cyclodextrin. An unspecified amount of the 1-MCP/α-cyclodextrin complex was weighed into a dish and then place in a Tedlar bag fitted with a valve, and sealed. Water was injected into the bag via the valve to release 1-MCP. A headspace sample was collected from the bag using a gastight syringe and analyzed by gas chromatography with flame ionization detection (see below) to verify the 1-MCP content. An unspecified volume (determined by calculation) of the 1-MCP stock gas was then injected into apple-containing chambers to produce a 1-MCP concentration of 1.2

ppm (1200 ppb or 1.2x the maximum label rate). After exposure of the apples to 1-MCP for 24 hours, the chambers were vented to remove all atmospheric 1-MCP.

Apple Sampling: Apples were stored in the chamber until sampled. Samples consisted of three apples each collected from the top, middle, and bottom of the chamber. A minimum of two samples (6 apples) were collected from each treatment chamber.

Sample Handling/Processing: Each three-apple sample was immediately placed into separate containers and homogenized with 250-mL of saturated ammonium sulfate. In the same sample set, an untreated control sample was homogenized and two homogenates were fortified with 1-MCP. Each homogenizer is sealed and airtight during homogenization. A valve in the top of the homogenizer permits analysis of headspace gas or the introduction of fortification spikes.

Sample Analysis: A 3-mL sample of headspace gas over each homogenate was collected with a gastight syringe and transferred to sealed scintillation vials containing liquid scintillation cocktail. Volatile radioactive residues absorbed by the cocktail were measured by LSC analysis. After sampling, headspace gas in the homogenizers was vented. Graphitized carbon and Celite were then added to the homogenate and the mixture was blended. After drying and sieving, radioactive residues absorbed from the homogenates were analyzed by LSC following combustion. Total radioactive residues (TRR) measured in the headspace gas and in the homogenates were combined and calculated as equivalent µg/kg or ppb 1-MCP using the measured TRR and the specific activity of ^{14}C-1-MCP. No raw data or sample calculations were submitted by the registrant.

'Red Delicious' apples 0°C temperature regime were sampled at 4, 24, 48, 72, and 1 week posttreatment (Table 1); 'Gala' apples in the 0°C temperature regime were sampled at 24 hours, 1 week, and 2 weeks posttreatment (Table 2); 'Gala' apples in the ambient temperature regime were sampled at 24 hours and 1 week posttreatment, but data were only available for the 24-hour sampling interval at the time of his report (Table 3).

Results and Discussion: The TRR found in 'Red Delicious' and 'Gala' apples are presented below. Tables 1a, 2a, and 3a list the TRR that are uncorrected for the mean analytical method recovery percentage. Tables 1b, 2b, and 3b list the TRR that are corrected for the mean analytical method recoveries reported for the respective sampling intervals.

Table 1a. TRR in 'Red Delicious' apples following a 24-hour treatment with 1.2 ppm (1200 ppb) 1-MCP for 24 hours at 0°C; values are uncorrected for the mean method recovery for each sample set.

PTI*	TRR (ppb ^{14}C-1-MCP)					Fortification Data			
	Sample Location in Chamber					ppb ^{14}C-1-MCP	% recovery	ppb ^{14}C-1-MCP	% recovery
	Top	Middle	Bottom	Mean	Std. Dev.				
4 hr	3.9	3.0	4.4	3.8	0.7	1	87.2	10	87.2
24 hr	4.2	2.4	4.2	3.6	1.0	1	95.4	10	86.3
48 hr	1.5	4.0	2.4	2.6	1.3	1	72.5	10	90.0
72 hr	3.7	2.5	1.1	2.4	1.3	1	16.6**	10	86.0
1 week	3.5	1.8	4.0	3.1	1.2	1	84.7	10	103
Mean	3.4	2.7	3.2	3.1	-	-	85.0	-	90.5
std. dev.	1.1	0.8	1.4	1.1	-	-	9.5	-	7.2

* Posttreatment Interval

** This low recovery was identified with a footnote in the table submitted by the registrant, but no footnote was listed below the table to explain this anomalous value.

Table 1b. TRR in 'Red Delicious' apples following a 24-hour treatment with 1.2 ppm (1200 ppb) 1-MCP for 24 hours at 0°C; values are corrected for the mean method recovery for each sample set.

PTI*	TRR (ppb ^{14}C-1-MCP)***				Fortification Data	
	Sample Location in Chamber				ppb ^{14}C-1-MCP	Mean % recovery***
	Top	Middle	Bottom	Mean		
4 hr	4.5	3.4	5.1	4.3	1, 10	87.2
24 hr	4.6	2.6	4.6	3.9	1, 10	90.9
48 hr	1.9	4.9	3.0	3.3	1, 10	81.3**
72 hr	4.3	2.9	1.3	2.8	1, 10	86.0
1 week	3.7	1.9	4.3	3.5	1, 10	93.9
Mean	-	-	-		-	85.9

* Posttreatment Interval

** Mean calculated without the anomalous 16.6% recovery from apples fortified with 1 ppb ^{14}C-1-MCP

*** Corrected TRR values and mean method recoveries were calculated by the Agency reviewer

In 'Red Delicious' apples, TRR (corrected for method recoveries; Table 1b) ranged 1.9-5.1 ppb ^{14}C-1-MCP throughout the 1-week sampling period (Table 1b). The highest observed residue value (5.1 ppb) was in a sample collected from the bottom of the treatment chamber at 4 hours posttreatment. Overall, there were no trends in TRR relative to sample location in the test chamber or sampling interval. Radioactive residues declined slightly, but not significantly, between 4-hours and 1-week posttreatment. Uncorrected TRR values are listed in Table 1a.

Table 2a. TRR in 'Gala' apples following a 24-hour treatment with 1.2 ppm (1200 ppb) 1-MCP for 24 hours at 0°C; values are uncorrected for the mean method recoveries for each sample set.

PTI*	TRR (ppb ^{14}C-1-MCP)					Fortification Data			
	Sample Location in Chamber					ppb ^{14}C-1-MCP	% recovery	ppb ^{14}C-1-MCP	% recovery
	Top	Middle	Bottom	Mean	Std. Dev.				
24 hr	5.6	3.9	4.7	4.7	0.9	1	97.4	10	86.6
1 week	3.4	6.4	4.5	4.8	1.5	1	79.6	10	76.7
2 week	5.9	5.0	4.5	5.2	0.7	1	82.0	10	86.0
Mean	5.0	5.1	4.6	4.9	1.0	-	86.3	-	83.1
std. dev.	1.4	1.2	0.1	1.0	-	-	9.7	-	5.5

* Posttreatment Interval

Table 2b. TRR in 'Gala' apples following a 24-hour treatment with 1.2 ppm (1200 ppb) 1-MCP for 24 hours at)°C; values are corrected for mean method recoveries for each sample interval.

PTI*	TRR (ppb ^{14}C-1-MCP)**				Fortification Data	
	Sample Location in Chamber				ppb ^{14}C-1-MCP	Mean % recovery**
	Top	Middle	Bottom	Mean		
24 hr	6.1	4.2	5.1	5.1	1, 10	92.0
1 week	4.4	8.2	5.6	6.1	1, 10	78.2
2 week	7.0	6.0	5.4	6.1	1, 10	84.0
Mean	-	-	-		-	84.8

* Posttreatment Interval

** Corrected TRR values and mean method recoveries were calculated by the Agency reviewer.

In 'Gala' apples, TRR (corrected for mean method recoveries; Table 2b) ranged 4.2-8.2 ppb ^{14}C-1-MCP throughout the 2-week sampling period. The highest observed residue value (8.2 ppb) was in a sample collected from the middle of the treatment chamber at 1 week posttreatment. Although there were substantial differences in TRR amongst replications at different sampling intervals, there were no apparent trends relative to sample location in the test chamber or sampling interval. Radioactive residues increased slightly, but not significantly, between 4-hours and 2-weeks posttreatment. Uncorrected TRR values are listed in Table 2a.

Table 3a. TRR in 'Gala' apples following a 24-hour treatment with 1.2 ppm (1200 ppb) 1-MCP for 24 hours at ambient temperature; values are uncorrected for the mean method recoveries for each sample set.

PTI*	TRR (ppb ^{14}C-1-MCP)					Fortification Data			
	Sample Location in Chamber					ppb ^{14}C-1-MCP	% recovery	ppb ^{14}C-1-MCP	% recovery
	Top	Middle	Bottom	Mean	Std. Dev.				
24 hr	2.6	2.2	7.7	4.2	3.1	1	94.1	10	86.0

* Posttreatment Interval

Table 3b. TRR in 'Gala' apples following a 24-hour treatment with 1.2 ppm (1200 ppb) 1-MCP for 24 hours at ambient temperature; values are corrected for the mean method recoveries for each sample set.

PTI*	TRR (ppb ^{14}C-1-MCP)				Fortification Data	
	Sample Location in Chamber				ppb ^{14}C-1-MCP	Mean % recovery
	Top	Middle	Bottom	Mean		
24 hr	2.9	2.4	8.6	4.6	1, 10	90.1

* Posttreatment Interval

** Corrected TRR values and mean method recoveries were calculated by the Agency reviewer.

In 'Gala' apples, TRR (corrected for mean method recoveries; Table 3b) ranged 2.4-8.6 ppb ^{14}C-1-MCP at the 24-hour posttreatment sampling interval. The study author stated that samples were collected and analyzed at other sampling intervals, but the data were not ready for presentation in this report. The highest observed residue value (8.6 ppb) was in a sample collected from the bottom of the treatment chamber at 24-hours posttreatment. TRR were substantially higher in the apples collected from the bottom of the test chamber at 24 hours posttreatment. Uncorrected TRR values are listed in Table 3a.

Verification/Supporting Data: The registrant verified that the experimental protocol for generating 1-MCP and its introduction into the test chambers was accurate via five experiments wherein 1-MCP stock was generated and transferred to an empty treatment chamber. Concentrations of gaseous 1-MCP were determined for the stock mixture and in the treatment chambers via a GC/FID method using isobutene as a calibration standard (see Table 4). The method is described below. Stability of 1-MCP in the storage chambers was measured in three of the five aforementioned experiments by measuring 1-MCP concentrations at 24-hours post-introduction and compared to the initial concentration (see Table 5). The number of chamber volumes of air needed to completely vent 1-MCP from the chambers after the exposure period was also determined (see Table 6).

Analytical Method for GC/FID Analysis of non-radiolabeled 1-MCP in Headspace Gas:

Headspace gas collected from the stock mixture and the test chambers in support of an accuracy test (Table 4), in a stability test (Table 5), and in venting test (Table 6) was analyzed for 1-MCP by GC/FID equipped with a Porabond Q column. Data collected form the aforementioned analyses are presented below. Isobutene was used a calibration standard. Standards of 1-MCP (a gas at ambient temperatures; boiling point = 11°C) are not available because the condensed liquid is unstable and must be stored at low temperatures. Additionally, at high concentrations in the gaseous state, 1-MCP is flammable and self-condenses into dimers and polymers. Isobutene (MW = 56) was chosen as a calibration standard because it has the same number of double bonds and carbon atoms as 1-MCP (MW = 54) and is expected to have equivalent GC responses. No sample chromatograms were submitted. This method is similar to the method previously submitted to the Agency by the registrant.

Table 4. Accuracy of MCP Chamber Charging. Calculated Concentrations of 1-MCP vs. Measured Concentrations of 1-MCP determined by GC/FID.

Experiment No.	Calculated 1-MCP Conc. (ppm)	Measured 1-MCP Conc. (ppm)	Percentage Recovery
1	1.10	1.06	96
2		0.95	86
3		0.97	88
4	1.20	1.12	93
5		1.13	94

Table 5. Stability of 1-MCP in a Test Chamber Over 24 Hours.

Experiment No.	Initial 1-MCP Conc. (ppm)	24-Hr 1-MCP Conc. (ppm)	Percentage Change
1	1.06	1.02	-4
2	0.95	0.93	-2
3	0.97	0.93	-4

Table 6. Chamber Vent Test. Concentration of 1-MCP Remaining in a Test Chamber After Multiple Changes in Chamber Air Via Venting.

Experiment No.	Vent Flow (L/min)	Vent Time (min)	Chamber Volumes Purged	1-MCP Conc. (ppm)
1	40	15	6	<0.01
2	20 20	16 24	3.2 4.8	0.05 <0.01
3	40	12	4.8	<0.01
4	40	16	6.5	<0.01

The data presented in Tables 4, 5, and 6 above demonstrate that the concentration of 1-MCP was maintained at levels reasonably close to the calculated levels of exposure during the exposure period (Table 4), that ^{14}C-1-MCP was stable in the treatment chambers during the exposure period (Table 5), and that 1-MCP could be reduced to <0.01 ppm in the test chamber following a single 15-minute air purge at 40 L/min (6 volumes of air replaced) or two 20-min air purges at 20 L/min (8 volumes of air replaced).

Study Deficiencies: Minor; do not affect study conclusions. No raw data or sample chromatograms presented for analytical methods. No data presented for sampling intervals longer than 2 weeks posttreatment.

ATTACHMENTS

Pages 20-39 - *Access to FIFRA health and safety data is restricted under FIFRA

13544

R141539

Chemical: Cyclopropene,1-methyl-

PC Code:
224459

HED File Code: 41500 BPPD Tox/Chem
Memo Date: 5/3/2001
File ID: DPD273531
Accession #: 000-00-9002

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