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

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

DATE: October 3, 2007

SUBJECT: Science Review in Support of Section 3 Registration of 1-Methylcyclopropene (EPA Symbol #: 71297-X), Containing 3.8% of 1-Methylcyclopropene (Active Ingredients). Review of Product Chemistry, Acute Toxicity, Nature of Residue Studies, and Non-Target Organism and Plant Studies

Decision Number: 373522
DP Number: 339988
EPA File Symbol Number: 71297-X
Chemical Class: Biochemical
PC Code: 224459
Active Ingredient Tolerance Exemptions: 40 CFR 180.1220
MRID Numbers: 47024912, 47024914 -15, 47024917 – 24, 47024928 - 34, 47024941-42, 47024944, 47024946 - 47, 47024949 - 50, 47024954, 47088601-20 & 47108201-07

FROM: Manying Xue, Chemist
BPB/BPPD (7511P)

THROUGH: Russell S. Jones, Ph.D., Senior Biologist /s/ 10/03/2007
BPB/BPPD (7511P)

TO: Driss Benmhend, Regulatory Action Leader
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CONTAINS CONFIDENTIAL BUSINESS INFORMATION

ACTION REQUESTED:

AgroFresh, Inc., a subsidiary of Rohm and Haas Company, has submitted an application for a Section 3 registration for the product AFxRD-038, a powder formulation containing 3.8% of the active ingredient 1-methylcyclopropene (1-MCP). AFxRD-038 is a formulated product produced from the EPA registered Manufacturing Use Product, SF, (EPA Registration No. 7 1297-4). The new use product, AFxRD-038, is proposed for preharvest use.

An exemption from the requirement of a tolerance has been established for residues of 1-Methylcyclopropene in/on fruits and vegetables when used as a post harvest plant growth regulator, *i.e.*, for the purpose of inhibiting the effects of ethylene (40 CFR 180.1220).

This petition proposes to extend the tolerance exemption for preharvest use of AFxRD-038 on pome fruit (Crop Group 11), kiwifruit, walnuts, fruiting vegetables, (Crop Group 8), corn, cotton, soybean and sunflower.

In support of this petition, the petitioner has submitted product chemistry studies of 1-Methylcyclopropene, active ingredient (MRIDs 47108201- 02, 47108205, 47108208, 47024907, 47024938, 47024942, 47088601- 08, 47088613 -15), acute toxicity studies (MRIDs 47024928-33), nature of residue studies (47088611-12 & 47108203), two-week inhalation range-finding (MRID 47024934), ecological effects (47024918-24, 47024941, 47024944, 47024946-47, 47088609-10, 47088617-20), basic Confidential Statements of Formula (CSF, dated 12/21/06), and proposed label, etc..

BPPD has reviewed and evaluated the submissions for 1-Methylcyclopropene (EPA Symbol #: 71297-X). The decisions are made to reflect the current OPP's policies.

RECOMMENDATIONS AND CONCLUSIONS:

1. The submitted product chemistry studies for the EP product, AFxRD-038 is **UNACCEPTABLE** based on OPPTS guidelines 830 Series, but upgradeable if the registrant resolves the deficiencies: 1) clarifies that SmartFresh Technology High AI Powder and Manufacturing Use Product – SF (EPA Reg. No. 71297-4) is the source of the active ingredient and lists it and its registration number and supplier on the CSF; 2) lists the total weight on the CSF (Box 17); 3) explains the reason the expanded upper and lower certified limits of the inert ingredients and omits [redacted] as an inert ingredient; and 4) addresses explodability and performs one-year storage stability and submits the results upon completion.

2. The submitted acute toxicity studies (the six packets) are **ACCEPTABLE** under OPPTS guidelines 870.1100 - 1300 & 870.2400 – 2600 for the EP product AFxRD-038 (EPA Symbol #: 71297-X, 3.8% 1-methylcyclopropene, a.i.). Toxicities were determined as category IVs for acute oral toxicity, acute dermal toxicity, acute inhalation toxicity, acute eye irritation and acute dermal irritation. The dermal sensitization study showed that the EP product AFxRD-038 is not a dermal sensitizer.

3. The submitted range-finding study is designed to estimate toxicity or to provide information needed for dose selection for subsequent studies. It does not meet regulatory guidelines for numbers of animals per group or inclusion of both sexes of animals. 1-MCP, when administered as a gas via whole-body inhalation to male rats for 13 exposures over three weeks, had a No Observable Effect Level (NOEL) of 107 ppm. **However, no additional study is required.**

4. The estrogenic potential of the test material was determined to be improbable. The submitted structure-Activity Hazard Screen (Nonguideline) showed that the DEREK system calculateda Log Kp of -2.02, a Log P of 1.45, and a molecular weight of 54.092. No bacterial or mammalian alerts were found.

5. The submitted toxicokinetic study does not satisfy data requirement under OPPTS 870.7485. However, **no additional toxicokinetic study is required.** Metabolite identification studies were not conducted. The animals were observed and excreta collected over a 20-hour period rather than the recommended seven days. Because of the low absorption of the test material, however, the study duration could be considered acceptable. The study report appears to be presented from various modifications conducted over a period of years. References to later additions to the report were not included in the summary or conclusions of the study report. **However, the study will not affect the conclusion of risk assessment for this registration.**

6. The submitted waiver request for immunotoxicity is **ACCEPTABLE**.

7. The submitted nature of the residue study for 1-Methylcyclopropene (AFxRD-038) in apple, maize and tomato are not adequate to satisfy the OPPTS guideline 860.1300. Based on the guideline, the registrant should use the maximum exaggerated rate for apple metabolism study in order to identify and characterize the major metabolites. The characterization and identification of the metabolites were not conducted; and metabolic pathways for 1-methylcyclopropene were not proposed. No information of storage stability studies were conducted or reported. **However, the data of nature of residue studies are not required to support an exemption from the requirement of a tolerance for this petition.**

8. The submitted studies of ecological effects, aquatic invertebrate acute toxicity test, freshwater daphnids (OPPTS 850.1010), freshwater fish testing, Tier I (OPPTS 850.1075), avian acute oral toxicity (OPPTS 850.2100), Avian Dietary Toxicity (OPPTS 850.2200), honey bee acute contact toxicity (OPPTS 850.3020), and algal toxicity, Tiers I and II (OPPTS 850.5400) are **ACCEPTABLE**. The results are listed in the Table (Table 28).

9. The studies for non-target insects (the predatory mites, *Typhlodromus pyr*i, the beetles *Poecilus cupreus* and the parasitic wasp *Aphidius rhopalosiphi*) treated with 3.4% 1-Methylcyclopropene alpha cyclodextrin complex and 3.6% w/w 1-methylcyclopropene are **ACCEPTABLE**. The studies showed that there were no statistically significant differences in the survival or feeding behavior of the beetles in either test material group compared to the negative control group.

10a. Based on the screening level non-target organism studies there are no concerns for non-target and endangered birds.

10b. Based on the report bindings and lack of detectable leachates of 1-MCP in a soil adsorption/desorption isotherm study, there will be no exposure to fish and aquatic invertebrates when the product is used in accordance with approved labeling.

10c. Based on the submitted studies, BPPD has determined there will be no effects (NE) on endangered birds, fish, aquatic invertebrates.

11. The study for acute earthworm toxicity testing is **ACCEPTABLE**. AFxRD-038 applied at a concentration sufficient to produce an atmospheric concentration of 10 ppm v/v 1-methylcyclopropene had no effect on survival or weight of *E. fetida*. The poor recovery of active ingredient in the test vessels was likely due to absorption (and probably degradation) by the soil. Recovery of the active ingredient in other studies without soil but using a similar setup and the same analytical method (MRIDs 47024944 and 47088617) was acceptable.

12. The study is **ACCEPTABLE** for honey bee acute contact toxicity. In a laboratory study, adult honey bees (*Apis mellifera*) were exposed to a nominal atmospheric concentration of 10 ppm (v/v) 1-methylcyclopropene for 48 hours. The test also included a negative control group exposed to air only. At test end, there was no statistically significant difference in bee mortality in the test material group (8.3%) compared to the negative control group (10.0%), and no treatment-related differences in behavior of bees in the test material group compared to the negative control group. The 48-hour LD_{50} for bees exposed to 10 ppm 1-methylcyclopropene in air was >10 ppm and the no-observed-effect concentration was 10 ppm.

13. The study for soil adsorption/desorption isotherm (OPPTS 835.1220) is **ACCEPTABLE**. When radiolabeled 1-Methylcyclopropene (^{14}C-1-MCP) was applied to four soil types in column leaching experiments, less than 3% of the applied radioactivity was found to leach in each soil type thus demonstrating that the field use of 1-MCP should not result in significant leaching of 1-MCP residues to groundwater. Analyses of the soil layers from the column experiments indicate that 1-MCP binds rapidly and tightly to the soil types tested. No detectable amount of the parent 1-MCP was found in leachates from any of the soils tested. Thus, 1-MCP can be classified as a compound with low mobility in soil. The amount of applied ^{14}C-1-MCP was approximately ten times the maximum field application rate of 500 g a.i./h.

14. The submitted study for Ready Biodegradability (OPPTS 835.3110) is **ACCEPTABLE**. Test chambers from the control and treatment groups were analyzed for 1-MCP on Days 0, 7, 13, 21 and 28. The reference group was dosed with sodium benzoate at a nominal concentration of 20 mg C/L. The test inoculum was demonstrated to be viable by actively degrading more then 70% of the sodium benzoate after 7 days. Degradation of 1-MCP was not observed under the test conditions for the 28 day test. Average measured test substance treatment concentrations of 1-MCP on Days 7 and 28 were 0.506 and 0.507 mg/L, respectively.

STUDY SUMMARIES

Directions for Use (OPPTS 860.1200)

AgroFresh, Inc., a subsidiary of Rohm and Haas Company, has submitted a product label for the product AFxRD-038, a powder formulation containing 3.8% of the active ingredient 1-methylcyclopropene (1-MCP).

Target Uses

AFxRD-038 is proposed to be used for protecting fruits, vegetables and agronomic crops from the various effects of ethylene.

Use Directions

Application Instructions

Mixing Instructions

1. Fill the tank with approximately 2/3 of required water volume.

2. Measure the proper (See Use of Adjuvants below) volume of spray oil and surfactant for the total spraying volume. See Use of Adjuvants section below.
3. Add spray oil ONLY to the tank with agitator running. Do not add surfactant at this time to avoid foaming. The spray oil must emulsify and spray solution for application must look milky white before adding Harvista Technology.

4. The enclosed inner pouches containing Harvista Technology are water-soluble. Do not allow water soluble pouch to become wet prior to adding to the spray tank. Do not handle with wet hands. After opening the outer foil package, drop the unopened inner water soluble pouch into the spray tank. Multiple water soluble pouches may be required as directed in Use Rate Table above. The entire water soluble pouch will dissolve in the water.

5. Fill the spray tank to final required water volume and continue running the agitation system for 10 minutes after adding Harvista Technology water soluble pouches, DO NOT EXCEED 10 MINUTES OF AGITATION. Add the surfactant in the last few minutes of agitation.

6. Do not use agitator during spraying.

7. Initiate spray application as soon as possible after step 5 and no later than 60 minutes after preparation of tank mixture.

Use of Adjuvant

Harvista Technology must be used with a spray oil adjuvant. Use summer spray oil at a concentration of .0% (v/v) based on the volume of the spray solution plus an organosilicone surfactant at 0.05% v/v. To reduce foaming, minimize agitation and if necessary add a defoamer.

Spray Volume

Apply Harvista Technology in a sufficient amount of water to ensure good coverage of the tree canopy. Product efficacy requires that fruits and foliage receive uniform spray coverage. Use calibrated spray equipment to ensure uniform coverage of leaves and fruit.

Adjust water volumes based on tree size and spacing. In most cases, use a minimum of 200 gallons per acre or the equivalent of a dilute spray. Use larger droplets of 300 microns for better spray coverage. Excessive spray application volume can result in spray runoff and a reduction in product performance.

Spray Nozzle Requirements

Use flat-fan nozzles. Select nozzles and pressure combinations which deliver coarse (250-3 75 urn) spray droplets.
The summary of AFxRD-038 label is shown in Table 1.

Table 1. Summary of End-Use 1-Methylcyclopropene Product AFxRD-038

Crops	Application Timing	Rate	Restrictions
Apple	3-7 Days Before Anticipated Harvest (Fruit Ripening)	37.6 to 112.7 (1.43 to 4.28 oz ai/acre)	Do not apply more than 112.7 oz /acre per crop. Allow a minimum of 3 days between treatment and harvest
Pear	7 Days Before Anticipated Harvest (Fruit Ripening)		
Kiwifruit	3-7 Days Before Anticipated Harvest (Fruit Ripening)	9.4 to 37.6 (0.36 to 1.43 oz ai/acre)	Do not apply more than 37.6 oz/acre per crop. Allow a minimum of 3 days between treatment and harvest
Walnut	At flowering, when 10-30% Flowers are receptive to pollen	18.8 to 37.6 (0.71 to 1.43 oz ai/acre)	Do not apply more than 37.6 oz/acre per crop.
Tomato, Fresh Market and Processed	At Bloom Timing Apply at the initiation of the 1st bloom period followed by a second application 4-7 days after 15t application or a second application at the initiation of the 2'' bloom period.	3.8 to 9.4 (0.14 to 0.36 oz ai/acre)	Do not apply more than 18.8 oz/acre per crop. Allow a minimum of one day between treatment and harvest.
Tomato, Fresh Market and Marked	Before Harvest Timing Apply 28 days before harvest or as fruit begins to color and apply 4 to 7 days after the first application.		
Peppers	At Bloom Timing Apply at the initiation of the 1st bloom period followed by a second application 4-7 days after 1 application or a second application at the initiation of the 2 bloom period.		
Corn - Field, pop, sweet (Includes seed production and forage or silage production)	. Apply to an actively growing corn crop between V5 and V12.	3.8 to 9.4 (0.14 to 0.36 oz ai/acre)	Do not apply more than 18.8 oz/acre per crop
Cotton	Apply to an actively growing cotton crop between Pin-head Square (PHS) stage and Early Bloom stage. A 2' application may be required 14 days following the 1 application.		
	Apply to an actively growing		

Soybean	soybean crop between early flower (V6/R1 stage) and beginning pod (R3 stage).		
Sunflower	beginning pod (R3 stage). Apply to an actively growing sunflower crop between 7-leaf (V7 stage) and beginning flowering (R2 stage).		

Conclusions:
The submitted label for the end-use product, AFxRD-038 (EPA Symbol #: 71297-X) has described the general information: first aid, direction for use, ect..

Product Properties (OPPTS 830 Series GLNs)

End Use Product, AFxRD-038

AgroFresh, Inc., a subsidiary of Rohm and Haas Company, has submitted product chemistry studies for the product AFxRD-038, a powder formulation containing 3.8% of the active ingredient 1-methylcyclopropene (MRIDs 47108201- 02, 47108205, 47108208, 47024907, 47024938, 47024942, 47088601- 08, 47088613 -15), and basic Confidential Statements of Formula (CSFs), dated 12/21/2006 (Table 2).

Table 2 lists the nominal concentrations and certified limits for the ingredients in the end-use product AFxRD-038. The certified limits for the ingredients are essentially within the OPPTS 830.1750-recommended ranges and are consistent with the results of the preliminary analysis.

TABLE 2. Nominal CSF concentrations and limits for AFxRD-038[a]

Ingredients (CAS number)	PC Code/ 40CFR	Purpose	Concentration (% by weight)		
			Nominal	Lower	Upper
Active Ingredient					
1-Methylcyclopropene, 96% pure (3100-04-7)	224459	a.i.	3.8%	3.4%	4.2%
Inert ingredient					
[redacted]					

[a] Data from CSF.

Physical and Chemical Characteristics

The product chemistry data base for AFxRD-038 is essentially complete. There are no reported impurities of toxicological concern. The Series 830 physical and chemical properties are given in Table 3.

TABLE 3. Physical and Chemical Properties for AFxRD-038			
Guideline Reference No.	Property	Description of Result	Methods
830.6302	Color	White at 21°C and ambient pressure	MRID 47088601; Visual inspection
830.6303	Physical State	Cotton wool like powder at 21°C and ambient pressure	MRID 47088601; Visual inspection
830.6304	Odor	Slight gasoline-like at 21°C and ambient pressure	MRID 47088601; "was made qualitatively"
830.6313	Stability	Not required for EP	
830.6314	Oxidation/Reduction: Chemical Incompatibility	Avoid contact with acids, alkalies, and strong oxidizing agents	MSDS for EP
830.6315	Flammability	Not flammable; ignition temperature 400°C (572°F). 1-MCP Formulation containing 3.3% a.i. is not highly flammable. In contact with the ignition source 1-MCP Formulation burned immediately and little pellets were observed which sustained burning. Only part of the test item was burning and the propagation was about 200 mm during 9 minutes.	MSDS for EP; MRID 47024942; EEC Directive 92/69, Part A.
830.6316	Explodability	Not addressed	
830.6317	Storage Stability	The active ingredient 1-MCP in AFxRD-038 formulation is stable [3.8 wt% (elevated temperature) vs 3.9 wt% (reference sample)] after 14 days at 54±2°C when stored in commercial packaging consisting of an inner PVA pouch and an outer foil pouch. One-year storage stability is not addressed.	MRID 47088602; AgroFresh Report AF-06-078
830.6319	Miscibility	Not addressed; not applicable	
830.6320	Corrosion Characteristics	The PVA pouch was slightly yellowed and brittle after 14 days at 54±2°C, the integrity of the PVA package was not compromised.	AgroFresh Report AF-06-078
830.6321	Dielectric Breakdown Voltage	Not addressed; not applicable	
830.7000	pH	10.6 at 21.1°C (1% w/w in water)	MRID 47088603; using pH meter and a lass electrode; CIPAC MT 75.3
830.7050	UV/Visible	Not required for EP; The absorption spectra in neutral (in water), acidic (hydrochloric acid), and alkaline (sodium hydroxide) media did not exhibit maxima. Therefore, the molar absorption coefficient and a bandwidth could not be calculated.	MRID 47088604; using spectrophotometer
830.7100	Viscosity	Not addressed; not applicable	
830.7200	Melting Range	Not required for EP; "no melting could be observed" from 25°C to 400°C (preliminary test) and 100°C to 230°C (main test)	MRID 47088605; using differential scanning calorimeter
830.7220	Boiling Range	Not required for EP; "did not boil" at 25°C to 400°C at 101.2 kPa.	MRID 47088605; using differential scanning calorimeter

830.7300	Bulk Density	0.2729 and 0.2586 g/mL (pour density) 0.3411 and 0.3428 g/mL (tap density)	MRID 47088606; "fall vertically through a distance of 25 mm" and "after 50 taps"
830.7370	Dissociation Constant in Water	Not required for EP	
830.7520	Particle Size	Approximately < 0.5 µm to < 225 µm (particle size distribution); < 24.7 µm (mass median diameter)	MRID 47088607; using laser diffraction method
830.7550	Partition Coefficient	Not required for EP	
830.7840	Water Solubility	Not required for EP; Between 50.7 g/L and 51.1 g/L (not corrected for the purity of the test item); The water solubility of 1-MCP was estimated to be approximately 137 mg/L at room temperature.	MRID 47088608; by a simple flask method (defined amount of test item mixed with water and stirred); 1-MCP: MRID 47088615; 1-MCP gas released from formulation and saturated in water, then GC analysis
830.7950	Vapor Pressure	Not required for EP; The calculated vapor pressure of α-cyclodextrin, EDTA, and 1-methylcyclopropene are 1×10^{-29} Pa, 2×10^{-7} Pa, and 2×10^{5} Pa, respectively, at 25°C.	MRIDs 47088613 and 47088614; based on EEC directive 02/69, Part A, and OECD guideline No. 104.

Classification: Unacceptable, but upgradeable. To upgrade to acceptable, the registrant must resolve the deficiencies: 1) clarifies that SmartFresh Technology High AI Powder and Manufacturing Use Product – SF (EPA Reg. No. 71297-4) is the source of the active ingredient and lists it and its registration number and supplier on the CSF; 2) corrects the amount of the a.i. (Box 13 a) on the CSF; 3) lists the total weight on the CSF (Box 17); 4) addresses the formation of impurities; 5) explains the reason the expanded upper and lower certified limits of the inert ingredients and omits [redacted] as an inert ingredient; and 6) addresses explodability and performs one-year storage stability and submits the results upon completion.

Acute Toxicity (OPPTS 870.1100 - 1300 & 870.2400 – 2600)

AgroFresh, Inc., a subsidiary of Rohm and Haas Company, has submitted acute toxicity studies using the EP product AFxRD-038 (EPA Reg. No. 71297-A, 3.8% 1-methylcyclopropene, a.i.) as test material for acute oral toxicity, acute dermal toxicity, acute inhalation toxicity and skin sensitization studies (MRIDs: 460249-28 through-33). The studies were conducted at Product Safety Laboratories, Dayton, NJ.

Acute oral toxicity study (OPPTS 870.1100)

Test Animals: Three female Sprague-Dawley rats were received from Ace Animals, Inc., Boyertown, PA, and weighed 193-198 g on the day of dosing. The young adult animals, 10 or 11 weeks old, were housed individually in suspended stainless steel cages with

mesh floors. The animals were fed Purina Rodent Chow No. 5012. Filtered tap water was available *ad libitum*. The environmental conditions of the animal room were as follows: temperature, 19-22°C and photoperiod, 12 hour light/dark cycle. Relative humidity and air changes per hour were not reported.

Methods: The rats were ear-tagged: Nos. 6584, 6627, and 6628, acclimated for 15 or 20 days and fasted overnight prior to treatment. The test material (5000 mg/kg body weight) was dosed as a 30% w/w suspension in corn oil by gavage (Table 1). Due to the high volume of test suspension to be administered (15.83 mL/kg), each rat's dose was divided into two approximately equal portions, dosed two hours apart. Body weight was recorded prior to dosing, and on days 7 and 14. The test animals were observed for mortality and clinical signs of toxicity during the first several hours post-dosing and at least daily for 14 days. All animals were necropsied.

Acute Dermal Toxicity - Rats (OPPTS 870.1200)

Test Animals: Five male and five female Sprague-Dawley rats were received from Ace Animals, Inc., Boyertown, PA, were assigned to groups, and weighed 328-340 g (males) and 218-230 g (females) on the day of treatment. The young adult animals, 10-11 weeks old, were housed individually in suspended stainless steel cages with mesh floors. The animals were fed Purina Rodent Chow No. 5012 and filtered tap water was available *ad libitum*. The environmental conditions of the animal room were as follows: temperature, 19-22EC and photoperiod, 12 hour light/dark cycle. Relative humidity and air changes per hour were not reported.

Methods: Rats were ear-tagged: Male – Nos. 7015 to 7019; Female – Nos. 7020 to 7024 and acclimated for 22 days. The test material was mixed with mineral oil to prepare a dry paste (a 50% w/w mixture). The test material (5000 mg/kg body weight) was applied to a 2 inch x 3 inch gauze pad and placed on a shaved area of approximately 2 inch x 3 inch (approximately 10% of the body surface) of the dorsal trunk. The gauze pad and entire trunk were wrapped with Durapore tape. The coverings were removed after 4 hours (assumed to be a typographic error, should be 24 hours) and excess test material removed. The test animals were observed during the first several hours after treatment for mortality, signs of gross toxicity, and behavior changes, and daily thereafter for 14 days. The rats were weighed prior to treatment and on days 7 and 14. The rats were euthanized on day 14 and necropsied.

Acute Inhalation Toxicity (OPPTS 870.1300)

Test Material: AFxRD-038 (EPA Reg. No. 71297-A) is an end-use product (EP) used to protect fruits, vegetables, and agronomic crops from the various effects of ethylene. The active ingredient in the EP is 3.8% w/w 1-methylcyclopropene (1-MCP; 96% purity). The inerts are [redacted] 1-MCP, a gas at room temperature, is trapped in an alpha-cyclodextrin complex for ease in handling and controlled release. AFxRD-038 is a

powder which releases 1-MCP gas when mixed with water. The registrant's registered product, SmartFresh™ (EPA Reg. No. 71292-2), contains 3.3% 1-MCP.

Acute Eye Irritation - Rabbits (OPPTS 870.2400)

Test Animals: Three male young adult New Zealand White rabbits were received from Robinson Services, Inc., Clemmons, NC. The animals, 11-12 weeks old, were housed individually in suspended stainless steel cages with mesh floors. The animals were fed Pelleted Purina Rabbit Chow No. 5326. Filtered tap water was available *ad libitum.* The environmental conditions of the animal room were as follows: temperature, 19-23EC and photoperiod, 12 hour light/dark cycle. Relative humidity and air changes per hour were not reported.

Methods: Rabbits were ear-tagged: Nos. 15763, 15764, 15765 and acclimated for 13 days. The test material, 0.1 mL (0.03 g/eye/animal), was applied in the conjunctival sac of the right eye, and the eye held closed for approximately one second. The left eye served as control. The eyes were examined and scored 1, 24, 48 and 72 hours after test material instillation.

Primary Dermal Irritation - Rabbits (OPPTS 870.2500)

Test Animals: Three male young adult New Zealand White rabbits were received from Robinson Services, Inc., Clemmons, NC. The animals, 10 weeks old, were housed individually in suspended stainless steel cages with mesh floors. The animals were fed Pelleted Purina Chow No. 5326. Filtered tap water was available *ad libitum.* The environmental conditions of the animal room were as follows: temperature, 19-21EC and photoperiod, 12 hour light/dark cycle. Relative humidity and air changes per hour were not reported.

Methods: The rabbits were ear-tagged: Nos. 15724, 15725, and 15726 and acclimated for five days. The fur on the dorsal trunk of each rabbit was clipped on the day prior to treatment. The test material was moistened with mineral oil to prepare a 50% w/w mixture (dry paste). The rabbits were treated with 0.5 g of test material (1.0 g test mixture) applied on a 1 inch x 1 inch gauze pad and applied to a 6 cm^2 clipped intact dose site. The pad and entire trunk were wrapped with a semi-occlusive Micropore tape and Elizabethan collars were placed on the rabbits. The covering and the collar were removed four hours later and the site cleansed to remove any residual test material. The animals were observed at least once daily for gross toxicity and behavior changes during the study. Dermal examination was recorded at 1, 24, 48, and 72 hours after removal of the patch.

Skin Sensitization (OPPTS 870. 2600)

Test material: AFxRD-038 (EPA Reg. No. 71297-A) is an end-use product (EP) used to protect fruits, vegetables, and agronomic crops from the various effects of ethylene. The active ingredient in the EP is 3.8% w/w 1-methylcyclopropene (1-MCP) that is 96% pure. The inerts are [redacted]

[redacted] 1-MCP, a gas at room temperature, is trapped in an alpha-cyclodextrin complex for ease in handling and controlled release of 1-MCP gas. AFxRD-038 is a powder which releases 1-MCP gas when mixed with water. The registrant's registered product SmartFresh™ (EPA Reg. No. 71292-2) contains 3.3% 1-MCP.

The reviewer finds the information submitted in support of using surrogate toxicity data for 1-Methylcyclopropene Alpha-Cyclodextrin Complex (3.3% a.i.) for a dermal sensitization study of AFxRD-038 as acceptable. The reviewer has evaluated MRID 45458607 (BPPD assignment No. 131) previously and found that 1-Methylcyclopropene Alpha-Cyclodextrin Complex (3.3% a.i.) was not a dermal sensitizer.

The results for acute studies from the submitted studies and literature reports are summarized in Table 4.

TABLE 4 Acute Toxicity Profile - Test Substance

Guideline No.	Study Type	MRID(s)	Results	Toxicity Category
870.1100	Acute oral [rat]	47024928	LD_{50} = >5000 mg/kg for female	IV
870.1200	Acute dermal [rat]	47024929	LD_{50} = >5000 mg/kg for males, females, and combined	IV
870.1300	Acute inhalation [rat]	47024933	The inhalation LC_{50} for males, females, and combined for 1-MCP was > 2.5 mg/L.	IV
870.2400	Acute eye irritation [rabbit]	47024930	The maximum average score was 10.0 one hour after test material instillation. AFxRD-038 was minimally irritating.	IV
870.2500	Acute dermal irritation [rabbit]	47024931	The primary irritation index was 0.3. AFxRD-038 was slightly irritating.	IV
870.2600	Skin sensitization [guinea pig]	47024932	surrogate toxicity data for 1-Methylcyclopropene Alpha-Cyclodextrin Complex (3.3% a.i.) support this registration	not dermal sensitizer

Conclusions:

The packet classification is **ACCEPTABLE** for the acute oral toxicity study with rats under OPPTS guideline 870.1100 for the EP product AFxRD-038 (EPA Reg. No. 71297-A, 3.8% 1-methylcyclopropene, a.i.). The oral LD_{50} for female rats was greater than 5000 mg/kg for both products. This places AFxRD-038 in TOXICITY CATEGORY IV.

The packet classification is **ACCEPTABLE** for the acute dermal toxicity study with rats under OPPTS guideline 870.1200. The dermal LD_{50} for males, females, and combined

was greater than 5000 mg/kg forAFxRD-038. This places AFxRD-038 in TOXICITY CATEGORY IV.

The packet classification is **ACCEPTABLE** for the acute inhalation toxicity study under OPPTS guideline 870.1300. The submitted information in support of using surrogate toxicity data on 1-MCP gas (95.8% active ingredient) for an acute inhalation toxicity study for AFxRD-038 indicates that AFxRD-038 is in TOXICITY CATEGORY IV.

The packet classification is **ACCEPTABLE** for the acute eye irritation study with rats under OPPTS guideline 870.2400 for the EP product AFxRD-038. Corneal opacity and iritis were not noted on any rabbit during the study. Positive conjunctival irritation was noted on all rabbits one hour after test material instillation with resolution by 24 hours. The maximum average score was 10.0 one hour after test material instillation. AFxRD-038 was minimally irritating and is in TOXICITY CATEGORY IV.

The packet classification is **ACCEPTABLE** for the acute dermal irritation study with rats under OPPTS guideline 870.2500 for the EP product AFxRD-038. Very slight erythema was noted on 3/3 rabbits one hour after patch removal with clearance on two rabbits by 24 hours and on one rabbit by 48 hours. The primary irritation index was 0.3. AFxRD-038 was slightly irritating and is in TOXICITY CATEGORY IV.

The information submitted in support of using surrogate toxicity data for 1-Methylcyclopropene Alpha-Cyclodextrin Complex (3.3% a.i.) for a dermal sensitization study of AFxRD-038 as **ACCEPTABLE** under OPPTS guideline 870.2600. The reviewer has evaluated MRID 45458607 (BPPD assignment No. 131) previously and found that 1-Methylcyclopropene Alpha-Cyclodextrin Complex (3.3% a.i.) was not a dermal sensitizer.

Two-Week Inhalation Range-Finding – Rats

AgroFresh, Inc., a subsidiary of Rohm and Haas Company, has submitted an inhalation range-finding study in rats. The test Material is 95.80% 1-Methylcyclopropene (1-MCP). Sixteen Crl:CD®BR male rats (source not reported) were assigned to groups and weighed 184.3-219.4 g (males) on the day of treatment.

Results:

All rats survived the study. No treatment-related clinical signs of toxicity were noted at any exposure level. All rats gained weight during the study and no effect on mean body weight was noted at any exposure level.

Conclusions:

The submitted range-finding study is designed to estimate toxicity or to provide information needed for dose selection for subsequent studies. It does not meet regulatory guidelines for numbers of animals per group or inclusion of both sexes of animals. 1-MCP, when administered as a gas via whole-body inhalation to male rats for 13 exposures over three weeks, had a No Observable Effect Level (NOEL) of 107 ppm.

Structure-Activity Hazard Screen (Nonguideline)

AgroFresh, Inc., a subsidiary of Rohm and Haas Company, has submitted a structure activity hazard screen study (MRID 47088618). In the study, 1-Methylcyclopropene was analyzed by the DEREK system; a predictive computer program that identifies the toxic potential of the compound using structure-activity relationships based on the Log Kp, Log P, and the molecular weight.

The result showed that DEREK system calculated a Log Kp of -2.02, a Log P of 1.45, and a molecular weight of 54.092. No bacterial or mammalian alerts were found. The estrogenic potential of the test material was determined to be improbable.

Toxicokinetic – Rat (OPPTS 870.7485)

AgroFresh, Inc., a subsidiary of Rohm and Haas Company, has submitted toxicokinetic study (MRID 47088616) using1-methylcyclopropene ^{14}C-radiolabeled at the C-3 carbon as test material. The studies were conducted at Rohm and Haas Co., Toxicology Department, Spring House, PA 19477-0904.

In a toxicokinetic study, 1-methylcyclopropene (1-MCP) in an alpha cyclodextrin complex (3.16% a.i., (sublots of Lot No. 1034.00 labeled on carbon 3 of the ring) was administered to groups of three male and three female cannulated Crl:CD®BR rats by inhalation at concentrations of 100 ppm or 1000 ppm (equivalent to 221 and 2212 mg/m^3 or 0.221 or 2.212 mg/L, respectively) for four hours. During exposure, whole blood and/or plasma samples were collected. At the end of exposure, three male and three female rats exposed to 1000 ppm were sacrificed. Three rats/sex/ exposure group were placed into metabolism cages and additional blood samples were drawn until 20 hours after exposure. At sacrifice, the liver, kidney, spleen, lungs, and fat were collected and the amount of radioactivity determined in these tissues and organs, as well as the residual carcass of all rats in the study.

Results:

Average exposure concentration: Shown in Table 5 are the average 1-MCP exposure concentrations to male and female rats over four hours. All results were within 14% of nominal.

TABLE 5. Average concentration of 1-MCP in atmosphere to male and female rats exposed for four hours.

Group	Sex	Number	Nominal (ppm)	Actual (ppm)	Percent of nominal	Percent of Target Dose[a]
1	Male	4	1000	1042 ± 24.5	104	107.2 ± 1.5
1	Female	2	1000	994 ± 78.8	99.4	102.3 ± 4.6
2	Male	3	100	103 ± 15.3	103	111.2 ± 15.9
2	Female	3	100	87 ± 8.3	87	91.8 ± 8.9
3	Male	3	1000	1078 ± 55.9	108	109.9 ± 5.74
3	Female	3	1000	1007 ± 51.9	101	102.9 ± 4.1

Data derived by reviewer from pages 97-102 of MRID 47088616
[a]Represents the total dose of radiolabeled 1-MCP the rats were exposed to.
Groups 3 and 3A are presented combined

Exposure assumptions: The approximate volume of the exposure bags when fully expanded was 30L. Assuming a rat weighing between 200 and 300 grams breathes at a rate of 0.2 L/min, the rat would inhale/exhale approximately 48 liters of air, or approximately 1.6 times the volume in the bag, during the four-hour exposure.

Absorption: If assumed that the percent of dose recovered in the carcass, tissue, and excreta represents absorbed test material, then rats exposed to 100 ppm (Group 2) test material had ~5% of the absorbed dose (males 6%, females 4%) while rats exposed to 1000 ppm (Group 3) had ~1.9% (males 2.4%, females 1.4%) 20 hours after treatment (Table 6). No significant sex-related differences in absorption were found. The administered dose (~1.6%) could be considered absorbed by male and female rats exposed to 1000 ppm (Group 1) with four hours of exposure.

TABLE 6. Percent of ^{14}C-1-MCP dose recovered from each fraction after four hours inhalation exposure

Group	Sex	Carcass	Tissue[a]	Excreta	Chamber Air	Total Recovery
Group 1 (1000 ppm)	Male	1.6	--	--	89.0	90.8
	Female	1.5	--	--	98.7	100.4
	Average	1.6	--	--	93.9	95.6
Group 2 (100 ppm)	Male	1.4	0.3	4.3	84.5	90.6
	Female	1.0	0.2	2.8	87.6	91.6
	Average	1.2	0.2	3.6	86.1	91.1
Group 3 (1000 ppm)	Male	0.6	0.1	1.7	92.6	94.7
	Female	0.3	0.1	1.0	92.4	93.9
	Average	0.5	0.1	1.4	92.5	94.3

Data derived by reviewer from page 132 of MRID 47088616
[a] Includes liver, kidney, spleen, and lung
Groups 3 and 3A are presented combined

Tissue distribution: As shown in Table 7, tissue accumulation of the radiolabel was low regardless of exposure concentration or sex. No significant tissue deposition site for the radiolabel was found.

TABLE 7. Distribution of radiolabel (percent of dose) in tissues 20 hours after inhalation treatment with 1-MCP

Group	Sex	Liver	Kidney	Spleen	Lung	Tissue	Carcass
Group 2 (100 ppm)	Male	0.07	0.05	0.03	0.010	0.24	1.44
	Female	0.06	0.05	0.03	0.04	0.18	1.05
Group 3 (1000 ppm)	Male	0.02	0.02	0.01	0.02	0.07	0.54
	Female	0.01	0.01	0.01	0.01	0.04	0.35

Data derived by reviewer from pages 37 and 41 of MRID 47088616
Groups 3 and 3A are presented combined

Toxicokinetics: The concentrations of ^{14}C-1-MCP in whole blood and plasma during and after exposure to the radiolabeled test material are shown in Table 5. Absorption of the radiolabeled test material was low during the exposure period and decreased upon removal of the animal from the exposure chamber. Radiolabel was detected in the whole blood and plasma within 15 minutes of the start of exposure in high-dose male and female rats. During exposure to 1000 ppm radiolabeled test material (Group 1), the whole blood concentration steadily climbed in a linear fashion reaching a peak of ~10.9 and 9.8 μg/g by the end of exposure in males and females, respectively. In Group 2 male and female rats, the concentration of radiolabel in the blood and plasma was similar, suggesting similar absorption between the sexes at both exposure concentrations. In

addition, similar T_{max}, C_{max}, and $T_{½}$ were found between the sexes. Group 3 results for T_{max}, C_{max}, and $T_{½}$ were also similar between the sexes although a bi-phasic elimination pattern was suggested in female rats (Tables 8 and 9). The difference between the AUC of the two exposure groups suggests that absorption was approaching saturation at 1000 ppm. The relatively long T½ in both sexes at both exposure concentrations suggests that elimination is slow.

TABLE 8. Concentration of radioactivity in plasma and whole blood of male and female rats exposed by inhalation to ^{14}C-1-MCP

Group	Matrix	Sex	Hour of exposure						Hours after exposure		
			0.25	0.50	1	2	3	4	4	8	20
Group 1 (1000 ppm)	Whole Blood	Male	3.8760 (9.2)	4.6251 (11.0)	5.9579 (14.2)	7.4833 (17.8)	9.1491 (21.8)	10.9172 (26.0)	-	-	-
Group 1 (1000 ppm)	Whole Blood	Female	4.1998 (10.1)	5.1492 (12.3)	5.7916 (13.9)	7.7946 (18.7)	8.9911 (21.5)	9.8431 (23.6)	-	-	-
Group 2 (100 ppm)	Plasma	Male	-	-	1.719(14.0)	2.1521 (17.6)	2.1452 (17.5)	2.5785 (21.1)	2.2339 (18.2)	-	1.4169
		Female	-	-	1.3199 (14.9)	1.5257 (17.2)	1.7229 (19.4)	1.9595 (22.1)	1.3307 (15.0)	--	1.0126 (11.4)
Group 2 (100 ppm)	Whole Blood	Male	-	-	1.1032 (13.2)	1.4736 (17.6)	1.7592 (21.0)	1.9625 (23.4)	1.335 (15.9)	-	0.7514 (9.0)
		Female	-	-	1.2279 (14.3)	1.5617 (18.2)	1.8262 (21.2)	2.0662 (24.0)	1.2259 (14.2)	-	0.6954 (8.1)
Group 3 (1000 ppm)	Plasma	Male	4.0684 (5.6)	5.907 (8.2)	6.3079 (8.7)	7.2458 (10.0)	10.0425 (13.9)	10.8923 (15.1)	8.9872 (12.4)	9.3664 (12.9)	9.5540 (13.2)
		Female	NS	NS	4.8750 (10.3)	6.0636 (12.8)	7.7216 (16.3)	10.1165 (21.4)	9.9893 (21.1)	NS	8.5168 (18.0)
Group (1000 ppm)	Whole Blood	Male	3.9813 (6.4)	5.2058 (8.3)	6.2722 (10.0)	8.7395 (13.9)	10.0437 (16.0)	11.1998 (17.9)	6.4541 (10.3)	6.2462 (10.0)	4.5539 (7.3)
		Female	NS	NS	6.2983 (13.5)	8.0740 (17.4)	9.6260 (20.7)	10.8793 (23.4)	6.9212 (14.9)	NS	4.6946 (10.1)

Data derived by reviewer from pages 34-35 and 38-39 of MRID 47088616
Results expressed as µg equivalent of ^{14}C-1-MCP/g matrix with percent of total recovered in parenthesis
NS = Not sampled
Groups 3 and 3A are presented combined

TABLE 9. Toxicokinetic parameters of male and female rats exposed to 1-MCP for four hous

Group	Matrix	T_{max} (Hours)	C_{max} (ppm)	$T_{½}$ (Hours)	AUC (ppm*hr)	T_{max} (Hours)	C_{max} (ppm)	$T_{½}$ (Hours)	AUC (ppm*hr)
		Male				Female			
Group 2 (100 ppm)	Plasma	4	2.58	23.5	45.3	4	1.96	24.4	30.2
	Whole Blood	4	1.96	15.6	28.1	4	2.07	14.2	27.0
Group 3 (1000 ppm)	Plasma	4	10.9	14.4	220	4	10.1	ND	210
	Whole Blood	4	11.2	5.0	157	4	10.9	6.1/18.8[a]	155

Data from pages 106-107 of MRID 47088616
[a]Initial phase/Overall
ND = An accurate value could not be determined
Groups 3 and 3A are presented combined

Excretion: The urinary and fecal excretion of ^{14}C-1-MCP is shown in Table 10. Both sexes of animals in both exposure groups eliminated the radiolabeled test material predominately in the urine with elimination in the feces secondary. Elimination in the excreta was <5% of the administered dose over the 24-hour study period, implying absorption was minimal.

TABLE 10. Urinary and fecal excretion (percent of dose) of radiolabeled 1-MCP in male and female rats					
Group	**Sex**	**Time**	**Urine**	**Feces**	**Ratio**
Group 2 (100 ppm)	Male	0-4	1.34	0.57	2.35
		4-24	2.03	0.38	5.34
		0-24	3.37	0.96	3.51
Group 2 (100 ppm)	Female	0-4	0.92	0.28	3.29
		4-24	1.40	0.20	7.00
		0-24	2.31	0.48	4.81
Group 3 (1000 ppm)	Male	0-4	0.77	0.06	12.8
		4-24	0.75	0.14	5.36
		0-24	1.51	0.20	7.55
Group 3 (1000 ppm)	Female	0-4	0.28	0.04	7.00
		4-24	0.58	0.10	5.80
		0-24	0.86	0.15	5.73

Data from page 108 of MRID 47088616
Groups 3 and 3A are presented combined

Conclusions:

The submitted toxicokinetic study does not satisfy data requirement under OPPTS 870.7485. Metabolite identification studies were not conducted. The animals were observed and excreta collected over a 20-hour period rather than the recommended seven days. Because of the low absorption of the test material, however, the study duration could be considered acceptable. The study report appears to be presented from various modifications conducted over a period of years. References to later additions to the report were not included in the summary or conclusions of the study report. **However, the study will not affect the conclusion of risk assessment for this registration.**

Immunotoxicity (OPPTS 880.3550) – Waiver Request

AgroFresh, Inc., a subsidiary of Rohm and Haas Company, has submitted a waiver request for immunotoxicity for 1-Methylcyclopropene and presented the following rationale: 1-MCP does not induce dysfunction or inappropriate suppressive or stimulatory responses in components of the immune system based on the current toxicological database for 1-MCP. In a 1-MCP three-month inhalation study in rats, no effects on thymus weight and no effects on the histopathology of the thymus, bone marrow or spleen that would be attributed to an impact on the immune system were seen. The changes observed in the spleen were secondary to a mild regenerative anemia. No effects were noted on white blood cell differential parameters. No treatment-related changes in serum total protein, albumin, globulin, or albumin to globulin ratio indicative of a primary antibody response were observed. When 1-MCP was tested in the Magnusson-Kligman Maximization test, 1-MCP exhibited no evidence of delayed contact hypersensitivity in the guinea pigs.

Conclusion:

The submitted waiver request for immunotoxicity is acceptable.

Nature of the Residue—Plants (OPPTS 860.1300)

AgroFresh, Inc., a subsidiary of Rohm and Haas Company, has submitted nature of the residue studies for the residues of 1-methylcyclopropene in apple, tomato and maize (MRIDs 47088611-12, 47108203).

Apple (MRID 47088612)

AFxRD-038, an aqueous formulation containing 1-methylcyclopropene ^{14}C-radiolabeled at the C-4 carbon (a.i. specific radioactivity 89.7 mCi/g or 3.32 MBq/mg), was applied with a hand-held sprayer to a 10-year old apple tree at a rate of 300 g 1-MCP/ 1867 L/ ha.(4.28 oz/A). During treatment, the tree was covered with polyethylene. The plastic was perforated after treatment and all but the top cover was removed the next day. A single application was made at the BBCH 86-87 growth stage (30-40% fruit ripeness), and apples were collected from top, middle, and bottom of the tree canopy 3 and 7 days later. Leaves were collected from the whole tree 3 and 30 days after application, and soil was collected at a depth of 1-2 cm beneath the tree one day after application. Collected samples were homogenized and subjected to radioanalysis within a day after collection. Juice was extracted from apples with a juicer. Samples were not stored prior to analysis and storage stability was not addressed.

Maize (MRID 47088611)

AFxRD-038, an aqueous formulation containing ^{14}C-1-methylcyclopropene radiolabeled at the C-4 carbon (a.i. specific radioactivity 90.2 mCi/g or 3337 MBq/g), was applied to maize plants at a rate of 50 g 1-MCP/ 400L/ha(0.71 oz/A). Plants were grown outdoors in local clay soil inside containers 40 cm deep with a surface area of 1 m^3, and imbedded into the ground to soil level. The plants were covered with polyethylene and treated by over-top spraying; the plastic was perforated after treatment and removed the next day. A single application was made at either the V12 vegetative stage or the R2 reproductive stage, with pre-harvest intervals of 80 and 52 days, respectively. The maize kernels, cob, and stover were harvested and immediately homogenized for radioanalysis. The kernels were further extracted using hexane and water, and oil was obtained from the hexane phase. Samples were not stored and storage stability was not addressed.

Tomato (MRID 47108203)

45 mL aliquots of formulation were applied at a concentration that would deliver the equivalent of 25 g 1-MCP per hectare (9.4 oz product/acre) the maximum single application rate on the label. This procedure determined an average spray efficiency of 86.3%. Each plant had 10 sample tomatoes on the plant. A tomato sample consisted of either an individual tomato or a composite of smaller tomatoes that weighed up to approximately 250 g. A total of 10 tomatoes were collected from each plant. Individual tomatoes were homogenized in a blender; then the tomato homogenate was filtered to produce a filter cake which was allowed to partially dry. The dried tomato filter cake was ground and allowed to air dry overnight before residue analysis. All leaves were removed from the plants, frozen and ground for analysis. Likewise, stems were removed from the pots, cut into small pieces and ground for analysis. Soil was collected from the

top surface of the pot and from the floor of the spray chamber. Soil was homogenized before residue analysis. Fortifications were prepared for each sample type by spiking control samples of each matrix.

Analytical Methodology

Radioactivity was quantified by LSC with Packard LSC instruments equipped with DPM and luminescence options, and the counts were corrected for quenching. All samples were counted at least in duplicate for up to 20 minutes. The scintillant used was 10 mL Irga Safe Plus (Packard Instruments). The volume of the added aqueous or organic sample solutions was ≤ 5 mL. Plant samples (≤ 250 mg) and soil samples (200 mg) were combusted in oxygen gas at 900°C using an OX sample oxidizer (Zinsser Analytic, Germany). The evolved $^{14}CO_2$ was absorbed in 9 mL ethanolamine: methoxyethanol (1:3 v/v) and the resulting material subjected to LSC in 10 mL scintillation fluid.

To validate the analytical method for 1-MCP, recoveries were determined in triplicate from matrices fortified with known amounts of ^{14}C-1-MCP: 0.003-0.006 mg/kg for apple and 0.001 mg/kg for soil samples. ^{14}C-1-MCP gas was released as a gas from the capsules by ultrasonication in 2 mL water in sealed glass bottles, and was injected into sealed glass containers containing control plant samples. After incubation for 16 hours at 20°C, the spiked plant samples were analyzed similarly to the treated samples.

For apples, the LOD was 0.0012 ppm and the LOQ was 0.0018 ppm. For leaves, the LOD was 0.0060 ppm and the LOQ was 0.0090 ppm. For soil, the LOD was 0.0015 ppm and the LOQ was 0.0023ppm. For all matrices, the LOD was twice the LSC background (15 dpm), and the LOQ was thrice the LSC background.

For maize kernels, cob, and stover, the LOD was 0.0008 ppm and the LOQ was 0.0011 ppm. For soil, the LOD was 0.00015 ppm and the LOQ was 0.0002 ppm. For all matrices, the LOD was twice the LSC background, and the LOQ was thrice the LSC background.

For tomato, the limit of quantitation (LOQ) for each sample type was experimentally determined by fortifications to be 1 ppb and the calculated limit of detection (LOD) was 0.3 ppb.

Results

Apple

TRRs were determined for the fresh apple, leaves, and soil, as shown in Table 11. Residue levels were greatest in the leaves (0.212-0.379 ppm), followed by the soil (0.017 ppm). If the soil had been taken from 10 cm depth instead of the 1-2 cm as in this study, it is likely residue levels would have been <0.01 ppm. Residues were the lowest in the apples (0.003-0.004), with no differences found in apples collected from the top, middle, or bottom of the tree. Reside levels in apple juice were comparable to those in fresh apples, indicating a lack of concentration during processing.

TABLE 11 Total Radioactive Residues (TRRs) in Apples, Leaves, Juice, and Soil			
Matrix	Timing and Application Number	PHI (days)	Fresh Weight ppm ± S.D. [1]
Apples	BBCH 86-87; Top, 1 application BBCH 86-87; Middle, 1 application BBCH 86-87; Bottom, 1 application	3 3 3	0.004 ± <0.001 0.004 ± <0.001 0.004 ± <0.001
	BBCH 86-87; Top, 1 application BBCH 86-87; Middle, 1 application BBCH 86-87; Bottom, 1 application	7 7 7	0.003 ± <0.001 0.004 ± 0.001 0.004 ± <0.001
Apple juice	Extracted from composite sample of apples from top, middle, and bottom	(apples: 3 days)	0.003
		(apples: 7 days)	0.003
Leaves	BBCH 86-87; 1 application	3	0.212
	BBCH 86-87; 1 application	30	0.379
Soil, top 2 cm	BBCH 86-87; 1 application	1	0.017

Data from pp. 12, 23, and 26-28 of MRID 47088612.
[1] The sample residues are presented in terms of mg parent equivalents per kg fresh weight tissue. The standard deviation (S.D.) was calculated for 4 individually sampled apples. The leaves and soil were each combined into one composite sample.

The total radioactive residues (TRR), in ppm parent equivalents per fresh weight, for apples and apple juice, were 0.003-0.004 ppm at both pre-harvest intervals (PHI). No differences were seen in apple residue levels from the top, middle, or bottom of the tree canopy. Soil residues were also low (0.017 ppm), and would likely have been <0.01 ppm in a 10 cm deep sample. Residues were greatest in the leaves (0.212-0.379 ppm).

Maize

TRRs were determined for the fresh and dry maize RACs kernels, cob, and stover, as shown in Table 12. Residue levels for all maize matrices were lower in the V12 samples than in the R2 samples, and were greatest in the stover in each case (~0.06 ppm vs. 0.006-0.04 ppm for kernels and cob). Soil residues at a depth of 0-10 cm were below the LOQ for the V12 soil samples (<0.0009 ppm) and the R2 soil samples (<0.0008 ppm). At a depth of 10-20 cm, residues were below the LOD for both plots. Data were not presented for a depth of 20-30 cm, but would be expected to also be <LOD.

TABLE 12. Total Radioactive Residues (TRRs) in Maize Kernels, Cob, and Stover.				
Matrix	Timing and Applic. No.	PHI (days)	Fresh Weight ppm ± S.D. [1]	Dry Weight ppm ± S.D. [1]
Kernels: V12 sample	V12 vegetative stage, 1 application	82	0.0056 ± 0.0008	0.0085 ± 0.0012
R2 sample	R2 reproductive stage, 1 application	50	0.0370 ± 0.0046	0.0540 ± 0.0062
Cob: V12 sample	V12 vegetative stage, 1 application	82	0.0102 ± 0.0025	0.0214 ± 0.0061
R2 sample	R2 reproductive stage, 1 application	50	0.0204 ± 0.0044	0.0411 ± 0.0096
Stover: V12 sample	V12 vegetative stage, 1 application	82	0.0599 ± 0.0110	0.2466 ± 0.0282
R2 sample	R2 reproductive stage, 1 application	50	0.0670 ± 0.0210	0.2563 ± 0.0799

Data from pp. 17, 23, 25, 28, and 29 of MRID 47088611.
[1] The sample residues are presented in terms of mg parent equivalents per kg fresh or dry weight tissue. The standard deviation (S.D.) was calculated for 8 plants.

None of the radioactive residues were characterized or identified; with the exception that radioactivity was measured at several stages of processing kernels into oil (Table 13.). OPPTS 860.1300 guidelines call for characterization and/or identification of

residues in matrices with radioactivity levels of ≥0.01 ppm, which in this case would qualify all except the V12 sample kernels.

Oil was extracted from the maize kernels by soaking them in hot water, followed by hexane/water extraction of the resulting solids. The recovery of radioactivity in the aliquots of the hot water and the subsequent extraction fractions (aqueous and hexane), as determined by combustion analysis, are shown in Table 13. The total recovered was 133% for the V12 sample and 124% for the R2 sample. Residues in the final oil samples were 0.044 ppm (1.2% TRR) in the V12 sample and 0.159 ppm (0.74% TRR) in the R2 sample. This represented an approximately 8-fold and 4-fold concentration of radioactivity for the V12 and R2 samples, respectively, relative to the fresh kernel.

TABLE 13. Radioactivity in Maize Kernel Fractions After Treatment With ^{14}C-Labeled 1-Methylcyclopropene and Extraction with Hexane.

Fraction	Kernel V12 sample		Kernel R2 sample	
	TRR = 0.0056 ppm fresh weight		TRR = 0.0370 ppm fresh weight	
	%TRR	ppm	%TRR	ppm
Water phase after soaking kernels	12.6	0.00070	4.4	0.00162
Water phase - after extraction with hexane	9.3	0.00052	5.4	0.00201
Hexane phase - after extraction with hexane	1.2	0.00007	0.9	0.00033
Total extractable[1]	23.1	0.00129	10.7	0.00396
Total unextractables	110	0.0357	113	0.0053
Total recovery of radioactivity[2]	133	--	124	--
Oil, extracted from kernels in hexane phase[3]	1.2	0.044 (7.9x)	0.74	0.159 (4.3x)

Data taken from pp. 25-26 of MRID 47088611.
[1] Calculated by the reviewer by adding the water and hexane phase values.
[2] Calculated by the reviewer as total extractable + total unextractable radioactivity obtained by combustion analysis.
[3] Calculated by the reviewer as the fraction of initial radioactivity in fresh kernels. The value in parentheses is the concentration factor, obtained by dividing the fresh weight TRR by the oil TRR.

The fortification studies showed acceptable mean recoveries for kernels (117% at 0.0034 ppm), oil (80.1% at 0.160 ppm; 85.0% at 0.044 ppm), cob (118.5% at 0.0034 ppm; 112.4% at 0.0125 ppm), and soil (93.4% at 0.001 ppm). The recovery for stover was poor (55.0% at 0.0032 ppm; 63.9% at 0.0657 ppm), however, acceptable recoveries were obtained using overripe stover from remaining maize plants that was analyzed later (93.5% at 0.0806 ppm), and in a previous experiment using green stover (97.3% at 0.550 ppm; 102.4% at 0.890 ppm).

Tomato

The maximum residue levels of ^{14}C-1-MCP determined under the two treatment conditions for each sample component are reported in Table 14. Tomato results are from 10 samples taken for each treatment. When treated under typical field conditions, the maximum residue level of ^{14}C-1-MCP in tomatoes was below 1 ppb with the treatment of 8 km/hr wind. In the worst case scenario which was the sealed chamber, the maximum residue level in tomatoes was 2.2 ppb. When treated under typical field conditions, the maximum residue levels in the nonedible portions of the plant (stem and leaves) were

30.2 ppb and 183.5 ppb, respectively. When treated under typical field conditions, residues in the soil were low, 74.9 ppb.

TABLE 14. Summary of Residue Results

Sample Component	ppb Residue – Sealed Chamber			ppb Residue – 8 km/hr Wind		
	Treatment 1	Treatment 2	Average	Treatment 3	Treatment 4	Average
Tomato	1.2	2.2	1.7	<1.0	<1.0	<1.0
Leaf	130.2	183.5	156.8	181.1	150.3	165.7
Stem	18.9	30.2	24.6	13.3	15.5	14.4
Soil	62.7	74.9	68.8	14.6	10.6	12.6

Table reproduced from pg. 27, MRID 47108203

Detailed residue data for ^{14}C-1-MCP are presented in Table 15 for leaf, stem and soil.

TABLE 15. Detailed residue data for leaf, stem and soil.

	Treatment 1			Treatment 2			
Sample Component	Fresh Wt. (g)	Total DPM	Residue ppb	Fresh Wt. (g)	Total DPM	Residue ppb	Average Residue
Sealed Chamber							
Leaf	92.48	1.32×10^7	130.2	67.65	1.51×10^7	183.5	156.8
Stem	126.44	3.05×10^6	18.9	196.39	3.52×10^6	30.2	24.6
Soil	2889	3.35×10^7	62.7	2286.6	3.17×10^7	74.9	156.8
8 km/hr Wind							
	Treatment 3			**Treatment 4**			
Sample Component	Fresh Wt. (g)	Total DPM	Residue ppb	Fresh Wt. (g)	Total DPM	Residue ppb	Average Residue
Leaf	244.51	1.49×10^7	181.1	186.37	1.55×10^7	150.3	165.7
Stem	140.7	2.71×10^6	13.3	110.23	2.34×10^6	15.5	14.4
Soil	2214.7	5.97×10^6	14.6	2257.7	4.44×10^6	10.6	12.6

Table reproduced from pg. 29, MRID 47108203

Results of the distribution of the applied ^{14}C-1-MCP in various environmental compartments are presented in Table 4. Using the sealed chamber, 100% of the applied ^{14}C-1-MCP was accounted for. The majority of the applied ^{14}C-1-MCP resided in the atmosphere where it will rapidly dissipate based upon results of typical field conditions study with 8 km/hr wind. Likewise, the distribution of absorbed ^{14}C-1-MCP is presented in Tables 16 and 17.

TABLE 16. Distribution of Radioactivity in Environmental System Compartments Based on Applied Radioactivity

System Environmental Compartment	% of Applied dpm	
	Sealed Chamber[1]	8 km/hr Wind[1]
Chamber Air (at 4 hrs)	64.4%	0.00%
Tomato	0.5%	0.2%
Leaf	9.9%	11.2%
Stem	2.3%	1.8%
Soil	22.9%	3.8%
Accountability	100%	16.7%

[1]Average of 2 treatments
Table reproduced from pg. 30, MRID 47108203

TABLE 17. Distribution of Radioactivity in System Environmental Compartments Based on Absorbed Radioactivity		
System Environmental Compartment	% of Absorbed dpm	
	Sealed Chamber[1]	8 km/hr Wind[1]
Tomato	1.5%	1.4%
Leaf	27.8%	65.5%
Stem	6.5%	10.8%
Soil	64.2%	22.3%

[1]Average of 2 treatments
Table reproduced from pg. 31, MRID 47108203

Fortified controls were prepared and analyzed to demonstrate radioactive recovery for each sample type. The following average fortification recoveries calculated across fortification levels are presented in Table 18.

TABLE 18. Average Fortification Recoveries				
Sample Type	Tomato	Leaf	Stem	Soil
% recovery	95.7	91.0	75.2	93.0

Table reproduced from pg. 7, MRID 47108203

Conclusions:

For Apples:

The submitted nature of the residue study for 1-Methylcyclopropene (AFxRD-038) in apple is not adequate to satisfy the OPPTS guideline 860.1300. Based on the guideline, the registrant should use the maximum exaggerated rate for apple metabolism study in order to identify and characterize the major metabolites. The characterization and identification of the metabolites were not conducted; and metabolic pathways for 1-methylcyclopropene were not proposed. No information of storage stability studies were conducted or reported. However, the study is not required for application of an exemption from the requirement of a tolerance.

With a single spray application of apple trees with 300 g radiolabeled 1-MCP/ ha (4.28 oz/A) as the formulation ADxRD-038, the total radioactive residues (TRR), in ppm parent equivalents per fresh weight, for apples and apple juice, were 0.003-0.004 ppm at both pre-harvest intervals (PHI). No differences were seen in apple residue levels from the top, middle, or bottom of the tree canopy. Soil residues were 0.017 ppm, and would likely have been <0.01 ppm in a 10 cm deep sample. Residues were 0.212-0.379 ppm in the leaves.

For Maize:

The submitted nature of the residue study for 1-Methylcyclopropene (AFxRD-038) in maize is not adequate to satisfy the OPPTS guideline 860.1300. The characterization and identification of the metabolites were not conducted; and metabolic pathways for 1-methylcyclopropene were not proposed. No information of storage stability studies were conducted or reported. The total radioactive residues (TRR), in ppm parent equivalents

per fresh weight, for the kernels, cob, and stover were, respectively 0.0056, 0.0102, and 0.0599 ppm for the V12 stage samples, and were 0.0370, 0.0204, and 0.0670 ppm for the R2 stage samples. Radioactivity levels were 2 to 4-fold greater on a dry weight basis. None of the radioactive residues were identified and most were not characterized. Residues were partially characterized during the processing of kernels to oil (soaking them in hot water followed by water:hexane extraction). The majority of the radiolabel in kernels was unextractable (110% of kernel fresh weight), and the hexane phase, which contained the oil, represented 1.2% of the kernel fresh weight TRR.

For Tomato:

The submitted nature of the residue study for 1-Methylcyclopropene (AFxRD-038) in tomato is not adequate to satisfy the OPPTS 860.1300. The characterization and identification of the metabolites were not conducted; and metabolic pathways for 1-methylcyclopropene were not proposed. No information of storage stability studies were conducted or reported. The maximum residue level of ^{14}C-1-MCP in tomatoes was below 1 ppb with the treatment of 8 km/hr wind. In the worst case scenario which was the sealed chamber, the maximum residue level in tomatoes was 2.2 ppb. When treated under typical field conditions, the maximum residue levels in the nonedible portions of the plant (stem and leaves) were 30.2 ppb and 183.5 ppb, respectively. When treated under typical field conditions, residues in the soil were low, 74.9 ppb. The characterization and identification of the metabolites were not conducted; and metabolic pathways for 1-methylcyclopropene were not proposed. However, the study is not required for application of an exemption from the requirement of a tolerance.

Ecological Effects (OPPTS 850 Series GLNs)

AgroFresh, Inc., a subsidiary of Rohm and Haas Company, has submitted ecological effect studies: acute toxicity on daphnids and fish, acute oral toxicity and dietary toxicity on avian, honey bee acute contact toxicity, algal toxicity (Tiers I and II), vegetative vigor (Tiers I and II), seedling emergence (Tiers I and II) and waiver requests for vegetative vigor and seedling emergence studies.

Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids (OPPTS 850.1010)

Test Material

1. 3.6% 1-methylcyclopropene in cyclodextrin/dextrose carrier (MRID 47088609)
2. 3.3% 1-methylcyclopropene α-cyclodextrin complex (MRID 47024914)

A 48-hour static test with 3.6% 1-methylcyclopropene in cyclodextrin/dextrose carrier was conducted to determine the acute toxicity of the test material to *Daphnia magna*. The daphnids were obtained from in-house cultures maintained by the testing facility. Adult daphnids were cultured for 29 days prior to collection of the juveniles used in the test. The adults showed no sign of disease or stress during the culture period. The culture water was moderately-hard well water that was passed through a sand filter and pumped to a storage tank for aeration. Prior to use, the water was filtered to 0.45 μm and UV sterilized. The dilution water used in the bioassay was from the same source as the culture water. During

the four weeks immediately preceding the test, the specific conductance, hardness, alkalinity, and pH of the water were 300-305 µmhos/cm, 136-140 mg/L (as $CaCO_3$), 176-180 mg/L (as $CaCO_3$), and 8.0-8.2, respectively.

A 48-hour static test was conducted to determine the acute toxicity of the test material to *Daphnia magna*. *Daphnia magna* neonates received an aqueous exposure to 1-methylcyclopropene α-cyclodextrin complex (3.3% a.i.) at a mean measured concentration of 0.776 mg a.i./L. Control daphnids were exposed to dilution water only. No immobilization or adverse effects were observed in any of the treated or control daphnids after 24 hours. At 48 hours, one daphnid in the control group (3.3%) was dead, and one daphnid in the test material group (3.3%) was immobile.

Results:

The result for using 3.6% 1-methylcyclopropene in cyclodextrin/dextrose carrier: the measured concentration of 1-methylcyclopropene in the test material solutions ranged from 0.637 to 0.738 mg/L (78.5 to 90.9% of nominal), with a mean of 0.678 mg/L. No immobilization or adverse effects were observed in any of the treated or control daphnids. The 48-hour EC_{50} was >0.678 mg a.i./L, and the NOEC was 0.678 mg/a.i./L.

The result for using 3.3% 1-methylcyclopropene α-cyclodextrin complex, the measured concentration of 1-methylcyclopropene in the test material solutions ranged from 0.738 to 0.861 mg/L (91.4 to 104.8% of nominal), with a mean of 0.766 mg/L. No immobilization or adverse effects were observed in any of the treated or control daphnids after 24 hours. At 48 hours, one daphnid in the control group (3.3%) was dead, and one daphnid in the test material group (3.3%) was immobile. The 48-hour EC_{50} was >0.776 mg a.i./L, and the NOEC was 0.776 mg/a.i./L.

Conclusion:

The 48-hour EC_{50} was >0.678 mg a.i./L and >0.776 mg a.i./L, and that the 48-hour no-mortality/immobility concentration and the NOEC were 0.678 mg/a.i./L and 0.776 mg/a.i./L, respectively.

Freshwater Fish Testing, Tier I (OPPTS 850.1075)

Test Material

1. 3.6% 1-methylcyclopropene in a cyclodextrin/dextrose carrier (MRID 47088610)
2. 3.3% 1-Methylcyclopropene alpha cyclodextrin complex (MRID 47024915)

Test Methods

For using the 3.6% 1-methylcyclopropene in a cyclodextrin/dextrose carrier, a 96-hour static renewal acute toxicity test was conducted to determine the toxicity of the test material to rainbow trout (*Oncorhynchus mykiss*). Juvenile trout from the same hatch were

obtained from Thomas Fish Company (Anderson, CA) and held for approximately 4 weeks prior to the test in water from the same source as used in the test. During the holding period, the fish were fed a commercial salmon starter diet (Zeigler Brothers, Inc., Gardners, PA) daily until two days prior to test start. The water used for holding and for the test was moderately-hard well water from the test facility site. It was filtered to 25 µm, pumped to a storage tank, and aerated. Prior to use, it was filtered to 0.45 µm. During the 14 days preceding the test, the water temperature ranged from 12.0 to 13.5°C, the pH from 8.2 to 8.6, and the dissolved oxygen from 9.8 to 10.6 mg/L (≥91% of saturation). The test vessels were four-liter glass bottles containing a magnetic stir bar. They were filled with approximately four liters of dilution water (25 mL headspace) and the test substance was added directly to the dilution water to provide a nominal concentration of 22.9 mg/L (0.811 mg a.i./L). The nominal concentration was selected based on calculations indicating a 1000 ppm (v/v) headspace concentration was equivalent to an aqueous phase concentration of 0.811 mg a.i./L. The bottles were immediately sealed with a Teflon®-lined screw cap and stirred for one hour.

For using the 3.3% 1-Methylcyclopropene alpha cyclodextrin complex, a 96-hour static renewal acute toxicity test was conducted to determine the toxicity of the test material to rainbow trout (*Oncorhynchus mykiss*). Juvenile trout were obtained from Thomas Fish Company (Anderson, CA) and held for 25 days prior to the test in water from the same source as used in the test. The fish were acclimated to test conditions for approximately 50 hours prior to test start. During the holding period, the fish were fed a commercial diet (Zeigler Brothers, Inc., Gardners, PA) until the acclimation period began. The water used for holding and for the test was moderately-hard well water from the test facility site. During the four weeks prior to the test start, the specific conductance ranged from 310-315 µmhos/cm, the hardness from 128-132 mg/L (as $CaCO_3$), the alkalinity from 176-178 mg/L (as $CaCO_3$), and the pH from 8.1-8.3. The well water was filtered to 45 µm, pumped to a storage tank, and aerated. Prior to use, it was re-filtered. During the holding period, the water temperature ranged from 11.7 to 12.8°C, the pH from 8.2 to 8.5, and the dissolved oxygen from 9.1 to 10.4 mg/L. The test vessels were four-liter glass bottles containing a magnetic stir bar. They were filled with approximately four liters of dilution water (2 mL headspace) and 0.100 g of the test substance was added to provide a nominal concentration of 25 mg/L (0.825 mg a.i./L). The nominal concentration was selected based on calculations indicating a 1000 ppm (v/v) headspace concentration was equivalent to an aqueous phase concentration of 0.825 mg a.i./L. The test vessels were immediately sealed with a Teflon® septa and a screw cap, and stirred for one hour.

All samples of the test solutions were collected from each test vessel at test start, on test day 1 and at test end. The samples were collected at mid-depth using a gas-tight syringe inserted through the septa, and were analyzed for the active ingredient as soon as possible without storage. Analysis was by gas chromatography with flame ionization detection, a method supplied by the study sponsor and validated by the test facility. The method parameters were supplied in Appendices 4.1 and 4.2 of MRID 47024915. The instrument was a Hewlett-Packard Model 5890 with a Varian CP-PoraBOND Q fused silica column (0.32 mm id x 10 m). Isobutylene gas was used as the analytical standard.

Results

For using the 3.6% 1-methylcyclopropene in a cyclodextrin/dextrose carrier: The average wet weight (blotted dry) of seven negative control fish ranged from 0.22 to 0.38 g, with a mean of 0.29 g. The loading rate (total wet weight/L of test solution) was 0.51 g fish/L. The measured concentration of 1-methylcyclopropene in the test material solutions at test start ranged from 0.703 to 0.759 mg/L (86.7 to 93.6% of nominal). The measured concentration in old solutions collected on Day 1 and at test end ranged from 0.703 to 0.802 mg/L (86.7 to 98.8% of nominal), indicating the active ingredient was stable in the closed vessel system. The overall mean measured concentration was 0.750 mg/L. No mortality or abnormal behavior were observed in any of the treated or control fish. The 96-hour LC_{50} was >0.750 mg a.i./L, and the no-mortality concentration and the NOEC were 0.750 mg/a.i./L.

For using the 3.3% 1-Methylcyclopropene alpha cyclodextrin complex: The average wet weight (blotted dry) of seven negative control fish ranged from 0.27 to 0.49 g, with a mean of 0.35 g. The loading rate (total wet weight/L of test solution) was 0.61 g fish/L. The measured concentration of 1-methylcyclopropene in the test material solutions at test start ranged from 0.929 to 1.045 mg/L (114.6 to 125.3% of nominal). The measured concentration in old solutions collected on test day 1 and at test end ranged from 0.898 to 1.012 mg/L (110.8 to 124.5% of nominal), indicating the active ingredient was stable in the closed vessel system. The overall mean measured concentration was 0.966 mg/L. No mortality or abnormal behavior were observed in any of the treated or control fish. The 96-hour LC_{50} was >0.966 mg a.i./L, and the no-mortality concentration and the NOEC were 0.966 mg/a.i./L.

Conclusions:

The 96-hour LC_{50} for rainbow trout exposed to the test material was >0.750 mg a.i./L and >0.966 mg/L, and the 96-hour no-mortality concentration and NOEC were 0.750 mg/a.i./L and 0.966 mg/L. No carrier control was used in the test. The reviewer notes that the concentration of active ingredient (3.3% and 3.6%) in the test material used in the tests was slightly lower than that listed on the product label (3.8%).

Avian Acute Oral Toxicity (OPPTS 850.2100)

Test Material

AFxRD-038, 3.6% w/w 1-methylcyclopropene (MRID 47024917)

Test Methods

The study was conducted to evaluate the acute toxicity of the test material when administered as a single oral dose to northern bobwhite (*Colinus virginianus*). Apparently healthy northern bobwhite were obtained from Trace Pheasantry, Inc., Douglas, PA, and maintained separately by sex at the testing facility for an acclimation period of seven weeks prior to the test. The chicks received antibiotics in their drinking water for seven

consecutive days after arrival at the test facility. The pen-reared birds were from the same hatch and phenotypically indistinguishable from wild birds. At test start five males and five females were indiscriminately assigned to each of the treatment and control groups. Each test group contained one pen of females and one pen of males. The test birds were approximately 22 weeks old and weighed from 193 to 242 g at test start.

Throughout acclimation and testing, the birds were fed a game bird ration formulated to the test facility's specifications (composition of the ration is provided in Appendix II of MRID 47024917). Feed and water (Easton, MD municipal supply) were provided *ad libitum* during acclimation and testing, except for a 17-hour fasting period prior to dosing. No antibiotics were administered during the test.

Results

All the birds survived the test, and all control birds were normal in appearance and behavior. One male in the test material group was observed head shaking, salivating, and swallowing frequently about eight minutes after dosing, but no regurgitation was seen. The same bird had a slight ruffled appearance for about four and one-half hours after dosing. A second male in the test material group also exhibited a slight ruffled appearance about two hours after dosing. Both birds had recovered within six hours after dosing and had normal appearance and behavior for the remainder of the test. There were no treatment-related effects on body weight or feed consumption in either the material or control groups.

Conclusions:

The study is **acceptable**. In an acute oral toxicity study, young northern bobwhite (*Colinus virginianus*) were administered a single nominal oral dose of 2250 mg AFxRD-038 (a.i., 3.6% w/w 1-methylcyclopropene)/kg body weight in a gelatin capsule and monitored for 14 days. Control birds received an empty gelatin capsule only. All birds survived the test, and there were no treatment-related effects on body weight or feed consumption. The acute oral LD_{50} for northern bobwhite given a single nominal oral dose of AFxRD-038 was determined to be >2250 mg/kg and the no-mortality level was 2250 mg/kg.

Avian Dietary Toxicity (OPPTS 850.2200)

Test Material

AFxRD-038, 3.6% w/w 1-methylcyclopropene (MRID 47024918).

Test Methods

A dietary LC_{50} study was conducted to evaluate the toxicity of the test material to northern bobwhite (*Colinus virginianus*). Apparently healthy northern bobwhite was obtained from the testing facility's in-house flock. The pen-reared birds were from the same hatch and phenotypically indistinguishable from wild birds. Since the birds were

immature, they could not be differentiated by sex. The birds were acclimated to the caging and facilities from the day of hatch until test start. At test start the birds were 10 days old and weighed from 16 to 24 g.

Throughout acclimation and testing, the birds were fed a game bird ration formulated to the test facility's specifications (MRID 47024918). From hatch until test start the birds received a vitamin mix in their water. Feed and water were provided *ad libitum* during acclimation and testing. The birds were not given any antibiotic during acclimation or testing.

Results

No mortality or signs of toxicity were seen in any birds in the formulation blank group or any of the test material groups. One bird in the negative control group was found dead on day 6. Gross necropsy showed a small amount of hemorrhaging in the left lung and subscapular hemorrhaging at the anterior part of the kidney. This death was attributed to injury, possible sustained in the body weight collection on day 5. A second negative control bird was found dead on day 8. Gross necropsy for the second bird was unremarkable. All other control birds had normal appearance and behavior throughout the test. Two birds in the formulation control group showed clinical signs attributed to injury.

Although statistical analysis was not performed, there appeared to be no treatment-related effects on body weight or feed consumption of any of the test material groups compared to the control groups.

Recovery of the test material in the day 1 samples of the 562 and 5620 ppm diets was 92% and 94% of nominal, respectively, verifying the concentrations of the test material in the diets. The concentration of AFxRD-038 in the day 0 samples of the 562 and 5620 ppm diets was 519 ± 12 ppm (cv=2.31%) and 5280 ± 106 (cv=2.01%), respectively, indicating the test material was distributed homogeneously in the diet. Recovery of the test material in the day 1 samples of the 562 and 5620 ppm diet was 96% and 102% of nominal, respectively, indicating the test material was stable in the diet under ambient conditions. Absence of the active ingredient in the formulation blank diet and the negative control diet was confirmed.

Conclusions:

The study is **acceptable**. In a dietary LC_{50} study, northern bobwhite (*Colinus virginianus*) chicks were administered nominal concentrations of 560, 1000, 1780, 3160, or 5620 ppm of AFxRD-038 (a.i., 3.6% w/w 1-methylcyclopropene) in prepared diet for five days, followed by three days of untreated diet. The test also included a formulation blank control (AFxRD-038 without active ingredient) and a negative control (feed only). Analysis of the diets confirmed the concentration, homogeneity, and stability of the test material in the diets. No mortality or signs of toxicity were seen in any birds in the formulation blank group or any of the test material groups. Two incidental mortalities occurred in the negative control group. There were no treatment-related effects on body weight or feed consumption of any of the test material groups compared to the control

groups. The dietary LC_{50} for northern bobwhite exposed to AFxRD-038 in the diet was determined to be >5620 ppm, the highest dose tested. The no-mortality concentration and the no-observed-effect concentration were each 5620 ppm.

Avian Dietary Toxicity (OPPTS 850.2200)

Test Material

AFxRD-038, 3.6% w/w 1-methylcyclopropene (MRID 47024919).

Test Methods

A dietary LC_{50} study was conducted to evaluate the toxicity of the test material to the mallard (*Anas platyrhynchos*). Apparently healthy three-day-old ducklings were obtained from Whistling Wings, Inc., Hanover, IL. The pen-reared ducks were from the same hatch and phenotypically indistinguishable from wild ducks. Since the ducks were immature, they could not be differentiated by sex. The ducks were acclimated to the caging and facilities from the day of receipt until test start (five days). At test start the ducks were 8 days old and weighed from 69 to 106 g.

Throughout acclimation and testing, the ducks were fed a game bird ration formulated to the test facility's specifications (composition of the ration is provided in Appendix II of MRID 47024919). Feed and water (Easton, MD municipal supply) were provided *ad libitum* during acclimation and testing. The ducks were not given any antibiotic during acclimation or testing.

Results

There was no mortality, and all birds were normal in appearance and behavior throughout the test. Although statistical analysis was not performed, there appeared to be no treatment-related effects on body weight or feed consumption of any of the test material groups compared to the control groups.

Recovery of the test material in the day 1 samples of the 562 and 5620 ppm diet was 92% and 94% of nominal, respectively, verifying the concentrations of the test material in the diets. The concentration of AFxRD-038 in the day 0 samples of the 562 and 5620 ppm diets was 519 ± 12 ppm (cv=2.31%) and 5280 ± 106 (cv=2.01%), respectively, indicating the test material was distributed homogeneously in the diet. Recovery of the test material in the day 1 samples of the 562 and 5620 ppm diet was 91% and 88% of nominal, respectively, indicating the test material was stable in the diet under ambient conditions. Absence of the active ingredient in the formulation blank diet and the negative control diet was confirmed.

Conclusions:

The study is **acceptable**. In dietary LC_{50} study, mallard (*Anas platyrhyncos*) ducklings were administered nominal concentrations of 560, 1000, 1780, 3160, or 5620 ppm

AFxRD-038 (a.i., 3.6% w/w 1-methylcyclopropene) in prepared diet for five days and given untreated diet for an additional three days. The test also included a formulation blank control (AFxRD-038 without active ingredient) and a negative control (feed only). Analysis of the prepared diets verified the concentration, homogeneity, and stability of the test material in the diet. No mortality or signs of toxicity were seen in any birds, and there were no treatment-related effects on body weight or feed consumption. The dietary LC_{50} for mallards exposed to AFxRD-038 in the diet was determined to be >5620 ppm, the highest dose tested. The no-mortality concentration and the no-observed-effect concentration were each 5620 ppm.

Waiver Request for Seedling Emergence (OPPTS 850.4225)

Registrant's Justification

In an enclosed residue field trial, ^{14}C-1-methylcyclopropene formulated as AFxRD-038 was applied to maize at an application rate of 50 g a.i./ha (18.8 oz product/A) (Mamouni, 2006a). Application took place at the V12 and R2 developmental stages. Soil cores were collected at depths of 0-10 cm and 10-20 cm in each two-square-meter plot. The time interval between application and sampling was not provided in MRID 47024921. Soil concentrations of 1-methylcyclopropene were below the LOQ (1 µg/kg) at the 0-10 cm depth, and below the LOD (0.33 µg/kg) at the 10-20 cm depth.

In an additional field trial using a closed system, an apple tree was treated with ^{14}C-1-methylcyclopropene at a rate of 300 g a.i./ha (113 oz product/A) (Rosenwald, 2006). Soil was collected from a four-square-meter by 2 cm deep area under the tree, homogenized, and analyzed for 1-methylcyclopropene. The time interval between application and sampling was not provided in MRID 47024921. The 1-methylcyclopropene concentration in the soil was 20µg/kg. The maize and apple tree studies were conducted under worst-case conditions, with closed systems limiting volatilization, but soil deposition of 1-methylcyclopropene was negligible in both cases.

A soil column leaching study was conducted using duplicate columns of four distinct soil types (MRID 47108204). The application rate was equivalent to 0.5 kg/ha, ten times the application rate to corn and 0.2 kg/ha higher than that used for apples. Twenty cm of $CaCl_2$-simulated rain was then applied to each column. Following the elution, most of the radioactivity remaining in the soil was non-extractable in acetonitrile. Eighty-seven to 102% of the applied radioactivity remained in three of the soils, and 96% remained in the fourth soil. Extractable radioactivity was less than 2% of that applied for all four soils, demonstrating that 1-methylcyclopropene is tightly bound in the soil.

Observations from many field trials on a variety of crops and soil types have shown that 1-methylcyclopropene did not affect the germination or growth of non-target plants such as weeds or ground cover.
These studies show that negligible levels of 1-methylcyclopropene would be deposited on soil under realistic use conditions, and any 1-methylcyclopropene that is deposited would be tightly bound to the soil and not available to germinating seeds.

Conclusion:

Based on the information provided, the reviewer believes the requested waiver for Seedling Emergence requirements can be waived. The reviewer has not seen all the studies cited in the waiver request, but assumes they have been submitted to the Agency. The reviewer found a separately submitted seedling emergence study (MRID 47088620) to be acceptable.

Algal Toxicity, Tiers I and II (OPPTS 850.5400)

Test Material

AFxRD-038, 3.6% 1-methylcyclopropene in a cyclodextrin/dextrose/EDTA carrier (MRID 47024923)

Test Methods

A 96-hour static toxicity test was conducted to determine the toxicity of the test material to the freshwater green alga *Pseudokirchneriella subcapitata.* The original algal cultures were obtained from the University of Toronto culture collection and were maintained at the test facility. The algal cells were cultured and tested in freshwater algal medium. Algal cells used in the test were obtained from test facility cultures that had been actively growing in culture medium for at least two weeks prior to test start, and were last transferred to fresh medium three days prior to test start.

Freshwater algal medium was prepared by adding reagent-grade chemicals to purified test facility well water to create stock nutrient solutions, which were then added to purify well water (NANOpure® water). Composition of the medium is detailed in Appendix 2 of MRID 47024923. The medium was supplemented with sodium bicarbonate, and the pH was adjusted to 7.5 ±1 using 10% HCl. The medium was sterilized by filtration (0.22 μm) prior to use.

Results:

The temperature ranged from 23.4 to 23.8°C during the test. The test solution pH ranged from 7.5 to 7.7 at test start, 8.9 to 9.9 at 72 hours, and 10.1 to 10.6 at test end, increasing with increased algal cell density.

No undissolved test material was seen in any treated replicates during the test. The measured concentrations ranged from 74 to 89% of nominal at test start and from 65 to 84% of nominal at 96 hours.

At test end, there were no signs of adherence of cells to the test vessels or of aggregation/flocculation of algae in any control or treatment group. There were no noticeable changes in cell morphology in any of the test material groups compared to the control.

Results for the mean cell density, mean area under the growth curve, and mean growth rate are given in Tables 19, 20, and 21, respectively. There were no statistically significant differences ($p \leq 0.05$) between the treated groups and the negative control group for any parameter. Growth parameters of the 0.576 mg a.i./L group (the only group compared statistically) were significantly reduced compared to the formulated blank control. The reduced growth was not believed to be treatment-related, but was attributed to a stimulatory effect on growth in the blank control as a result of the slightly higher content (3.6%) of cyclodextrin/dextrose/EDTA in the blank relative to the 0.576 mg a.i./L group.

TABLE 19. Mean cell density of *P. subcapitata* cultures exposed to AFxRD-038 for 96 hours

Mean measured concentration (mg/a.i./L)	72 hours			96 hours		
	Mean cell density (cells/mL)	Percent inhibition relative to the:		Mean cell density (cells/mL)	Percent inhibition relative to the:	
		Blank control	Negative control		Blank control	Negative control
Negative control	226,570	--	--	482,593	--	--
Blank control	524,633	--	--	919,235	--	--
0.039	244,246	53	-7.8	524,977	43	-8.8
0.088	242,267	54	-6.9	562,797	39	-17
0.144	213,170	59	5.9	540,290	41	-12
0.306	376,092	28	-6.6	714,355	22	-48
0.576	462,363	12*	-104	817,500	11*	-69

*Significantly different from the blank control ($p \leq 0.05$). Only the 0.576 mg a.i./L concentration was tested for significance.
Data from p. 29, MRID 47024923

TABLE 20. Mean area under the curve for *P. subcapitata* cultures exposed to AFxRD-038 for 96 hours

Mean measured concentration (mg/a.i./L)	0-72 hours			0-96 hours		
	Mean area	Percent inhibition relative to the:		Mean area	Percent inhibition relative to the:	
		Blank control	Negative control		Blank control	Negative control
Negative control	7,976,520	--	--	16,366,476	--	--
Blank control	18,706,800	--	--	35,913,216	--	--
0.039	8,612,844	54	-8.0	17,723,512	51	-8.3
0.088	8,541,612	54	-7.1	18,082,384	50	-10
0.144	7,494,108	60	6.0	16,415,628	54	-0.30
0.306	13,359,324	29	-6.7	26,324,688	27	-61
0.576	16,465,056	12*	-106	31,703,412	12*	-94

*Significantly different from the blank control ($p \leq 0.05$). Only the 0.576 mg a.i./L concentration was tested for significance.
Data from p. 30, MRID 47024923

TABLE 21. Mean growth rate of *P. subcapitata* cultures exposed to AFxRD-038 for 96 hours

Mean measured concentration (mg/a.i./L)	0-72 hours			0-96 hours		
	Mean growth rate	Percent inhibition relative to the:		Mean growth rate	Percent inhibition relative to the:	
		Blank control	Negative control		Blank control	Negative control
Negative control	0.0529	--	--	0.0476	--	--
Blank control	0.0646	--	--	0.0543	--	--
0.039	0.0539	17	-1.9	0.0485	11	-1.9
0.088	0.0539	17	-1.8	0.0492	9.5	-3.4
0.144	0.0518	20	2.1	0.0488	10	-2.5
0.306	0.0600	7.2	-13	0.0517	4.8	-8.6
0.576	0.0629	2.7*	-19	0.0531	2.2*	-12

*Significantly different from the blank control ($p \leq 0.05$). Only the 0.576 mg a.i./L concentration was tested for significance.
Data from p. 31, MRID 47024923

Conclusions:

The study is **acceptable**. In a 96-hour static toxicity bioassay, the freshwater green alga *Pseudokirchneriella subcapitata* was exposed in culture medium to AFxRD-038 (a.i., 3.6% 1-methylcyclopropene in a cyclodextrin/dextrose/EDTA carrier) at mean measured active ingredient concentrations ranging from 0.039 to 0.576 mg a.i./L. The test also included a negative control (culture medium only) and a formulation blank control (cyclodextrin/dextrose/EDTA only). There was no statistically significant difference between any treated group and the negative control group for the growth parameters of cell density, area under the growth curve, or growth rate. Growth parameters in the formulation blank were slightly (but statistically significantly) greater than in the highest test material group, but the difference was not considered treatment-related. The 72- and 96-hour EC_{50} values for each growth parameter were >0.576 mg a.i./L, and the 72- and 96-hour NOAEC for each growth parameter was 0.576 mg a.i./L.

Algal Toxicity, Tiers I and II (OPPTS 850.5400)

Test Material

3.3% 1-Methylcyclopropene alpha cyclodextrin complex (MRID 47024924)

Test Methods

A 96-hour static bioassay was conducted to determine the toxicity of the test material to the freshwater green alga *Selanastrum capricornutum.* The nominal test material concentration used in the test was 25 mg/L (approximately 0.825 mg a.i./L). The nominal concentration was selected based on calculations indicating a 1000 ppm (v/v) headspace concentration was equivalent to an aqueous phase concentration of 0.825 mg a.i./L.

The original algal culture was obtained from the University of Toronto culture collection and was maintained at the test facility. The algal cells were cultured and tested in freshwater algal medium. Algal cells used in the test were obtained from test facility cultures that had been actively growing in culture medium for at least two weeks prior to test start.

Results:

The temperature ranged from 23.4 to 24.3°C during the test. The test solution pH ranged from 7.6 to 7.7 at test start and from 10.5 to 10.6 at test end, increasing with increased algal cell density.

No undissolved test material was seen in any treated replicates during the test. The measured concentrations ranged from 117.7 to 123.7% of nominal at test start and from

89.9 to 108.1% of nominal at 96 hours. The overall mean measured concentration of active ingredient was 0.838 mg/L.

At test end, there were no signs of adherence of cells to the test vessels or of aggregation/flocculation of algae in any control or treatment group. There were no noticeable changes in cell morphology in any of the test material groups compared to the control.
Results for the mean cell density, mean area under the growth curve, and mean growth rate are given in Tables 22, 23, and 24, respectively. There were no statistically significant differences ($p \leq 0.05$) between the treated and control groups for any of the growth parameters.

TABLE 22. Mean cell density of *S. capricornutum* cultures exposed to 1-methylcyclopropene for 96 hours

Mean measured concentration (mg/a.i./L)	72 hours		96 hours	
	Mean cell density (cells/mL)	Percent inhibition	Mean cell density (cells/mL)	Percent inhibition
Negative control	283,333	--	578,333	--
0.838	243,333	14	606,667	-4.9

Data from p. 28, MRID 47024924

TABLE 23. Mean area under the growth curve of *S. capricornutum* cultures exposed to 1-methylcyclopropene for 96 hours

Mean measured concentration (mg/a.i./L)	72 hours		96 hours	
	Mean area	Percent inhibition	Mean area	Percent inhibition
Negative control	10,020,000	--	20,240,000	--
0.838	8,580,000	14	18,660,000	7.8

Data from p. 29, MRID 47024924

TABLE 24. Mean growth rate of *S. capricornutum* cultures exposed to 1-methylcyclopropene for 96 hours

Mean measured concentration (mg/a.i./L)	72 hours		96 hours	
	Mean growth rate (cells/mL/hr)	Percent inhibition	Mean growth rate (cells/mL/hr)	Percent inhibition
Negative control	0.0556	--	0.0495	--
0.838	0.0538	3.2	0.0500	-1.0

Data from p. 30, MRID 47024924

Conclusions:

The study is **acceptable**. In a 96-hour static toxicity bioassay, the freshwater green alga *Selanastrum capricornutum* was exposed in culture medium to 3.3% 1-methylcyclopropene in a cyclodextrin/dextrose carrier at mean measured concentration of 0.838 mg a.i./L. The test also included a negative control of culture medium only. There was no statistically significant difference ($p \leq 0.05$) between any treated group and the negative control group for algal cell density, area under the growth curve, or growth rate.

The 72- and 96-hour EC_{50} values for each growth parameter were >0.838 mg a.i./L, and the 72- and 96-hour NOAEC for each growth parameter were each 0.838 mg a.i./L.

Honey Bee Acute Contact Toxicity (OPPTS 850.3020)

Test Methods

A laboratory limit test was conducted to evaluate the toxicity of the test material to the honey bee (*Apis mellifera*). The bees were obtained as a frame of pupae (supplier not identified) that was held in a clear acrylic box for seven days in an environmental chamber at 31°C. Bees that emerged during the seven-day period were allowed to feed on honey *ad libitum* prior to the test (MRID 47024920).

The test chambers were stainless steel cylinders with perforations for ventilation. Each end of the cylinder was covered with a glass Petri dish. An inverted 20 mL glass vial containing 50% sucrose solution and covered with gauze to prevent leakage was inserted into the cylinder as a food source.

Results Summary

Results are given in Table 25. There was no statistically significant difference in mortality between the test material and control groups.

TABLE 25. Cumulative mortality of honey bees exposed to 10 ppm (nominal) 1-methylcyclopropene

Treatment	75 minutes	180 minutes	24 hours	48 hours
Negative control	0/60 (0%)	0/60 (0%)	2/60 (3.3%)	6/60 (10%)
1-Methylcyclopropene (10 ppm, nominal)	0/60 (0%)	0/60 (0%)	2/60 (3.3%)	5/60 (8.3%)

Data from p. 19, MRID 47024920

The measured concentration of 1-methylcyclopropene in the test material group samples was 10.2 ppm for a recovery of 102% of the nominal concentration. Absence of 1-methylcyclopropene in the control bags was confirmed.

Conclusions:

The study is **ACCEPTABLE** for honey bee acute contact toxicity. In a laboratory study, adult honey bees (*Apis mellifera*) were exposed to a nominal atmospheric concentration of 10 ppm (v/v) 1-methylcyclopropene for 48 hours. The test also included a negative control group exposed to air only. At test end, there was no statistically significant difference in bee mortality in the test material group (8.3%) compared to the negative control group (10.0%), and no treatment-related differences in behavior of bees in the test material group compared to the negative control group. The 48-hour LD_{50} for bees exposed to 10 ppm 1-methylcyclopropene in air was >10 ppm and the no-observed-effect concentration was 10 ppm.

Non-Target Insect Testing (OPPTS 880.4350)

Test Material

1. AFxRD-038, 3.6% w/w 1-methylcyclopropene (MRIDs 47024946 & 47024947)
2. 3.4% 1-Methylcyclopropene alpha cyclodextrin complex (MRIDs 47024941 & 47088617)

Test Insects

The parasitic wasp *Aphidius rhopalosiphi* (MRIDs 47024941 & 47024946), the predatory mite *Typhlodromus pyr*i (MRID 47088617) and the ground beetle *Poecilus cupreus* (MRID 47024947).

Test Methods

The effects of the test material on the survival and reproduction of the predatory mite *Typhlodromus pyr*i was conducted. The test organisms were originally obtained from BASF, Limburgerhof, Germany, as eggs and maintained at the test facility under environmental conditions similar to the test conditions. For the test, an egg cohort was left undisturbed until protonymphs were present (4-5 days). At test start, the protonymphs were <24 hours old (MRID 47088617).

The acute toxicity of the test material to the ground beetle *Poecilus cupreus* was conducted. The test organisms were obtained from BTL Sagerheide, Germany. Males and females were maintained separately in peat-filled boxes maintained under environmental conditions similar to the test conditions, and were fed punctured fly pupae (*Musca domestica*) twice weekly. At test start the beetles were 5.5 to 6 weeks old (MRID 47024947).

The effects of the test material on the survival and reproduction of the parasitic wasp *Aphidius rhopalosiphi* was conducted. The test organisms were obtained as mummies from PK Nutzlingszuchten, Welzheim and Katz Biotech AG, Baruth in Germany. During the culture period, honey and water were provided. Adults less than 48 hours old were used for the test (MRIDs 47024941 & 47024946).

Results:

The predatory mites, *Typhlodromus pyr*i were exposed in closed containers for seven days with 3.4% 1-Methylcyclopropene alpha cyclodextrin complex control group and an untreated control group. Then removed and observed for reproduction for an additional seven days. There were no statistically significant differences in the survival or reproduction of the mites in the treated group compared to the control groups.

The beetles *Poecilus cupreus* were maintained in moistened sand and fed fly pupae throughout the test with AFxRD-038 (a.i., 3.6% w/w 1-methylcyclopropene) applied at rates equivalent to 150 or 300 g a.i./ha. At test start, the beetles and the sand surface were sprayed with the test solutions, and monitored for 14 days. At test end, there were no

statistically significant differences in the survival or feeding behavior of the beetles in either test material group compared to the negative control group.

The parasitic wasp *Aphidius rhopalosiphi* were exposed in closed containers for 48 hourswith 3.4% 1-Methylcyclopropene alpha cyclodextrin complex and 3.6% w/w 1-methylcyclopropene; and surviving females were removed to open test chambers containing barley seedlings infested with aphids (*Rhopalosiphum padi*). After 24 hours of parasitization time, the females were removed and the test chambers were maintained for an additional 10 to 11days. The number of aphid mummies in the test chambers was then recorded. There were no statistically significant differences in the survival or fecundity of the parasitic wasps in the treated group compared to the control groups.

Conclusions:

The studies for non-target insects (the predatory mites, *Typhlodromus pyr*i, the beetles *Poecilus cupreus* and the parasitic wasp *Aphidius rhopalosiphi*) treated with 3.4% 1-Methylcyclopropene alpha cyclodextrin complex and 3.6% w/w 1-methylcyclopropene are **ACCEPTABLE**. The studies showed that there were no statistically significant differences in the survival or feeding behavior of the beetles in either test material group compared to the negative control group.

Waiver Request for Vegetative Vigor (OPPTS 850.4250)

Registrant's Justification

AFxRD-038 has been tested in hundreds of field trials around the world on numerous crops, soil types, and weather conditions. All the trials included observations for phytotoxicity and general plant growth. There were no phytotoxic effects from application to corn, soybeans, cotton, sunflower, or any other agronomic crop evaluated, even though the application rate was typically 100 g a.i./ha, four times the maximum single application rate on the proposed product label. There have been no phytotoxic effects from applications of 600 g a.i./ha on apple, pear, or other fruit trees. In an application at Colorado State University, a miscalculation resulted in an application rate of >1000 g a.i./ha, and no phytotoxic effects were noted.

Conclusion:

The submitted waiver request for Vegetative Vigor is acceptable based on the submitted vegetative vigor study (MRID 47088619).

Vegetative Vigor, Tiers I and II (OPPTS 850.4150, 850.4250)

Test Material

AFxRD-038, 3.6% w/w 1-methylcyclopropene (MRID 47088619).

Test Methods

A greenhouse study was conducted to determine the effects of AFxRD-038 on the vegetative vigor of ten species of non-target terrestrial plants grown in 11-cm by 10-cm deep round plastic pots (Table 26). The soil used was a sandy loam with an organic matter content of 2.2%. Limestone was added to buffer the pH to 7.3, and a slow-release fertilizer was added. The seeds used were not treated with fungicide, insecticide, or repellent prior to or during the test. After planting, the pots were maintained in the greenhouse. Seedlings were selected for the test based on visual similarity of their size and condition, then randomly assigned to the test groups.

The nominal concentrations of AFxRD-038 used in the study were 18.8, 37.5, 75.0, 150, and 300 g a.i./ha (4.28 oz ai/A), with an additional rate of 3.8 g a.i./A for cucumber only. These concentrations corresponded to nominal spray mixture concentrations of 93.8, 188, 375, 750, and 1500 ppm a.i., respectively, and 46.9 ppm a.i. for cucumber. The test also included a negative control group (well water only) and an adjuvant control group (0.38% adjuvant in well water). Seedlings were randomly assigned to the study groups. Each group consisted of six replicates containing five individually-potted plants each.

TABLE 26. Plant species treated with AFxRD-038

Species/variety/lot germination Seed source	Planting depth (mm)	Planting date Treatment date	Height and number of open leaves at treatment date
Monocots			
Onion (*Allium cepa*)/WI-609/>80% Wannamaker Seeds, St Matthews, SC	6	10/30/06 11/20/06	12-18 cm 1-2 leaves
Ryegrass (*Lolium perenne*) Manhattan 4 Perennial/90% Meyer Seed Co., Baltimore, MD	6	11/1/06 11/20/06	14-17 cm 4-5 leaves
Wheat (*Triticum aestivum*)/Polk/85% Johnny's Selected Seeds, Winslow, ME	20	11/8/06 11/22/06	23-35 cm 3 leaves
Corn (*Zea mays*)/Mandan Bride/94% Johnny's Selected Seeds, Winslow, ME	20	11/10/06 11/22/06	18-27 cm 3 leaves
Dicots			
Cabbage (*Brassica oleracea*)/Late Flat Dutch/85% Meyer Seed Co., Baltimore, MD	6	11/2/06 11/20/06	9-11 cm 2-3 leaves
Cucumber (*Cucumis sativa*)/Straight Eight/85% Meyer Seed Co., Baltimore, MD	20	11/6/06 11/22/06	5-8 cm 2 leaves
Soybean (*Glycine max*)/Williams 82/100% Missouri foundation Seeds, Columbia, MO	20	11/6/06 11/22/06	10-14 cm 3 leaves
Lettuce (*Lactuca sativa*)/Buttercrunch/99% Johnny's Selected Seeds, Winslow, ME	6	11/3/06 11/20/06	5-8 cm 3-4 leaves
Tomato (*Lycopersicon esculentum*)/Rutgers/90% Meyer Seed Co., Baltimore, MD	6	10/31/06 11/20/06	9-11 cm 3-4 leaves
Radish (*Raphanus sativus*)/Cherry Belle/85% Meyer Seed Co., Baltimore, MD	6	11/6/06 11/22/06	8-13 cm 2-4 leaves

Data from p. 17, MRID 47088619

Results:

The greenhouse temperature ranged from 15.95 to 30.69°C during the test, and the relative humidity ranged from 12.44 to 84.90%.

Since there were no statistically significant differences between the adjuvant and negative control results, the data for both controls were pooled and compared to the test material groups. There were no apparent treatment-related effects on plant condition or growth of any of the species tested. Sporadic signs of toxicity were seen, but there was no dose response, and these were considered incidental. The only statistically significant difference in growth of treated plants compared to the controls was a 5% decrease in height of soybeans in the 75.0 g a.i./ha group on day 7 only.

The mean measured active ingredient content in the samples collected from the 93.8 and 1500 ppm a.i spray solutions was 91.0 and 1902 ppm, for a mean recovery of 97 and 127% of nominal, respectively. Recovery of the intermediate concentrations ranged from 98 to 116% of nominal. The mean measured active ingredient content in the samples collected from the 46.9 and 1500 ppm a.i spray solutions on 11/22/06 was 48.3 and 1802 ppm, for a mean recovery of 103 and 120% of nominal, respectively. Recovery of the intermediate concentrations ranged from 101 to 116% of nominal. Absence of the active ingredient in the control spray solutions was confirmed for both application dates.

Conclusions:

The study is acceptable for the foliar application of AFxRD-038 at nominal application rates of up to 300 g a.i./ha resulted in no observed effects on either growth or condition of the ten species tested. The NOEC for all ten species was determined to be 300 g a.i./ha (4.28 oz ai/A) and the EC_{50} for all ten species was >300 g a.i./ha (4.28 oz ai/A). The concentration of active ingredient used in the test (3.6%) is slightly lower than that specified on the product label (3.8%).

Seedling Emergence, Tiers I and II (OPPTS 850.4100, 850.4225)

Test Material

AFxRD-038, 3.6% w/w 1-methylcyclopropene (MRID 47088620).

Test Methods

A greenhouse study was conducted to determine the effects of AFxRD-038 on the seedling emergence and growth of ten species of terrestrial plants. The soil used was a sandy loam with an organic matter content of 2.2%. Limestone was added to buffer the pH to 7.3, and a slow-release fertilizer was added. The seeds used were not treated with fungicide, insecticide, or repellent prior to or during the test.

Seeds were selected from a single size class within each species. The seed sources are given in Table 27. The seeds were planted in 16 cm x 12 cm deep plastic pots one day

prior to application of the test material. A template was used to gently compact the soil and leave ten uniform holes for planting. One indiscriminately selected seed was then placed in each hole, and the holes were closed by depressing the soil surface.

TABLE 27. Plant species treated with AFxRD-038

Species/variety/lot germination Seed source	Planting depth (mm)	Treatment date
Monocots		
Onion (*Allium cepa*)/WI-609/>80% Wannamaker Seeds, St Matthews, SC	6	11/29/06
Ryegrass (*Lolium perenne*) Manhattan 4 Perennial/90% Meyer Seed Co., Baltimore, MD	6	11/29/06
Wheat (*Triticum aestivum*)/Polk/85% Johnny's Selected Seeds, Winslow, ME	20	12/1/06
Corn (*Zea mays*)/Mandan Bride/94% Johnny's Selected Seeds, Winslow, ME	20	12/1/06
Dicots		
Cabbage (*Brassica oleracea*)/Late Flat Dutch/85% Meyer Seed Co., Baltimore, MD	6	12/1/06
Cucumber (*Cucumis sativa*)/Straight Eight/85% Meyer Seed Co., Baltimore, MD	20	12/1/06
Soybean (*Glycine max*)/Williams 82/100% Missouri foundation Seeds, Columbia, MO	20	11/29/06
Lettuce (*Lactuca sativa*)/Buttercrunch/99% Johnny's Selected Seeds, Winslow, ME	6	11/29/06
Tomato (*Lycopersicon esculentum*)/Rutgers/90% Meyer Seed Co., Baltimore, MD	6	11/29/06 1/5/07[a]
Radish (*Raphanus sativus*)/Cherry Belle/85% Meyer Seed Co., Baltimore, MD	6	12/1/06

Data from p. 17, MRID 47088620

[a]Due to inadequate emergence of control seedlings in 11/29/06 trial, the test for *L. esculentum* was repeated.

The nominal concentrations of AFxRD-038 used in the study were 18.8, 37.5, 75.0, 150, and 300 g a.i./ha. These concentrations corresponded to nominal spray mixture concentrations of 93.8, 188, 375, 750, and 1500 ppm a.i., respectively. The test also included a negative control group (well water only) and an adjuvant control group (0.38% adjuvant in well water). Each test group consisted of four replicates containing one pot of ten seeds each.

Results:

There were no apparent treatment-related effects on seedling emergence or growth of any of the species tested. Significant reductions relative to the pooled control groups were noted among *A. cepa* (emergence at 150 g a.i./ha), *T. aestivum* (emergence at 75.0 and 150 g a.i./ha),and *R. sativus* (survival at 300 g a.i./ha), but there was no dose response and the reductions were considered incidental to treatment. Although not statistically significant, dry weight of *L. esculentum* was reduced by 26% and 24% compared to the pooled controls at the 150 and 300 g a.i./ha application rates, respectively. There was also a non-significant reduction (12%) in mean height of *L. esculentum* at the 300 g a.i./ha application rate. Based on these results, the NOEC, EC_{25} and EC_{50} were estimated for the test species.

Conclusions

The study is acceptable. There was a slight, but apparently treatment-related effect on the dry weight of *L. esculentum*, resulting in a NOEC of 75.0 g a.i./ha, and an EC_{25} and EC_{50} of 256 and >300 g a.i./ha, respectively. There were no adverse effects on the other nine species. The NOEC for the other nine species was 300 g a.i./ha, and the EC_{25} and EC_{50} for those species was >300 g a.i./ha.

Table 28 Toxicity of Ecological Effect and Non-target Organism

Study Type/OPPTS Guideline	LD_{50}/LC_{50}/Results	Toxicity Category	MRID
Avian Acute Oral/OPPTS 850.2100	>2250 mg/kg	Practically non-toxic	47024917
Avian Dietary/OPPTS 850.2200	>5620 ppm	Practically non-toxic	47024918
Freshwater Fish LC50/OPPTS 850.1075	>0.750 mg and >0.966 mg ai/L	Highly toxic	47024915 47088610
Freshwater Invertebrate/OPPTS 850.1010	>0.678 mg a.i./L and >0.776 mg a.i./L	Highly toxic	47088609 47024914
Non-target Plants/OPPTS 850.4250	Waiver acceptable	No apparent affect	47088619
Non-target Plants/OPPTS 850.4150, 850.4250	EC_{50}: >300 g a.i./ha (4.28 oz ai/A)	No apparent affect	47088619
Non-target Plants/OPPTS 850.4100, 850.4225 (Seedling Emergence)	EC_{25} and EC_{50} : >300 g a.i./ha.	No apparent affect	47088620
Non-target Insects (Honey bee)	10 ppm	Practically non-toxic	47024920
Acute Earthworm Toxicity	10 ppm	Practically non-toxic	47024944

Acute Earthworm Toxicity Testing (Nonguideline)

Test Material

3.4% 1-Methylcyclopropene alpha cyclodextrin complex (MRID 47024944).

Test Methods

A laboratory study was conducted to determine the acute toxicity of the test material to the earthworm, *Eisenia fetida*. The test organisms were obtained from cultures maintained at the testing facility, started by organisms obtained from BBA, Braunschweig, Germany. The worms were cultured in a pesticide-free peat medium, and were fed frozen-treated horse manure, apple dregs, and lucern meal. Prior to the test, they were transferred to artificial soil for acclimatization. At test start, the worms were 6.5 to 7.5 months old and

had developed clitella. Body weight ranged from 301 to 596 mg, with a mean of 392 mg. The burrowing time of all worms was within 10 minutes, indicating they were healthy.

Results:

The temperature during the test ranged from 18.5 to 20.5°C, and the relative humidity measured in one quality control vessel and one test material vessel was >100%. On day 0, the pH was 5.81 and the mean soil moisture content was 40.3%. On day 14, the pH was 5.78 and the mean soil moisture content was 40.3%.

All earthworms survived the test and were healthy. Burrowing times on days 0 and 7 were <10 minutes. There was no statistically significant difference in body weight of any of the test groups.

Recovery of 1-methylcyclopropene in the quality control replicates ranged from 0 to 13% of nominal. The maximum recovery in the test material replicates was 15.4%, which occurred eight hours after the initial treatment. One week following treatment, no 1-methylcyclopropene was detected in the test material replicates. The study author stated that adequate recovery in other studies conducted at the test facility indicates the low recovery in the present study is likely due to the presence of the soil. Furthermore, the results were similar in the quality control and test material replicates. No 1-methylcyclopropene was detected in the untreated control or carrier control replicates.

Conclusions:

The study for acute earthworm toxicity testing is acceptable. AFxRD-038 applied at a concentration sufficient to produce an atmospheric concentration of 10 ppm v/v 1-methylcyclopropene had no effect on survival or weight of *E. fetida*. The poor recovery of active ingredient in the test vessels was likely due to absorption (and probably degradation) by the soil. Recovery of the active ingredient in other studies without soil but using a similar setup and the same analytical method (MRIDs 47024944 and 47088617) was acceptable.

Soil Adsorption/Desorption Isotherm (835.1220)

Test Material

The test material was ^{14}C-1-methylcyclopropene (MRID 471082-04).

Soil Preparation

Soils arrived in good condition at the test facility where they were sieved to 2 mm after which they were air dried for 2 weeks at room temperature. The soils were characterized for organic carbon content, pH, calcium carbonate, total nitrogen, cation exchange capacity, particle size and microbial biomass. This study was performed in glass columns equipped with a porous glass filter plate at the bottom.

Treatment and Sampling

For the formulation of the test substance, ^{14}C-1-MCP was mixed with a Dyne-Amic surfactant/EDTA solution and 0.5 mL of the solution added to 50 g soil in tubes to produce ^{14}C-1-MCP soil aliquots. The ^{14}C-1-MCP was applied at ten times the maximum field rate of 500 g a.i./ha. The ^{14}C-1-MCP soil aliquots (47- 58 g) were applied to the saturated soil columns (# 2,4,6,8 and 9-12) using a spatula. Following treatment, the columns were covered with a round filter paper and protected from light using aluminum foil in order to avoid photolysis.

For the reference substance, an aliquot of 1 mL^{14}C-atrazine solution was applied to a 50 g aliquot of each soil which was then applied to separate soil columns (# 1,3,5 and 7).

Leaching Procedure

A volume of about 400 mL 0.01 M $CaCl_2$ (equivalent to about 200 mm of rain) was delivered to the top of each saturated column over a period of two days with the aid of a peristaltic pump at room temperature. Leachates were collected at 24 and 48 hours and aliquots were measured by LSC to determine the total radioactivity.

Analysis of Soil Samples and Leachates

After percolation, the soil columns were removed from the glass-column and sectioned into five segments of about 6 cm each. The soil was extracted once with acetonitrile/water (4:1; v/v). The extractions were performed in a shaker at about 250 strokes per minute for about 30 minutes. The amount of solvent used was in general about 1 mL per g of soil. The individual extracts were centrifuged, quantified by LSD, separately concentrated under reduced pressure (at about 35°C) in a rotary evaporator and the radioactivity was remeasured by LSC. The samples were then submitted to HPLC analysis. TLC was also conducted on selected samples. The extracted soil samples were air-dried and the non-extractable radioactivity was determined by combustion and LSC. For a complete radioactive balance, the empty glass columns from the end of the experiment were rinsed with acetone and the radioactivity determined by LSC.

The radioactivity in the leachates was determined by direct liquid scintillation counting of 2 or 5 mL aliquots. Thereafter, an aliquot of each leachate was concentrated in a rotary evaporator at about 40°C. Using this method, about 50% of the radioactivity was found in the distillate, therefore a separate extraction was performed in order to determine the nature of the volatile radioactivity present. For this purpose, an aliquot of each leachate was taken, extracted by using reverse phase RP18 cartridges, eluted by methanol, and contained the volatile part. The resulting samples were directly analyzed by HPLC.

Results and Discussion

Distribution of the Radioactivity in the Leachates: Very low amounts of radioactivity were detected in the leachates during the 48-hour leaching period (equivalent to 200 mm rainfall). In the first 24-hours, between 0.1% and 0.4% of the applied radioactivity was

found in the leachates. Between 24 and 48 hours of leaching, slightly more radioactivity was detected (up to 2.2% of applied).

Distribution of the Radioactivity in the Soil Profile: After leaching, the soil columns were sectioned into five layers of equal length. The distribution of the radioactivity is in all eight soil columns after the leaching procedure. The results were very similar between the soil types. The top layer of all four soils contained virtually all of the radioactivity, accounting for between 84% and 100% of the applied amount in the Oakville, Waddesdon and Itingen soils and 59-62% of applied for Hesingue soil. Essentially the entire radioactivity in the top and following layers was non-extractable. Extractable radioactivity represented only 1 to 2 % of the total radioactivity in the columns. HPLC analyses of the soil layer extracts showed the absence of the parent compound 1-MCP and the presence of up to seven very minor fractions. The reference substance, atrazine, applied to separate soil columns was detected in all soil layers and was mobile in the column leaching experiment.

Conclusions:

The study for soil adsorption/desorption isotherm (OPPTS 835.1220) is acceptable. When radiolabeled 1-Methylcyclopropene (^{14}C-1-MCP) was applied to four soil types in column leaching experiments, less than 3% of the applied radioactivity was found to leach in each soil type thus demonstrating that the field use of 1-MCP should not result in significant leaching of 1-MCP residues to groundwater. Analyses of the soil layers from the column experiments indicate that 1-MCP binds rapidly and tightly to the soil types tested. No detectable amount of the parent 1-MCP was found in leachates from any of the soils tested. Thus, 1-MCP can be classified as a compound with low mobility in soil. The amount of applied ^{14}C-1-MCP was approximately ten times the maximum field application rate of 500 g a.i./h.

Ready Biodegradability (835.3110)

Test Material

1-methylcyclopropene from 1-MCP alpha-cyclodextrin complex, 3.3% a.i.(MRID 47024912).

Test Methods:

Test Medium: The test medium was modified biochemical oxygen demand (BOD) test dilution water and was prepared using high quality water, autoclaved and allowed to cool to room temperature.

Test Apparatus: The degradation test chambers were 125 mL serum bottles stoppered with Teflon lined septa and sealed with crimp seals. The inoculum viability test chambers were darkened 2-liter Erlenmeyer flasks stoppered with foam plugs. All chambers were identified by project number, test substance ID, test concentration and replicate. The test was conducted at 22°C.

Results and Discussion

The temperature range recorded during the test was 20 to 22°C. The result of the standard plate count performed on the inoculum was 2.5 x 10^4 CFU/mL. Elimination of 1-MCP from the treated test media was not observed. There was no degradation as the average measured concentrations on Days 7 and 28 were 0.506 and 0.507 mg/L, respectively. A moderate decline in 1-MCP from the sterile treatment test media was observed. Average measured concentrations in the sterile treatment test media on Days 7 and 28 were 0.645 and 0.478 gm/L, respectively. The test inoculum was demonstrated to be viable by actively degrading the reference substance sodium benzoate, 96% of the applied degraded by Day 13, 97% degraded by Day 21 and 100% degraded by Day 28. An average percent degradation of greater than 70% was observed on Day 7 of the test, thereby fulfilling the criteria for a valid test.

Conclusion:

The submitted study for Ready Biodegradability (OPPTS 835.3110) is **acceptable**. Test chambers from the control and treatment groups were analyzed for 1-MCP on Days 0, 7, 13, 21 and 28. The reference group was dosed with sodium benzoate at a nominal concentration of 20 mg C/L. The test inoculum was demonstrated to be viable by actively degrading more then 70% of the sodium benzoate after 7 days. Degradation of 1-MCP was not observed under the test conditions for the 28 day test. Average measured test substance treatment concentrations of 1-MCP on Days 7 and 28 were 0.506 and 0.507 mg/L, respectively.

Atmospheric Fate and Photolysis

AgroFresh, Inc., a subsidiary of Rohm and Haas Company, has submitted supplemental studies for atmospheric fate and photolysis (MRIDs 471082-06 & 471082-07).

Test Material

AFxRD-038 (1-Methylcyclopropene)

Conclusions:

1. The supplemental study for atmospheric fate is acceptable. Measurements related to two of the chemical removal processes of 1-MCP in the atmosphere were determined: reaction with OH and photolysis. The average rate of the reaction of 1-MCP with OH is measured as $k_{OH} = 2.7 \pm 1.3 \times 10^{-11}$ cm^3 s^{-1} at STP. Assuming a typical 12-hour average concentration of OH of 1.5 x 10^6 molecules/cm^3, the time over which 1-MCP would be reduced to 1/*e* (36.8%) of its concentration is calculated to be 6.4 hours. The reaction of 1-MCP with OH is the main removal mechanism. The absorbance of 1-MCP as a function of wavelength in the UV-visible spectrum has been measured. The absorbance results confirm that most of the UV absorption occurs at wavelengths less than 240 nm. Therefore, the photolysis pathway is expected to be negligible. Thus, when released to the atmosphere, 1-MCP is expected to degrade primarily by reaction with OH then with

secondary sinks through reaction with NO_3 and O_3. Photolysis of 1-MCP is expected to be limited.

2. The supplemental study for photolysis is acceptable. A photolysis technique using pulsed laser induced fluorescence was used to measure the rate coefficient for the reaction of the hydroxyl radical with 1-methylcyclopropene (1-MCP) at room temperature and at several pressures in He and air buffer gases. The unweighted average of eight measurements gives a rate coefficient of $k_{OH} = 2.7 \pm 1.3 \times 10^{-11}$ cm^3 s^{-1} at STP.

cc: D.Benmhend, R. S. Jones; BPPD Chron File; OHAD/ARS
M. Xue, BPPD, 10/03/07