UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Science Review in Support of a Petition (Petition No. 0F06144) from the Requirements of a Tolerance on All Food Commodities and Label Amendment for EthylBloc™ (EPA Reg. No. 071297-1) Containing 0.14% 1-Methylcyclopropene (Chemical No. 224459). DP Barcodes D266197, D266198, and D272185; Case Nos. 292962 and 063215; Submission Nos. S580023, S578833, and S591344 (MRID 450896-01).

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THRU: Freshteh Toghrol, Ph.D., Senior Scientist
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TO: Driss Benmhend, Regulatory Action Leader
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ACTION REQUESTED

AgroFresh, Inc. [formerly BioTechnologies for Horticulture, Inc (BTH, Inc.; a subsidiary of Rohm and Haas Company)] requests an exemption from the requirements of tolerances of residues of 1-methylcyclopropene (1-MCP) on food commodities. 1-MCP is the active ingredient in the end-use product, EthylBloc™ (EPA Reg. No. 071297-1) which contains 0.14% 1-MCP. EthylBloc™ is a powdered product that releases a gas (1-MCP) when mixed with water or a buffering agent. The end-use product is currently registered for non-food use on floral and nursery crops.

Subsequent to the AgroFresh, Inc.'s FQPA Notice of Filing for the tolerance exemption petition (published in the Federal Register on 6/21/2000), Valent BioSciences (formerly Abbott BioSciences) submitted comments (see letter from M. Tichon to EPA/PIRIB, dated 7/20/2000) regarding the petition (PP#0F06144). In a letter from S. L. Longacre to D. Benmhend (dated 12/19/2000), AgroFresh, Inc. responded to these comments. Both the Valent BioSciences

comments and the AgroFresh, Inc. responses will be discussed in this document in conjunction with the science review for the tolerance exemption petition.

In support of the of the tolerance exemption, the registrant has submitted: (i) a petition for an exemption from the requirement of tolerances for residues of 1-methylcyclopropene (1-MCP) on food commodities; (ii) a rationale for waivers of study requirements for registration of 1-MCP use on post-harvest fruits and vegetables; (iii) an assessment of risk criteria established in FQPA supporting an exemption from the requirements of tolerances for 1-MCP (MRID 450896-01); and (iv) a request for a label amendment to add indoor use on post-harvested fruits and vegetables.

CONCLUSIONS AND RECOMMENDATIONS

1. The submitted data support the petition for an exemption from the requirements of a tolerance for 1-methylcyclopropene (1-MCP), the active ingredient contained in the end-use product EthylBloc™ (EPA Reg. No. 071297-1) at a concentration of 0.14% of the product by weight.

2. The submitted data support the request for a label amendment to permit the indoor use of EthylBloc™ (EPA Reg. No. 071297-1) on post-harvested fruits and vegetables.

3. The submitted data demonstrate that residues of 1-MCP in treated food are likely to be extremely low to non-existent. However, to alleviate concerns regarding potential dietary exposure to 1-MCP in treated food commodities, the registrant should conduct residue studies (using apples) showing that 1-MCP and any potential metabolites are below detection limits in raw and processed apple commodities (including pulp, applesauce, and juice). BPPD further recommends the use of radioisotope techniques (with ^{14}C-1-MCP) combined with GC/MS to validate the analytical method and to determine the identities of any radioactive metabolites (if any).

4. The most likely route of exposure to 1-MCP (a gas) is via inhalation. The submitted data demonstrate that exposure of humans to 1-MCP via the inhalation pathway will be minimal. Exposure of humans to 1-MCP via oral, dermal, or eye pathways is highly unlikely. However, to alleviate concerns regarding potential inhalation toxicity, the registrant should conduct additional acute and chronic inhalation studies using 1-MCP concentrations up to and including 1000 ppm.

5. 2-chloro-3-methylpropene (CMP) is a potential contaminant that may be formed as a residual component of the initial reaction in the EthylBloc™ manufacturing process. CMP is a potential human health concern due to evidence of carcinogenicity in experimental animals; no data are available regarding the carcinogenicity (if any) of CMP in humans. To alleviate concerns regarding the potential presence of 3-chloro-2-methylcyclopropene (CMP), a potential human carcinogen, the registrant is required to

conduct air sample analyses for CMP in closed storage chambers treated with EthylBloc™.

6. In the initial review of data (see Memorandum from R.S. Jones to D. Benmhend, dated 12/23/1998) it was originally concluded that 1-MCP was not a mutagen based on a lack of significant data. Upon further review by BPPD, it was determined that three studies submitted in fulfillment of Subdivision M Guideline 152-19 for mutagenicity [the reverse mutation assay (MRID 44464709) and the mouse lymphoma forward mutation assay (MRID 44496118)] may not have adequately assessed the effects of 1-MCP. The test systems used water to prepare the test substance for use in cell culture systems and for oral gavage. Since 1-MCP is released from the product when it is mixed from water, it is likely that little or no 1-MCP was present in the culture media when the testing was initiated. To alleviate concerns regarding the potential for 1-MCP to be a mutagen, the registrant should redo the mutagenicity studies wherein the test systems are exposed to 1-MCP gas at concentrations up to and including 1000 ppm v/v in a closed system.

7. The submitted data demonstrate that it is highly unlikely that non-target organisms will be affected by 1-MCP. The product is not intended for use outdoors or in other non-enclosed areas. Outdoor venting of enclosed facilities following treatment of non-food and food commodities with the product is not likely to affect non-target organisms since 1-MCP will be rapidly dissipated in the external atmosphere.

BACKGROUND

EthylBloc™ (EPA Reg. No. 071297-1) is currently registered for non-food use on floral and nursery crops in enclosed, indoor areas. In support of this registration, the registrant has submitted acceptable product chemistry studies (Subdivision M Guidelines 151-10 to -17) and acute mammalian toxicity studies [152-10 to -16 (see Memoranda from R. S Jones to D. Benmhend, dated 12/23/1998 and 3/1/1999)]; it was also concluded that the compound is not a mutagen (152-19). No data for non-target organisms and ecological effects (154-6 to -11) were required because the product was not intended for use outdoors or in other non-enclosed areas. Subsequent to the registration, an amendment to reflect a correction in the amount of 1-methylcyclopropene (1-MCP, the active ingredient) listed on the label from 0.43% to 0.14% was requested. The amendment was requested because new and improved analytical methodology demonstrated that the actual 1-MCP content in the product was approximately 25% of the a.i. content listed on the label [see letter from S. Longacre to D. Benmhend (dated 3/8/2000)]. The label amendment, as well as a brief description of the new analytical methodology and two revised Confidential Statements of Formula (CSFs, dated 3/8/2000) were deemed acceptable by BPPD (see Memorandum from R. S. Jones to D. Benmhend, dated 3/9/2000).

More recently, the registrant requested an experimental use permit (EUP; EPA Reg. No. 71297-EUP-R) to permit the commercial indoor testing of EthylBloc™ on postharvest stored

apples (EPA File No. 71297 -1). Under the EUP, the registrant intended to use a maximum of 52.9 lbs of EthylBloc™ (equivalent to 0.074 lbs of 1-MCP) on 10.8 million lbs of postharvested apples. A maximum of six trials are currently in progress in three states [CA (2), PA (2), and WA (2)] and are being conducted in commercial apple storage facilities at each location. Apples were harvested from a total of 491 acres of apple trees. Treatments at each location were conducted in a room with a volume of approximately 2500 m^3 and containing approximately 1.8 million apples. Nominal concentrations of 1-MCP in each trial were 1000 ppb over a 24- to 48-hr treatment interval. Following treatment, the apples will remained in storage under refrigeration and low oxygen concentration for up to 9 months. During the 9-month storage interval, samples are being collected for analysis of various physiological parameters. This EUP was deemed acceptable by BPPD (see Memorandum from R. S. Jones to D. Benmhend, dated 9/28/2000) provided it included a crop destruct requirement. The crop destruct requirement was included because there were no established tolerances or tolerance exemptions for residues of 1-MCP on apples or other food commodities. In the EUP review of 9/28/2000, BPPD stated that the EUP could be amended at a later date to a non-crop destruct, after a permanent exemption from the requirement of tolerances had been established for 1-MCP.

A petition to establish a permanent exemption from the requirement of tolerances for residues of 1-MCP on food commodities was submitted to the Agency in April 2000 and is the subject of this review. It was noted in the EUP review of 9/28/2000, that since apples are also processed for juice, puree, applesauce, animal feed, etc, that residue data should be used to support the petition for a permanent tolerance exemption for 1-MCP and that these residue data should include data on all raw and processed apple commodities.

On 6/21/2000, a Notice of Filing of a Pesticide Petition to Establish a Tolerance for Certain Pesticide Chemicals in or on All Food Commodities appeared in the Federal Register Volume 65, Number 120; Docket Control No. PF-947), indicating that 1-MCP posed "no significant risk to humans or the environment." In a comment submitted to the Agency (see letter from M. Tichon, Valent Biosciences Corporation, dated 7/20/2000), it was argued that "the literature indicates risks of adverse effects posed by toxicity of and exposure to 1-MCP are considerably greater than represented in the notice of filing" and that "these risks argue against the issuance of the proposed tolerance exemption. In a letter from S. Longacre to D. Benmhend (dated 12/19/2000), Agrofresh, Inc. submitted a response to these comments including information supporting the registrant's contention that the active ingredient would not cause adverse effects on humans and wildlife when the product is used according to label directions.

The information submitted by AgroFresh, Inc. to support the petition (Petition No. 0F06144) from the requirements of a tolerance for 1-MCP on all food commodities and a label amendment for EthylBloc™ (EPA Reg. No. 071297-1), containing 0.14% 1-MCP as its active ingredient, the comments submitted by Valent BioSciences (dated 7/20/2000), and AgroFresh's response (dated 12/19/2000) to the Valent BioSciences comments, are discussed in detail below.

RISK ASSESSMENT FOR 1-MCP

1. Description of the Biopesticide

1-Methylcyclopropene (1-MCP) is the active ingredient of the end-use product (EP), EthylBloc™ (EPA Reg. No. 071297-1), and comprises 0.14% of the EP by weight. When the EP is added to water, 1-MCP is released as a gas. The active ingredient is structurally-related to naturally-occurring compounds found in plants.

2. Mode of Action

1-MCP is an inhibitor of ethylene, a naturally-occurring plant hormone. Ethylene activity is inhibited via the reversible binding of 1-MCP to ethylene receptors in plants, competitively excluding ethylene from the receptor sites, and subsequently counteracting the physiological effects of the phytohormone [see Sisler and Serek (1999), Bot. Bull. Acad. Sin., 40: 1-7]. Inhibition of ethylene activity counteracts many undesirable effects on post harvest fruit and vegetables such as accelerated ripening and softening of climacteric fruit, accelerated de-greening and softening of non-climacteric fruit, senescence, abscission, and other physiological disorders [Abeles et al. (1992), Ethylene in Plant Biology, Academic Press, NY]. Due to this non-toxic mode of action, BPPD has classified 1-MCP as a biochemical and considers this compound to be a plant growth regulator (see letter from W. Schneider, dated 12/3/96).

3. Proposed Uses/Application Rate

EthylBloc™ (EPA Reg. No. 071297-1), containing 0.14% 1-MCP by weight, is currently registered for indoor non-food use on floral and nursery crops. The registrant also intends to amend the registration to permit indoor food-use of the product on post-harvested fruit and vegetables in enclosed areas (expected to occur primarily in commercial food storage facilities, many of which are controlled atmosphere facilities which use relatively low oxygen levels and high carbon dioxide levels. No outdoor uses, or uses in unenclosed facilities are anticipated or permitted.

When the product is applied at the maximum proposed label use rate for postharvested food commodities (1.6 g product/m^3 in 25 mL water) approximately 1000 ppb, or 1 ppm (v/v) of 1-MCP will be released into the treated storage area. Depending upon the storage temperature, food commodities may be treated for at least 24 hours. Following the prescribed treatment interval, vapors of the active ingredient are vented outdoors.

4. Residue Chemistry (Nature and Magnitude of the Residue on Food Commodities)

Due to the low product application rate, residues of 1-MCP on food commodities are expected to be very low to nonexistent. Since the active ingredient is a gas, no solid or liquid residues of 1-MCP will be observed on treated food. Residues, if present, will be found on the ethylene

receptors within treated plant tissues, and the maximum concentration of 1-MCP will not exceed the concentration of ethylene receptors to which it will potentially be bound. In a review of the literature, Sisler [1991 (in The Plant Hormone Ethylene, A. K. Mattoo and J. C. Suttle, eds., CRC Press, Boca Raton, FL, p. 90)] listed the concentrations of ethylene-binding sites (receptors) reported for tissues of different plant species. The concentration of ethylene-receptors ranged from 1.9×10^{-9} to 6.8×10^{-9} mol/kg fresh weight in vegetative tissues (leaves, stems, roots, bean cotyledons, flower petals) and 7×10^{-11} to 4.8×10^{-9} mol/kg fresh weight in "edible" tissues (tomato fruit, apple pulp). Using a "worst-case" scenario wherein 100% of the ethylene receptors in plant tissue are saturated with 1-MCP, and the ethylene receptor concentrations listed by Sisler (1991), the registrant calculated the following theoretical maximum for 1-MCP residues in EthylBloc™-treated food commodities:

Leaf Tissue (highest reported concentration in *Ligustrum* leaves):

6.8×10^{-9} mole receptors/kg x 54 g 1-MCP/mole x 1.0 (100% saturation) = 3.7×10^{-7} g 1-MCP/kg (or 0.37 ppb);

and

"Edible" Tissue (highest reported concentration in tomato fruit):

7.0×10^{-11} mole receptors/kg x 54 g 1-MCP/mole x 1.0 (100% saturation) = 4.0×10^{-9} g 1-MCP/kg (or 0.004 ppb)

Therefore, assuming that 1-MCP occupies all ethylene receptors in a plant, the theoretical maximum residues in the edible portion of tomato fruit would not exceed 0.004 ppb. Using the same equation and Sisler's (1991) list, maximum 1-MCP residues would not exceed 0.002 ppb in apple pulp. These calculations overestimate the theoretical maximum that would be present in human food since the time interval between postharvest treatment of food commodities and their arrival at the consumer's table would permit 1-MCP to diffuse out of the treated food. Additionally, it is not certain that there would be a 100% saturation of ethylene receptors in treated tissues.

5. Toxicity Profile

The registrant submitted acceptable acute toxicity studies (152-10 to 152-16) for EthylBloc™ (see Memorandum from R. S. Jones to D. Benmhend, dated 12/23/98). Based on a lack of mortality observed in albino rats orally dosed with 5000 mg/kg of powdered product, the oral LD_{50} was >5000 mg/kg; tox category IV. Based on a lack of mortality observed in albino rabbits dermally dosed with 2000 mg/kg of powdered product, the LD_{50} was >2000 mg/kg; tox category III. **NOTE: 2000 and 5000 mg product/kg = 2.8 and 7.0 mg a.i., respectively.** Based on a lack of mortality observed in albino rats exposed to165 ppm of MCP gas for 4 hours, the LC_{50} was >165 ppm; tox category IV. Ocular instillation of 0.1 mL of powdered product caused mild to moderate eye irritation symptoms (redness, chemosis) which cleared by 72 hours posttreatment; tox category III. Dermal application of 0.5 g of powdered product did not cause

any dermal irritation symptoms up to 72 hours postdosing; tox category IV. Based on the data, the test substance is not considered to be a contact sensitizer. No hypersensitivity incidents have been reported. Approximately 4100 person hours of MCP exposure have been experienced by humans without any known MCP-induced health related problems being reported. Acute toxicity data are summarized in the table below:

Study (Guideline)	Results	Toxicity Category	MRID
Acute Oral Toxicity (152-10; OPPTS 870.1100)	Rat LD_{50} >5000 mg product/kg	IV	44464704
Acute Dermal Toxicity (152-11; OPPTS 870.1200)	Rabbit LD_{50}>2000 mg product/kg	III	44464705
Acute Inhalation Toxicity (152-12; OPPTS 870.1300)	Rat LC_{50} >165 ppm a.i.	IV	44464706
Primary Eye Irritation (152-13; OPPTS 870.2400)	Mild to moderate eye irritation symptoms in rabbits (redness, chemosis) following ocular instillation of product; cleared by 72 hours posttreatment	III	44464707
Primary Dermal Irritation (152-14; OPPTS 870.2500)	No dermal irritation symptoms in rabbits following dermal treatment with product up to 72 hours postdosing with product	IV	44464708
Hypersensitivity (152-15; OPPTS 870.2600)	Approximately 4100 person hours of MCP exposure have been experienced by humans without any known MCP-induced health related problems being reported.	Product not a sensitizer	44517005

Although acute oral toxicity, acute dermal toxicity, primary eye irritation, primary dermal irritation, and hypersensitivity studies were conducted with formulated product (containing 0.14% 1-MCP) and not pure active ingredient, it is emphasized that oral, dermal, and eye pathways are not likely routes of 1-MCP exposure to humans. Since the active ingredient is a gas, inhalation exposure is the most likely route of exposure for this active ingredient. The acute inhalation toxicity study was conducted using 165 ppm 1-MCP, well above the Toxicity Category IV limit dose of >2 mg/L (>2 ppm) for acute inhalation toxicity.

BPPD initially deemed mutagenicity studies (Subdivision M Guideline 152-19) submitted in support of the original registration to be acceptable. Based on a lack of statistically significant data obtained from a reverse-mutation assay study (MRIDs 44464709), a mouse lymphoma forward mutation study assay (MRID 44496118), and a mouse micronucleus study (MRID 44464711), 1-MCP was not considered a mutagen. Upon further review, it was noted that the three studies may not have adequately assessed the effects of 1-MCP. All three test systems used cell culture systems or dose preparation techniques wherein the test substance (EthylBloc™) was

mixed with water or water-based culture media prior to inoculation. Since 1-MCP is released from the product when it is mixed from water, it is likely that little or no 1-MCP was present in the culture media (or the oral dose in the case of the mouse micronucleus assay) when the testing was initiated. Therefore, the aforementioned tests may not be valid for use in assessing 1-MCP as a mutagen.

At this time, the Agency does not consider 1-MCP to be a potential mutagen based its lack of structural similarity with known mutagens or classes of mutagens. However, BPPD notes that the registrant has subsequently indicated that it is conducting additional mutagenicity studies to verify that 1-MCP is not a mutagen (see letter from S. Longacre to D. Benmhend, dated 2/2/2001). These studies include:

1. A mouse *in vivo* micronucleus assay conducted with 1000 ppm v/v 1-MCP gas; and

2. Three mutagenicity studies in which the agar plates or aqueous cell systems are exposed to an atmosphere of 1000 ppm v/v 1-MCP gas.

These studies will be submitted as part of a Section 3 and EUP applications for an alternate formulation of the product containing a higher percentage of active ingredient.

Toxicity of 3-Chloro-2-methylpropene (CMP): CMP is a potential contaminant that may be formed as a residual component of the initial reaction in the manufacturing process (see MRIDs 44464701 and 44517002; and Memorandum from R. S. Jones to B. Benmhend, dated 12/23/98). CMP is a potential human health concern. In its 9th Report on Carcinogens (Revised January 2001) the Environmental Health Information Service (EHIS) indicated that although there was sufficient evidence for the carcinogenicity of CMP in experimental animals, there were no data available on the carcinogenicity of CMP in humans. The EHIS statement was based on chronic feeding studies (2-year) using rats and mice (see summary below).

In a chronic dietary toxicity study conducted by NTP, (NTP, TR-300, 1986; NTIS No. PB86-247293/AS), male and female rats and mice were dosed (by oral gavage) with CMP, five days/week for 103 weeks at 75-200 mg CMP/kg body weight. The study authors reported that there was "clear evidence of carcinogenicity" for CMP based on the increased incidences of squamous cell neoplasms in the forestomach of rats and mice. BPPD notes that the primary route of CMP exposure to humans (if present in the end-use product) is via inhalation; oral ingestion of CMP via use of the end-use product is a highly unlikely route of exposure to humans.

A more recent chronic inhalation study was conducted by Katagiri, et al. (2000, Industrial Health 38: 309-318). In this study, BDF1 mice were exposed (via inhalation) to atmospheric CMP concentrations of 0, 50, 100, or 200 ppm five days per week for 104

weeks. The authors stated that "dose-related increases in the incidences of gastric mucosal hyperplasia and squamous cell papilloma were observed in both sexes, and squamous cell carcinoma was observed in only one male mouse in the 100 ppm group. An increased incidence of Harderian gland adenoma in female mice was also recognized. In the nasal cavity, eosinophilic exudate associated with atrophy of olfactory epithelia, respiratory metaplasia of olfactory epithelia and olfactory gland, and eosinophilic changes in respiratory and olfactory epithelia were increased in both sexes." No mortalities were reported and NOECs were not calculated.

Although the information reported for the inhalation study is sobering, BPPD notes that chronic inhalation exposure to CMP, at levels used in the aforementioned study (50-200 ppm), is highly unlikely to occur to either pesticide applicators or the general public when the end-use product is used according to label directions in indoor facilities.

If present, residual CMP formed during the manufacturing process for EthylBloc™, may be encapsulated in the inert materials of the end-use product. The registrant was aware of this potential problem and took steps to minimize contamination of the product by CMP.

[redacted]

BPPD calculates that if the end-use product, EthylBloc™, releases no more than approximately 1000 ppb 1-MCP into the atmosphere upon mixing with water, CMP concentration (if present) will not exceed 0.71 ppb. Additionally, the end-use product is only applied in closed, sealed storage facilities where there is minimal exposure to applicators and no exposure to the public. When the treated air of the storage facility is vented outside the treatment chamber, all gases will be rapidly diluted by the external atmosphere.

6. Aggregate Exposure

Dietary Exposure: Potential exposure to 1-MCP would be via food consumption. Residues of 1-MCP in treated food commodities (if present) are not likely to exceed a theoretical maximum of approximately 0.4 ppb assuming a 100% saturation of all ethylene-binding sites by 1-MCP. Any residues present are expected to decline in storage following the conclusion of treatment with the end-use product, and after the food is removed from storage, prior to consumption. Cooking and/or processing are expected to lower the potential for residues to occur on treated food. Since 1-MCP is a gas released only in closed, sealed storage facilities, there is no potential for exposure via drinking water.

Non-dietary Exposure: The end-use product is intended only for use in closed, sealed treatment chambers that humans will be prohibited from entering after treatment has been initiated. Applicators will be required to wear PPE These chambers will be vented at the end of the treatment interval prior to entry by humans. Concentrations of 1-MCP in the treated area will not exceed approximately 1000 ppb and air vented to the outside atmosphere will be rapidly diluted. Therefore, non-dietary exposure is likely to be extremely low to non-existent.

7. Cumulative Exposure

1-MCP has a non-toxic mode of action in post-harvested fruits and vegetables. Furthermore, acute toxicity testing (see above) has demonstrated that 1-MCP has no acute toxicity effects in mammals. Therefore, consideration of the cumulative effects of 1-MCP and other substances via a common mechanism of toxicity is not appropriate.

8. Endocrine Effects

There are no data available to suggest that 1-MCP would have any effects on endocrine systems.

9. Ecotoxicity/Non-Target Organisms

No data were submitted for non-target organisms/ecological effects (Subdivision M Guidelines 154-6 to 154-11), but none are required for EthylBloc™ at this time. The product is intended for indoor non-food and food use in enclosed areas, but is not intended for use outdoors or in other non-enclosed areas. If the registrant intends to use this product (or other products containing MCP as the active ingredient) on food crops/commodities, outdoors and/or in other non-enclosed areas, or in enclosed areas where non-target insects and plants may be exposed, additional non-target organism/ecological effects studies may be required. Outdoor venting of enclosed facilities following treatment of non-food and food commodities with the product is not likely to affect non-target organisms since 1-MCP will be rapidly dissipated in the external atmosphere.

COMMENTS TO THE NOTICE OF FILING FOR A PETITION TO ESTABLISH AN EXEMPTION FROM THE REQUIREMENT OF A TOLERANCE FOR 1-METHYLCYCLOPROPENE IN OR ON FOOD (FEDERAL REGISTER 65, 120, DATED 6/21/2000)

Valent BioSciences Corporation (formerly Abbott BioSciences) submitted comments (see letter from M. Tichon to EPA/PIRIB, dated 7/20/2000) regarding the tolerance exemption petition. In a letter from S. L. Longacre to D. Benmhend (dated 12/19/2000), AgroFresh, Inc. responded to these comments. The Valent BioSciences comments, the AgroFresh, Inc. responses, and BPPD comments are summarized below.

I. TOXICITY

A. Valent Comments: Valent (citing Daly et al., 2000 U. S. Patent 6,017,849, column 1, line 39) states that 1-MCP and related compounds have been characterized as "reactive gases and therefore highly unstable because of oxidation and other potential reactions." The commenter further states that 1-MCP irreversibly binds to ethylene receptors (citing Sisler et al., 1996) and that 1-MCP can undergo unintended reactions of "toxicological significance" in organisms other than plants due to the molecule being comprised of a "highly-strained, three-member ring."

B. AgroFresh Response: In response, AgroFresh states that 1-MCP gas is "flammable and unstable when concentrated" and has an explosive limit of 10, 000 ppm. The registrant further explains that 1-MCP is stabilized in alpha-cyclodextrin in the manufacturing process and this stabilization permits safe handling. The product is only used in closed storage facilities and 1-MCP concentrations will not exceed approximately 1 ppm (1000 ppb) during treatment. Although 1-MCP has a higher binding affinity to the ethylene receptors in plant tissue than ethylene (the basis for its action) the binding is non-covalent (i. e. it is reversible) as demonstrated by 1-MCP treated crops eventually regain sensitivity to ethylene.

C. BPPD Comment: Valent presented no quantitative data to support the statement that 1-MCP can undergo unintended reactions of "toxicological significance" in organisms other than plants due to the molecule being comprised of a "highly-strained, three-member ring." Furthermore, acute toxicity studies submitted in support of the registration of EthylBloc™ (containing 0.14% 1-MCP) demonstrated a lack of acute toxicity to the formulated product. Sisler and Serek (1999, Bot. Bull. Acad. Sin. 40: 1-7; and 1997, Physiologia Plantarum 100: 577-582) present data demonstrating that 1-MCP binding to ethylene receptors is reversible and non-permanent. Bananas, peas, and carnations regained sensitivity to ethylene (indicating loss of 1-MCP from the receptor) 7-12 days following treatment with 1-MCP. BPPD concurs with AgroFresh's response.

1. Potentiation of Other Compounds, Including Drugs

A. Valent Comments: The commenter proposes that 1-MCP may result in adverse drug reactions due to its close structural similarity to cyclopropane gas (used as an anesthetic). Citing

Morrow (1996, Anesthesia and Analgesia, 46:675-681; but no complete reference was provided for review) the commenter states that cyclopropane gas increased the toxicity of digitalis.

B. AgroFresh Response: AgroFresh states that anesthetics are commonly administered at concentrations >10, 000 ppm, whereas 1-MCP will only be present in closed treatment chambers at approximately 1 ppm. AgroFresh further states that "there are no plans to register and market 1-MCP as a pharmaceutical agent, the point of the comment is unclear/not relevant."

C. BPPD Comment: It is not evident how the anesthetic use of cyclopropane gas (at high concentration) and its potential to increase the toxicity of the pharmaceutical drug digitalis can be extrapolated to infer similar effects by exposure to 1-MCP. At its maximum intended use rate (1 ppm), atmospheric concentrations of 1-MCP will be several orders of magnitude lower than cyclopropane gas when it is used as an anesthetic. Furthermore, human exposure to atmospheric 1-MCP will be limited by its use in closed treatment chambers and applicators who will be wearing PPE. BPPD concurs with AgroFresh's response.

2. Hepatic Toxicity

A. Valent Comments: Valent suggests that exposure to 1-MCP may result in hepatic toxicity due to its structural similarity to 1-cyclopropanecarboxylic acid (CPCA). CPCA has been demonstrated to inhibit mitochondrial function that include fatty acid oxidation, gluconeogenesis and pyruvate metabolism (literature review in Ulrich et al., 1998; Toxicology 131: 33-47). CPCA is a mammalian metabolite formed from "panadiplon", a clinical drug whose development was cancelled due to hepatocellular toxicity in human volunteers (Ulrich et al., 1998; Toxicology 131: 33-47).

B. AgroFresh Response: A review of the Ulrich et al. (1998) article by AgroFresh concluded that metabolic inhibition of hepatic cell cultures occurred with panadiplon levels of 30-100 μM (equivalent to 335 g/mole) and could be achieved in humans only by ingesting an oral dose equivalent to 10-30 mg panadiplon/kg. The registrant further stated that rats administered oral doses of 5000 mg EthylBloc™ (equivalent to 7 mg 1-MCP/kg) demonstrated no toxic effects and that the levels of panadiplon used to show hepatic toxicity were far greater than any conceivable 1-MCP exposure.

C. BPPD Comments: The conclusions by Ulrich et al. (1998) were that "inhibition of mitochondrial activity in human hepatocytes by panadiplon suggests that inhibition of β-oxidation may have occurred in human patients", and suggested that the metabolite CPCA may have been responsible. The data were equivocal and the authors did not (except for inference) state that CPCA was directly responsible for the adverse effects observed in human volunteers that had been administered panadiplon (only that a panadiplon metabolite may be responsible). The data reported in the Ulrich et al. (1998) article were generated using isolated liver (or liver component) cell cultures, rather than from whole tissues from animals or humans that had been orally dosed with panadiplon or its metabolite CPCA. Thus it is difficult to directly extrapolate

these data to humans. Furthermore, Ulrich et al. (1998) also state that no cell deaths occurred in any of the experiments and that the effects of panadiplon and CPCA "may have no consequence" in a healthy individual. No unequivocal evidence has been presented to demonstrate that 1-MCP will adversely affect liver function due to its structural similarities to CPCA. Additionally, BPPD concurs with the registrant's conclusion that any conceivable exposure of 1-MCP to humans will be orders of magnitude lower than the concentrations of CPCA described in the Ulrich et al. (1998) article.

3. Carcinogenicity

A. Valent Comments: The commenter cites a study with Rainbow trout (Eisle et al., 1978; J. Environ. Path. Toxicol. 1:773-778) demonstrating that fatty acids containing a cyclopropene functional group (CFPA) have been shown to lower cytochrome P_{450} levels in these fish when fed 300 ppm cyclopropene (in the form of *Sterculia foetida* oil based in sterculic acid content) for 74 days. Citing Stryer (1981; Biochemistry, 2nd Edition, p. 475), the commenter further stated that cytochrome is involved in "metabolic processes associated with the activation of chemicals to their carcinogenic forms." The Eisle et al. (1978) study was also cited as reporting that CFPA fatty acids induced the enzyme that metabolizes benzo[a]pyrene in trout. Valent then cites Streitwesser and Heathcock (1981, Introduction to Organic Chemistry, 2nd Ed., pp. 1054-1055) to show that the metabolized form of benzo[a]pyrene is a carcinogen. Finally, Valent cites Sinnhuber et al. (1976; Fed Proc. 35: 505, Abstract #1662) to show that sterculic acid (a naturally-occurring CFPA) was reported to be a liver carcinogen. With the information obtained from the aforementioned references, Valent concluded that the cyclopropene moiety of 1-MCP could also act as a co-carcinogen.

B. AgroFresh Response: Agrofresh responded by stating the cytochrome P_{450} is " a group of isozymes that metabolizes endogenous fatty acids and steroids, in addition to metabolizing many xenobiotics in an effort to make them more soluble for conjugation and excretion." The registrant further states that cytochrome activity can be increased via exposure to high concentrations of fatty acids, but not specifically to the CFPA moiety. Citing Ames (1991; The Science of the Total Environment 104: 159-166), the registrant also states that many naturally-occurring chemicals (such as sterculic acid, a constituent of the Indian almond) may be carcinogenic in animals, but only at levels that far exceed "real world" exposures. The registrant also states that there is broad exposure to cyclopropanes and cyclopropenes present in many food commodities.

NOTE: The registrant concludes by stating that a battery of mutagenicity studies using EthylBloc™ (containing 0.14% 1-MCP) showed no mutagenic effects. However (as discussed above in the Risk Assessment, 5. Toxicity Profile), these studies may not have adequately assessed the mutagenic potential of 1-MCP because the test substances were prepared in water prior to the initiation of the studies, causing an outgassing of 1-MCP.

C. BPPD Comments: The Eisele et al. (1978) article specifically assessed the effects of cyclopropene fatty acids (CFPAs) on the activity of cytochrome P_{450} and other mixed function oxidases (MFOs) obtained from the liver of trout fed a CFPA at 300 ppm for up to 74 days. The study did not address any gross toxic or pathological effects (i. e. mortality, tumors, other carcinogenic, or mutagenic effects) in the fish at the end of the study. Therefore, no conclusions regarding mortality, carcinogenicity, or mutagenicity of cyclopropenes can be made based on the study. A literature review in the Discussion and Conclusions section of the article proposed that altered MFO function resulting from dietary exposure to CFPA might be the mechanism whereby cyclopropenes act as co-carcinogens. No data were presented to support this hypothesis. Furthermore, MFO activity in trout liver was altered by fatty acids containing a cyclopropene moiety, not by cyclopropenes alone.

This paper, and the other supporting documentation, do not support the hypothesis that 1-MCP is a potential carcinogen because it is a cyclopropene. Three-carbon, cyclic carbon compounds are widespread in nature (such as 1-aminocyclopropane carboxylic acid, the precursor to ethylene synthesis in plants) and there is broad human exposure to these compounds. BPPD concurs with the AgroFresh response.

4. Other Biological Effects

A. Valent Comment: Citing Dulayymi et al. (1996; Tetrahedron 52: 12509-12020) the commenter states that "insect pheromones in which the olefin group is replaced by a cyclopropene have been shown to cause long term disruption of insect mating behavior." The commenter further contends that this is evidence showing that molecules containing cyclopropene moieties can interfere with the normal functioning of biological functions.

B. AgroFresh Response: AgroFresh states that cyclopropene-containing molecules are "ubiquitous in nature and that aminocyclopropane carboxylic acid, the naturally-occurring precursor to the hormone ethylene is widespread in many fruits and vegetables that are consumed by humans and wildlife."

C. BPPD Comments: Insect pheromones (naturally- produced and their synthetic analogues) are complex compounds that affect insect behavior. Simple, cyclic, 3-C compounds have not been demonstrated to have pheromone-like affects. Although Valent did not provide a copy of the Dulayyami et al. (1996) article for review, and the information they report from the article may be correct, it is not clear how 1-MCP would replace olefin groups on pheromones under indoor or outdoor conditions. Based on the information presented, and BPPD's long experience with pheromone active ingredients, it is concluded that use of 1-MCP will not cause any pheromone-like effects nor act to disrupt insect mating behavior. Furthermore, 1-MCP will only be used in enclosed, indoor treatment facilities.

II. EXPOSURE

1a. Valent Comment: The commenter states that the mammalian and genotoxicity studies were conducted with the end-use product EthylBloc™ (containing 0.14% active ingredient) resulting in limit doses that did not exceed 5000 mg a.i./kg). They further state that the levels tested were not sufficient to assess the acute toxicity of 1-MCP. "Extreme pH or heat is used to facilitate the release of 1-MCP from the formulation. Therefore the actual dose of 1-MCP delivered to the test organism is unclear."

2a. Agrofresh Response: Agrofresh concurs with Valent that the acute doses did not exceed 7 mg a.i./kg, but disagreed the comment describing 1-MCP as "encapsulated" in alpha-cyclodextrin, preferring the terms "entrapment or caging" that minimizes exposure to 1-MCP, which would "otherwise have to be commercialized as a stabilized gas ... with increased hazards." Agrofresh also states that release of 1-MCP from alpha-cyclodextrin does not require extreme pH or heat.

3a. BPPD Comment: The acute inhalation toxicity test was conducted with 1-MCP at 165 ppm as measured by the registrant (see MRID 44464706); the test solution was heated to 40°C to facilitate release of 1-MCP gas.

1b. Valent Comment: Valent contends that "a very large margin of safety would be required" if an RfD were calculated for 1-MCP, and that a margin of safety (MOS) of 10,000 to 100,000 would be appropriate based on the rat acute oral toxicity study data. They further state that, given the reactivity of 1-MCP, it may bind to sites in plants other than the ethylene receptor and be metabolized. The commenter proposed that this uncertainty would require residue and metabolism studies.

2b. Agrofresh Response: "No effects were observed in the acute oral study ... which represents a theoretical maximum of 7 mg a.i./kg for the 0.14% formulation tested." The registrant goes on to reiterate previously submitted calculations showing that if 100% of all ethylene receptors are saturated with 1-MCP, the theoretical maximum residue concentration (in apple pulp) would be 0.004 ppb. Another calculation is provided, based on a worst-case scenario in which a 70 kg human consumes his entire daily diet (estimated at 15000 g/day) and that all food contains 0.004 ppb 1-MCP. Given these parameters, it was calculated that daily dietary exposure to 1-MCP is equivalent to 8.6×10^{-8} mg a.i./kg. Assuming that the 1-MCP NOEL is 7 mg/kg, the calculated MOS is >81,000,000.

The registrant further calculates another worst-case scenario wherein all 1-MCP in a treatment chamber is bound to the treated food commodity. This would increase the 1-MCP concentration to 9 ppb. Using this value, the presumed 7 mg/kg NOEL, and a 1500 g daily diet, the registrant calculated that daily dietary exposure would be equivalent to 1.9×10^{-4} mg 1-MCP/kg and the MOS is >36,000.

3b. BPPD Comment: BPPD concurs with the AgroFresh response and calculations demonstrating that dietary exposure, even under the unlikely worst-case scenarios, would be extremely low. Additionally, there are no data available to suggest that 1-MCP will bind to anything other than ethylene receptors in plants (see articles by Sisler et al.). It is not known if 1-MCP is metabolized in plant tissue and, if so, the nature of these supposed metabolites. However, to alleviate concerns regarding 1-MCP residues on treated food, BPPD is requiring the registrant to develop radioisotope techniques to determine whether any 1-MCP (and/or metabolites) remain on treated food after treatment.

1c. Valent Comment: Valent states that workplace exposure could be significant and that "storage, rooms, coolers, shipping containers, or trailers where fruits and vegetables will be gasses with 1-MCP may lack proper containment facilities" and that "females of childbearing age will be exposed in the workplace."

2c. AgroFresh Response: AgroFresh states that treatment areas must be enclosed and sealed tightly "or 1-MCP will leak out and will not be efficacious." The registrant further explains that the label restricts workers from being in treatment rooms during 1-MCP exposure and that controlled-atmospheric storage rooms have most of the oxygen removed (precluding human occupancy). Additionally "the very low use rates" and " label requirements of non-entry" into the treated storage rooms while treatment is occurring makes worker exposure practically non-existent.

The registrant goes on to calculate a worst-case scenario wherein a worker makes five-minute entries into a treatment room once/hour over an 8-hour day. Using data from the acute rat inhalation study (MRID 44464706; 165 ppm 1-MCP for 4 hours), and assuming a 250 g rat, 0.2 L rat volume, Universal gas constant of 24.45, a 70 kg human, 20.8 L human volume, and a 1-MCP molecular weight of 53 g/mole, the registrant calculated a NOEL of 69 mg/kg. At a maximum concentration of 1 ppm (1000 ppb) 1-MCP in the treatment room, an MOS equivalent to 2650 was calculated. If it was assumed that the worker was exposed to 1-MCP in the treatment room for the entire 8-hour day (also assuming sufficient oxygen was present an in violation of label restrictions), the registrant calculated a MOS equivalent to 233.

The aforementioned calculations (provided in detail by the registrant) demonstrate that even in the worst, worst-case scenario (a worker breathing 1-MCP at 1 ppm for 8 hours) there is an MOS >100. Short-term worker exposure (including to females of child-bearing age) will have an extremely large MOS.

3c. BPPD Comments: BPPD concurs with AgroFresh's response and theoretical calculations. When the product is used according to label directions, exposure to 1-MCP will be far less than the exposures for which the registrant presented the aforementioned calculations.

1d. Valent Comments: The commenter states the FQPA requires that "a reasonable certainty of no harm for a pesticide chemical residue be established before a tolerance is granted." Valent then disagrees with the FR notice, stating that the data "fail to demonstrate any level of confidence that exposure to 1-MCP residues will not cause harm." The commenter then goes on to reiterate a summary of the points made previously, indicating that: (i) literature indicates potential adverse effects and that (ii) true dietary toxicity effects have not been tested for the a.i. and residue analysis has not been conducted.

2d. AgroFresh Response: (i) "There are no literature references of potential adverse effects resulting from exposure to 1-MCP;" (ii) The registrant reiterates a summary of its responses to Valent comments showing its risk assessments demonstrate extremely large margins of safety for consumers and workers.

3d. BPPD Comments: BPPD concurs with the AgroFresh response. Although true limit doses (up to 5000 mg a.i./kg) were not used for acute oral toxicity testing, AgroFresh's calculations demonstrate that the dose used in the acute oral toxicity study (7 mg a.i./kg) was orders of magnitude higher than any worst-case scenario calculations for residues of 1-MCP on treated food. However, to alleviate concerns regarding 1-MCP residues on treated food, BPPD is requiring the registrant to develop radioisotope techniques to determine whether any 1-MCP (and/or metabolites) remain on treated food after treatment. The registrant is also being required to re-conduct the three study battery of genotoxicity/mutagenicity studies described in Subdivision M 152-19.

BPPD reiterates that, when EthylBloc™ (containing 0.14% 1-MCP as its active ingredient) is used according to label directions, exposure to humans and wildlife (by oral, dermal, inhalation, or eye pathways) is extremely low to non-existent.

At this time, BPPD concludes that the registrant has submitted sufficient data/evidence and scientific rationale to show that there is a reasonable certainty of no harm with the use of EthylBloc™ (containing 0.14% 1-MCP as its active ingredient) on food commodities stored in closed, sealed treatment facilities and applied according to label directions.

cc: F. Toghrol, R. S. Jones, D. Benmhend, BPPD Subject File
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R156545

Chemical: Cyclopropene,1-methyl-

PC Code: 224459
HED File Code: 41500 BPPD Tox/Chem
Memo Date: 2/21/2001
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