GRAS Notice (GRN) No. 1155 with amendments
https://www.fda.gov/food/generally-recognized-safe-gras/gras-notice-inventory

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August 25, 2023

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Dr.
College Park, MD 20740

Attention: Dr. Susan Carlson
Re: GRAS Notification of 1-Methylcyclopropene 1-MCP

Dear Dr. Carlson:

GRAS Associates, LLC, acting as the Agent for Fresh Inset S.A. (Poland), is submitting for FDA review Form 3667 and the enclosed CD, free of viruses, containing a GRAS Notification for 1-MCP. Along with Fresh Inset S.A.'s determination of safety, an Expert Panel of qualified persons was assembled to assess the composite safety information of the subject substance with the intended use in sliced melon, pineapple, apple, mushroom and shredded cabbage/coleslaw. The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document.

If additional information or clarification is needed as you and your colleagues proceed with the review, please feel free to contact me via telephone or email. I also authorize Amy Mozingo (amozingo@gras-associates.com), VP US Nutra Regulatory Sciences, GRAS Associates LLC to lead communications related to this submission.

We look forward to your feedback.

Sincerely,

William J. Rowe

President
Agent for Fresh Inset S.A.
GRAS Associates, LLC
1810 Grand Park Ave, Suite 500
North Bethesda, MD 20852
wrowe@nutrasource.ca
Enclosure: GRAS Notification for Fresh Inset S.A. – 1-MCP

GRAS ASSOCIATES

Food Safety Regulatory Services,
A Nutrasource Company

nutrasource
Pharmaceutical and Nutraceutical Services

GRAS Conclusion

of

1-Methylcyclopropene (1-MCP)

Food Usage Conditions for General Recognition of Safety

on behalf of

Fresh Inset S.A.

Wilenska 4/A017, 87-100 Torun, Poland

8/23/23

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FOREWORD

Fresh Inset S.A. (Fresh Inset) based our Generally Recognized as Safe (GRAS) assessment of 1-Methylcyclopropene (1-MCP) for the intended use on scientific procedures. The safety/toxicity of 1-MCP, history of use of 1-MCP and compositional details, specifications, and method of preparation of the subject ingredient were reviewed. In addition, a search of the scientific and regulatory literature was conducted through July 10, 2023[1], with particular attention paid to adverse reports, as well as those that supported conclusions of safety. Those references that were deemed pertinent to this review are listed in Part 7. The composite safety/toxicity studies, in concert with dietary exposure information, ultimately provide the specific scientific foundation for the GRAS conclusion.

At Fresh Inset's request, GRAS Associates, LLC ("GA") convened an Expert Panel to complete an independent safety evaluation of 1-MCP for the intended use. 1-MCP is used at two different concentrations in the Vidre+ technology. Vidre+ contains 1000 g 1-Methylcyclopropene (1-MCP) Technical (3.3% 1-MCP + 96.7 % α-cyclodextrin) and 1000 g Polyvinylpyrrolidone (PVP), a 50:50 (w/w) mix. Vidre+ Low Concentration contains 100 g 1-MCP Technical, 1000 g PVP and an additional 900 g α-cyclodextrin). These formulations can be considered as dry composite mixtures. Isopropyl alcohol is mixed with either Vidre+ formulation to prepare a homogeneous mixture that is applied to the surface of a food grade paper or polypropylene sticker. Different printing techniques may be used to apply the mixture, such as flexography, flood coating systems, or screen printing. During the printing process a thin layer of the Vidre+ or Vidre+ low concentration formulation is applied on the sticker surface. Stickers of various sizes will be prepared for subsequent placement inside containers of processed fresh produce. The mixture is applied to a 1 cm^2 sticker/liter volume of package to provide a total of 1 ppm of 1-MCP gas, which will be released into the closed container over 25-50 hours, depending on storage temperature and humidity. The purpose of the evaluation is to ascertain whether 1-MCP is generally recognized as safe, i.e., GRAS, under the intended conditions of use. In addition, Fresh Inset has asked GA to act as Agent for the submission of the GRAS notification.

[1] Two PubMed searches were conducted for safety and adverse event data. Search strings were as follows: 1) (1-Methylcyclopropene or "3100-04-7") and (rat or rats or dog or dogs or mice or mouse or human or humans) and 2) (1-Methylcyclopropene OR "3100-04-7") and (mutagen or mutation or chromosome or Ames). Regulatory information was obtained by searching websites affiliated with governmental agencies for the term 1-Methylcyclopropene.

PART 1. SIGNED STATEMENTS AND CERTIFICATION

Fresh Inset S.A. has concluded that 1-Methylcyclopropene, referred to as 1-MCP, and which meets the specifications described below, is GRAS in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic Act (FD&C Act). This determination was made in concert with an appropriately convened panel of experts who are qualified by scientific training and experience. The GRAS determination is based on scientific procedures as described in the following sections. The evaluation accurately reflects the intended conditions of food use for 1-MCP.

(a) This certification is signed by a responsible official of GRAS Associates, LLC.
(b) This GRAS dossier did not rely on any confidential information pivotal to establishing safety.

(c) (1) This Independent GRAS Assessment was conducted in accordance with Subpart E of 21 CFR Part 170.

(c) (2) Names and addresses of organizations;

Sponsoring Party:
Fresh Inset S.A.
Wilenska 4/A017, 87-100 Torun, Poland

As the Responsible Party, Fresh Inset S.A. accepts responsibility for the GRAS conclusion that has been made for 1-MCP as described in the subject safety evaluation.

Agent:
GRAS Associates, LLC
11810 Grand Park Avenue
Suite 500
North Bethesda, MD 20852

(c)(3) The name of the ingredient is 1-Methylcyclopropene (1-MCP).

(c)(4) The ingredient will be used as a component of Vidre+ or Vidre+ low concentration. Either of these formulations would be placed on the surface of a food grade sticker, which is placed inside of cut produce (sliced melon, pineapple, apple, mushroom and shredded cabbage/coleslaw) containers to extend shelf life of the product. Under typical storage conditions, 1-MCP will be released from the sticker as a gas into the air surrounding the packaged produce. The cut produce will be exposed to an estimated maximum of 4.125-9.086 µg 1-MCP per kg of produce.

(c) (5) The statutory basis for our conclusion of GRAS status is through scientific procedures in accordance with § 170.30(a) and (b).

(c) (6) It is our view that the ingredient is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on our conclusion that the notified substance is GRAS under the conditions of its intended use.

(c) (7) If FDA were to ask to see the data and information that are the basis for our conclusion of GRAS status, either during or after FDA evaluation of this notice, we agree to:

(i) make the data and information available to FDA; and
(ii) agree to both of the following procedures for making the data and information available to FDA.

(A) Upon FDA's request, we will allow FDA to review and copy the data and information during customary business hours at our address specified where these data and information will be available; and

(B) Upon request by FDA, we will provide FDA with a complete copy of the data and information either in an electronic format that is accessible for their evaluation or on paper.

(c) (8) None of the data and information in Parts 2 through 7 of this GRAS notice are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552 (e.g., as trade secret or as commercial or financial information that is privileged or confidential).

(c) (9) We certify that, to the best of our knowledge, this GRAS Assessment is a complete, representative, and balanced review that includes favorable information, as well as unfavorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of the substance.

(c) (10) Fresh Inset does not intend to add 1-MCP to any meat and/or poultry products that come under FSIS/USDA jurisdiction. Therefore, 21 CFR 170.270 does not apply.

(c) (11) Signature

Signature

Agent for Fresh Inset S.A.

William J. Rowe
President
GRAS Associates, LLC
11810 Grand Park Ave
Suite 500
North Bethesda, MD 20852

Date: August 23, 2023

PART 2. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT

A. Chemical Identity of Ingredient

1-Methylcyclopropene (1-MCP) is the common or usual name of the ingredient. 1-MCP is commonly used in the agriculture industry to extend the post-harvest life of fruits, vegetables, and flowers. Information about mode of action is provided in Part 2.A and use is provided in Part 6.A.

The structure of 1-MCP is shown in Figure 1.

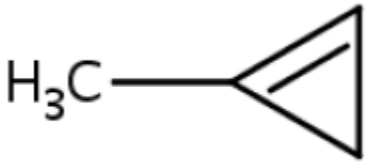

Figure 1. Structure of 1-Methylcyclopropene (1-MCP)[a]
[a] Health Canada (2017a)

Additional information about 1-MCP is as follows (FAO, 2010; Health Canada, 2017a). The material is a colorless gas at room temperature that volatilizes from moist surfaces and water.

Common or Usual Name:	1-Methylcyclopropene
Chemical Name:	Cyclopropene, 1-methyl-
Synonyms:	1-methyl-cyclopropene, methylcyclopropene
CAS Number:	3100-04-7
Molecular Formula:	C_4H_6
Molecular Weight (MW):	54.09 g/mole
Physical Form:	Colorless gas
Solubility in water at 20º C:	137 mg/L
Henry's Law Constant at 20º C:	4.38×10^9 Pa

1-MCP is chemically unstable and is seldom transported as a pure gas. Instead, it is complexed with α-cyclodextrin to improve stability, or it is generated *in situ* using preliminary prepared chemical components in a specialized gas generation device (EFSA, 2018). For the use described in this dossier, it is complex with α-cyclodextrin. This form is referred to as 1-MCP Technical in this document.

B. Manufacturing Processes

1-MCP is manufactured in the following manner. First, toluene is mixed together with sodium amide at a 15:1 ratio under an inert gas in a reactor equipped with a condenser and airtight stirrer. After bringing the mixture to reflux, 3-chloro-2-methylpropene (3-CMP) is added dropwise until all the

starting material is consumed. The escaping gases are passed through sulfuric acid whereupon 1-MCP is collected by condensation in a condenser cooled to -78°C.

1- MCP Technical (3.3% (33 g) 1-MCP + 96.7 % (967 g) α-cyclodextrin) is prepared as follows. 1-MCP is heated to its boiling point (48 to 54 ° F) and is stirred into a 20 L reactor containing α-cyclodextrin dissolved in a small amount of water. A precipitate is formed consisting of α -cyclodextrin saturated with 1-MCP to its limit capacity of about 5% w/w. The product is filtered off, washed and diluted with fresh α– cyclodextrin to adjust the content of 1-MCP to 3.3% (w/w).

1-MCP is used at two different concentrations in the Vidre+ technology. Vidre+ contains 1000 g 1-MCP Technical (3.3% 1-MCP + 96.7 % α-cyclodextrin) and 1000 g polyvinylpyrrolidone (PVP), in a 50:50 (w/w) mix. Vidre+ Low Concentration contains 100 g 1-MCP Technical, 1000 g PVP and an additional 900 g α-cyclodextrin. The concentrations of 1-MCP in Vidre+ and Vidre+ Low Concentration are therefore 1.65% and 0.165%, respectively.

The α-cyclodextrin has been notified as GRAS to FDA (FDA, 2004) and the PVP meets Food Chemicals Codex (FCC) specifications. PVP is approved as a secondary direct food additive per 21 CFR 173.55. 1-MCP is manufactured under controls to have more stringent specifications for contaminants than FAO specifications (see Table 1 in Part 2.C).

PVP (and additional α-cyclodextrin in case of Vidre+ low concentration) are mixed in a food grade plastic container with a 12 L volume using a mechanical stirrer (120 rpm; 30 minutes). These preparations are considered dry composite mixtures.

To prepare wet blended composite mixtures with appropriate viscosity for printing, 2680 mL (2093 g) of FCC grade isopropanol is added to 2 kg of each dry composite mixture. The mixture is stirred with a mechanical stirrer (240 rpm); (due to the viscosity, an adhesive mixing tip is recommended). The dry mix is added gradually, in portions, under stirring with a mechanical stirrer (240 rpm). When the isopropanol has absorbed the added portion of dry mixture, another portion of dry mixture is added. The procedure is repeated until all dry mixture is added. After all of the dry mixture has been added, the container is covered with a plastic lid with a center drilled hole. A mixing tip is added through the hole and all ingredients are mixed together for 30 minutes. A homogenous mixture without lumps should be obtained. After mixing, the container is closed with a plastic lid without a center drilled hole and set aside until all air bubbles disappear.

After extended storage in one position, the mixture may separate. The mixture is stirred gently using a mechanical stirrer (120 rpm) until a homogenous consistency is obtained before use. When mixing, over-aeration should be avoided. The composite mixture is ready for use after stirring. The mixture is printed onto food grade stickers using an appropriate printing technique, such as flexography, flood coating systems, or screen printing.

Nevertheless, in each type of printing machine used, the wet composite mixture is applied onto the outside surface of a food grade, paper or polypropylene sticker to create a thin layer of the composite mixture that adheres to the sticker substrate. Samples from the printed material are taken and then

analyzed for 1-MCP content according to the analytical method (see Appendix 1). If the product does not meet the quality control standard, it is not utilized.

The Vidre+ mixture is applied to the sticker at a rate of 1 g of 1-MCP Technical[2] per square meter of paper, which corresponds to 33 mg 1-MCP/m^2. A sticker size of 1 cm^2 would be used for a 1 liter package to provide 1 µL 1-MCP gas, or 1 ppm. A 1 cm^2 sticker would contain 1/10,000th of the amount of 1-MCP coated on a square meter surface, or 3.3 µg.[3] Instructions for use with the sticker will give specific guidelines to use one sticker of the proper size for each package. Stickers will be placed/adhered to the inside of lids of clamshell containers or inside plastic produce bags.

Vidre+ Low Concentration will be applied at the same rate as Vidre+, which corresponds to 0.33 µg 1-MCP/cm^2 sticker, because the concentration of 1-MCP in Vidre+ Low Concentration is 1/10th of Vidre+. Vidre+ Low Concentration will be used for small packages < 2 L in size due to the 1/10th concentration allowing the use of a bigger sticker.

C. Product Specifications

1. Specifications for Fresh Inset's 1-MCP Compared to FAO Specifications

The Food and Agriculture Organization of the United Nations (FAO) has developed specifications for 1-MCP "Technical Concentrate" (FAO, 2010). The specifications include a description, identity test, tolerance for concentration, and limits for relevant impurities, as well as methods of analysis for each parameter. The specifications are shown in Table 1.

Table 1. Specifications for Fresh Inset's 1-MCP Compared to FAO Specifications

Physical and Chemical Parameters	FAO 1-MCP Specifications		Fresh Inset 1-MCP Technical Specifications	
	Specification	Method	Specification	Method
Description	Homogeneous powder mixture of 1-MCP at a concentration of 3.3% together with related manufacturing impurities, in the form of a complex with alpha-cyclodextrin, together with any other necessary co-formulants. It shall be in the form of a powder free from visible extraneous matter and added modifying agents except for the diluents.	None mentioned	Powder mixture of 1-MCP at a concentration of 3.3% together with related manufacturing impurities, in the form of a complex with alpha-cyclodextrin	None mentioned

[2] Since the composite mix is 50/50 mix of "1-MCP Technical" and PVP, and the composite (50:50) mix is mixed with isopropanol and then coated on the paper, the paper is actually coated with 2 grams of the 50/50 mix (1 gram of 1-MCP Technical and 1 gram of PVP) per square meter.
[3] 1.65% = 16,500 µg/g x 2 g/m^2 = 33,000 µg/m^2 = 33,000 µg/10,000 cm^2 = 3.3 µg/cm^2

Physical and Chemical Parameters	FAO 1-MCP Specifications		Fresh Inset 1-MCP Technical Specifications	
	Specification	Method	Specification	Method
Identification	The active ingredient shall comply with an identity test and, where the identity remains in doubt, shall comply with at least one additional test.	Note 1	Retention time matches that of 1-MCP in GC/MS	Note 1
1-MCP Content	Up to 33 g/kg ± 10% of the declared content (equivalent to up to 3.3 ± 0.3 %)	Note 1	3.330 ± 0.0155 %	Note 1
3-CMP	≤ 0.05% of the 1-MCP content	Note 2	≤ 0.02% of the 1-MCP content	Note 2
1-CMP	≤ 0.05% of the 1-MCP content	Note 2	≤ 0.02% of the 1-MCP content	Note 2
Methylidine Cyclopropane (X-O isomer)*	None	None	≤ 1.96% of the 1-MCP content	Note 1

g – Grams; GC/MS – Gas chromatography/mass spectroscopy; kg – Kilograms; 1-MCP – 1- Methylcyclopropene; 1-CMP – 1-Chloro-2-methylpropene; 3-CMP – 3-Chloro-2-methylpropene
* IUPAC name for methylene cyclopropane
Note 1: Methods for the identification and determination of 1-MCP content were presented at the CIPAC Meeting in 2009 and provisionally adopted as CIPAC methods. See Appendix 1 for method
Note 2: The independent laboratory validated capillary GC-FID method (CIPAC/4667) for the determination of the relevant impurities 3-chloro-2-methylpropene and 1-chloro-2- methylpropene in 1-MCP was adopted by CIPAC in 2009. See Appendix 1 for method.

2. Demonstration of Batch Compliance with Specifications

The compositions of five non-consecutive lots of Fresh Inset's 1-MCP are compared with the specifications in Table 2. Specification/product data sheets for the 1-MCP are found in Appendix 2.

Table 2. Lot Conformance With Specifications for 1-MCP

Physical & Chemical Parameters	Fresh Inset S.A.'s Specifications for 1-MCP	1-MCP Representative Lots				
		Batch #01/07052019	Batch #02/24052019	Batch #03/1006019	Batch #04/2507019	Batch #05/1208019
1-MCP Content	3.330 ± 0.0155 %	3.331	3.307	3.338	3.350	3.326
3-CMP	≤ 0.02% of the 1-MCP content	0.00427	0.00526	0.00479	0.00587	0.00600
1-CMP	≤ 0.02% of the 1-MCP content	0.00138	0.00199	0.00121	0.00127	0.00124
Methylidine Cyclopropane (X-O isomer)*	≤ 1.96% of the 1-MCP content	0.0466	0.0457	0.0467	0.0468	0.0466

1-MCP – 1- Methylcyclopropene; 1-CMP – 1-Chloro-2-methylpropene; 3-CMP – 3-Chloro-2-methylpropene
* IUPAC name for methylene cyclopropane

D. Physical or Technical Effect

1-MCP irreversibly binds to the ethylene receptors present on the membranes of the cells of parts of plants, blocking their ability to bind to the ethylene molecules. The binding of 1-MCP to the ethylene receptors makes them insensitive to ethylene (no binding available) and also affects the synthesis of new ethylene (no positive feedback from the receptor-ethylene complex for the synthesis of additional ethylene) (Health Canada, 2017a). Consequently, effects related to ethylene such as fruit softening and development of superficial scald disorder are inhibited (Health Canada, 2004).

Fresh Inset S. A. intends to market its 1-MCP containing Vidre+ stickers for use as an ethylene inhibitor for cut produce. The stickers will be placed/adhered to the insides of packages that will be directly handled by consumers. They will be placed on the inside lids of clamshell packaging or inside plastic bags. Under typical storage conditions, the active ingredient, 1-MCP, would be released from the Vidre+ sticker as a gas into the air surrounding the packaged produce. The intent of the Vidre+ products will be to mitigate the action and formation of ethylene in a controlled fashion, which should extend shelf life of the cut produce. The humidity required for releasing the 1-MCP gas from the stickers (85%) would be reached in the containers[4] and the 1-MCP gas would treat processed produce over a maximum 50 h release period after application of the stickers to packaging as shown in Figure 2.

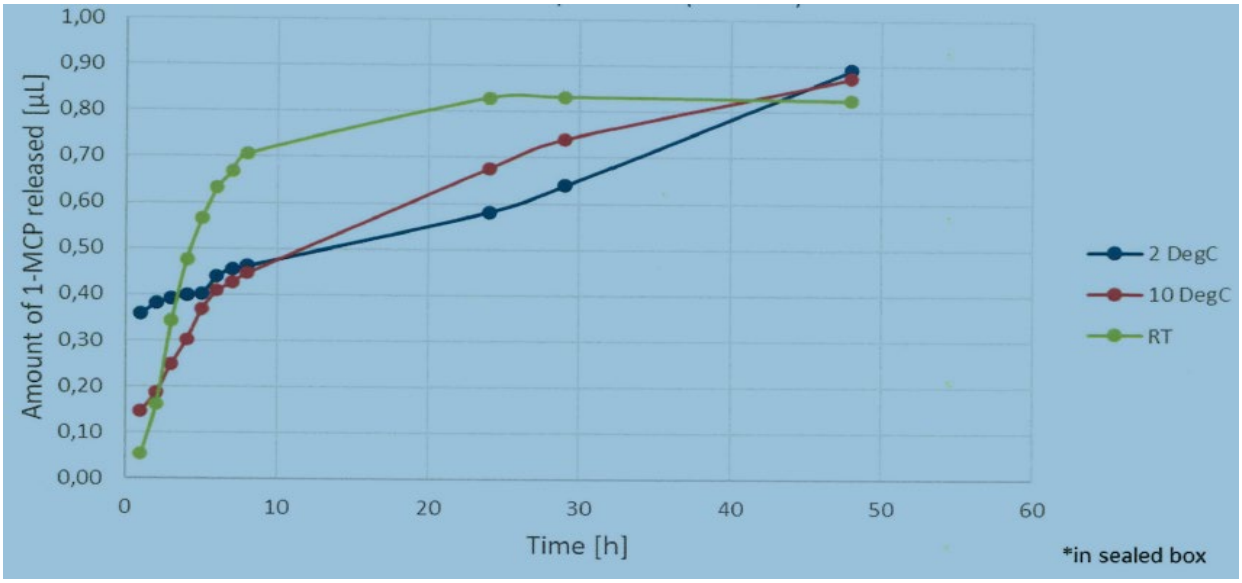

Figure 2. Comparison of 1-MCP Release from Vidre+ Labels at Different Temperatures (RH = 85%)

[4] Packaging is designed to maintain humidity in fruit and vegetable containers at 80-95% to prevent shriveling. (https://www.containerhandbuch.de/chb_e/scha/index.html?/chb_e/scha/scha_16_02.html). Accessed August 18, 2023.

E. Stability

1. Stability Data for 1-MCP

The EPA has stated that 1-MCP is stable between 0 and 37 ºC, under artificial sunlight, and in aqueous solution (EPA, 2008a).

PART 3. DIETARY EXPOSURE

A. Estimate of Dietary Exposure to the Substance

For the purpose of estimating consumption of the substance, it will be assumed that consumers of the produce in the containers will consume all of the 1-MCP that would be released onto the produce from the stickers. Types of produce that will be exposed to 1-MCP from the stickers are shown in Table 3.

Table 3. Cut Produce That Will Be Exposed To 1-MCP

Produce and Food Container	Pack Volume (L)	Pack Weight (kg)	L/kg Produce	Amount of 1-MCP on Produce (µg/kg) *
Sliced Melon in Clamshell	1.25	0.454	2.7533	9.086
Pineapple Rings or Chunks in Clamshell	1.75	0.794	2.2040	7.273
Sliced Apple in Bag	0.5	0.4	1.25	4.125
Sliced Mushrooms in Clamshell	1.25	0.454	2.7533	9.086
Shredded Cabbage Coleslaw in Bag	1.0	0.4	2.5	8.25

*3.3 µg/Liter volume x L/kg produce
kg – Kilograms; L – Liters; µg – Micrograms

The daily intake of 1-MCP is estimated using the maximum amount of 1-MCP that would be released into each produce container and the estimated consumption of the produce. The dietary exposures were calculated using the Creme Food Safety® model (https://www.cremeglobal.com). The model includes food consumption data included in the 2017-2020 National Health and Nutrition Examination Survey (NHANES) survey (CDC, 2020). Calculations for this intake analysis were completed using deterministic (single points) input data. Output calculation types include daily average intakes, acute exposures, as well as population statistics such as mean, percentiles, standard errors, and confidence intervals. Results are output for “Consumers Only” (i.e., consumers of the food / substance of interest). Results of the exposure assessment are given in absolute terms (µg/day) as well as relative to the consumer’s body weight (µg/kg body weight /day).). The per unit of body weight exposure is calculated on a subject level using the body weight recorded by the NHANES data. The results for “Consumers Only” are shown in Table 4 and a detailed report is provided in Appendix 3.

Table 4. Summary of US Consumer Only Estimated Daily Intake from the Proposed Use of Fresh Inset's 1-Methylcyclopropene (1-MCP) Based on 2017-2020 NHANES Data

Population Group	N	Intake (µg/day)		Intake (µg/kg bw/day)	
		Mean	90th Percentile	Mean	90th Percentile
Children, female (2-5 yr)	176	0.58	1.46	0.0341	0.0767
Children, male (2-5 yr)	166	0.71	1.66	0.0412	0.1067
Children, female (6-11 yr)	206	0.54	1.02	0.0177	0.0334
Children, male (6-11yr)	211	0.84	1.85	0.0248	0.0678
Teenage, female (12-18 yr)	144	0.60	1.50	0.0104	0.0215
Teenage, male (12-18 yr)	154	0.81	2.03	0.0132	0.0432
Adults, Female	1064	0.63	1.41	0.0088	0.0197
Adults, Male	806	0.84	1.60	0.0092	0.0170
All Ages	3053	0.71	1.48	0.0133	0.0286

bw – Body weight; kg – Kilogram; NHANES – National Health and Nutrition Examination Survey; yr – Year; µg – Micrograms

On a daily intake basis, teenage males are estimated to consume the most 1-MCP per day from the intended use (2.03 µg/day) while male children (2-5 years) are the highest consumer on a body weight basis (0.1067 µg/kg bw/day). This estimate is conservative in that it assumes all produce consumed has been exposed to 1-MCP.

To assess cumulative intake, the intended use by Fresh Inset on the select cut fruit and vegetables is substitutive for uses specified in GRN 585 (See Part 6.A.1). As mentioned in Part 6, 1-MCP has been approved for direct or post-harvest use on crops in the United States. Therefore, there could be some background exposure to 1-MCP from use on crops, which should be added to estimated exposure to 1-MCP from the intended use to determine cumulative exposure. EPA has stated that exposure from direct use on crops is "extremely low" and has not provided a numerical exposure estimate for this use (EPA, 2008a). As 1-MCP is volatile, it is expected that intake from direct use on crops would be negligible. EPA has determined that the worst-case scenario for exposure from post-harvest use (using the 0.004 ppm average residue concentration found in treated apples and assuming that concentration is present in 100% of the diet regardless of crops treated) indicates that a daily diet of 1.5 kg/day could contain 0.006 mg (6 µg) 1-MCP (EPA, 2002). For the general population (assuming an average body weight (bw) of 70 kg), this would represent a daily intake of 0.09 µg 1-MCP/kg bw. To account for the highest consumer (males 2-5 years) from the intended use, and assuming that this age group would eat 1 kg of food per day (4 µg 1-MCP) and a 15 kg body weight, the daily intake from background would be 0.27 µg 1-MCP/kg bw.

Use of 1-MCP on stickers should likely be substitutive for other uses, as stated in GRN 585 (FDA, 2016). For most fruits that are harvested and processed for sale as "fresh fruit" or "fresh vegetables", processing occurs soon after harvest with little to no time in a storage facility before entering the supply chain. The only exception to this could be sliced apples where the apple processor will buy

apples from a packer who has likely stored the apples for some time. Any stored apples have a high probability that 1-MCP was used in the storage. So, apples would be a case where the fruit may have more than one exposure to 1-MCP, but that would not be the case for the other target crops. Therefore, by adding the intake of 0.27 µg 1-MCP/kg bw from post-harvest use on apples to the highest EDI intake a body weight basis (0.1067 µg/kg bw/day) from the intended use, highest cumulative exposure of 1-MCP on a body weight basis is estimated to be 0.38 µg/kg bw/day (the value for male children 2-5 years). On an absolute basis, highest cumulative 1-MCP exposure is estimated to be 8.03 µg/day (the value for teenage males).

B. Estimated Dietary Exposure to Any Other Substance That is Expected to be Formed In or On Food

No substances other than 1-MCP Technical (and its possible contaminants, see Part 3.C.) and other components of the Vidre+ sticker could conceivably contact the food housed in the containers.

C. Dietary Exposure to Contaminants or Byproducts

Possible contaminants include 1-chloro-2-methylpropene (1-CMP), 3-CMP and methylene cyclopropane, all of which have been found in 1-MCP. Specifications for these substances are included to limit exposure. Total highest estimated intakes of 1-CMP, 3-CMP and methylene cyclopropane are 76 pg/kg bw/day, 76 pg/kg bw/day and 7.4 ng/kg bw/day, respectively, based on specification limits for these substances (maximum of 0.02% of the 1-MCP content for 1-CMP and 3-CMP and 1.96% of the 1-MCP content for methylene cyclopropane)[5] and estimated highest daily consumption of 1-MCP in terms of body weight (0.38 µg/kg bw/day, for 2-5 year old male children). In terms of absolute intake and using 8.03 µg/day as the intake of 1-MCP (the value for teenage males) and the specification limits for 1-CMP, 3-CMP and methylene cyclopropane, estimated intakes of these substances are 1.6 ng/day, 1.6 ng/day and 157.4 ng/day, respectively.

1-CMP and 3-CMP are carcinogenic to rodents and as such, cannot be permitted to be added to food per the Delaney clause. This clause, enacted in 1958, requires that the FDA cannot approve the use of any food additive that has been found to induce cancer in humans or animals (FDA, 2018). Based on a strict application of the Delaney clause, no amount of 1-CMP or 3-CMP would be permitted to be added to food. The FDA, however, considers a one-in-a-million cancer risk to be acceptable (Cheeseman et al., 1999) and has established a threshold of regulation (also called a threshold for toxicological concern or TTC), beneath which the risk for cancer would be acceptable.[6] The TTC for mutagenic substances of 0.15 µg/day (150 ng/day) is appropriate for 1-CMP and 3-CMP because both of these substances are mutagenic (EFSA, 2012; IARC, 1995a; IARC, 1995b). The TTC is higher than the estimated cumulative exposure to 1-CMP and 3-CMP calculated for the highest group of potential consumers of foods containing 1-MCP on an absolute basis (teenage males). Further, the

[5] Concentrations of these contaminants are not listed in the EPA approval documents for post-harvest use; therefore, it is assumed that the concentration limits are the same as those for Fresh Inset's 1-MCP.
[6] 21 CFR 170.39

TTC based on the average body weight of a 2-year-old male child[7] (8.3 ng/kg bw/day) is also higher than the estimated cumulative exposure to 1-CMP and 3-CMP calculated for 2-year-old male children.

Renwick et al. (2010) examined the rodent carcinogenicity data for 1-CMP and 3-CMP and determined that the lowest reliable benchmark dose (BMDL) for the purpose of risk assessment for either 1-CMP or 3-CMP was the $BMDL_{10}$ for nasal carcinomas in male rats of 11 mg/kg-bw/day 1-CMP (after correction for the 5 days/week dosage schedule). The $BMDL_{10}$ is the lower bound of the BMDL for a 10% incidence rate, determined from dose-response modelling. This dose is considerably higher than the estimated cumulative exposure to 1-CMP or 3-MCP from use of 1-MCP as indicated in this GRAS dossier.

According to Toxtree version 3.1.0[8], methylene cyclopropane is a Cramer Class II substance. The TTC for Cramer Class II substances is 90 µg/day (EFSA, 2012). The TTC is higher than the estimated exposure to methylene cyclopropane calculated for the highest group of potential consumers of foods containing 1-MCP on an absolute basis (teenage males). Further, the TTC based on the average body weight of a 2-year-old male child[9] (5 µg/kg bw/day) is also higher than the estimated exposure to methylene cyclopropane calculated for 2-year-old male children.

It is possible that consumers of the produce could be exposed to isopropanol from the substance leaching from the sticker. Based on 2093 g of isopropanol being added to 2 kg of each dry composite mixture, and the mixture being applied at a rate of 2 g/m^2 of paper, the isopropyl concentration would be 2.093 g $/m^2$. A sticker size of 1 cm^2 (1/10,000 m^2) would be used for a 1 L package, which would contain approximately 209 µg isopropyl alcohol. As shown in Part 3.A, the biggest package size is 1.75 L, for pineapple rings or chunks in a clamshell container. Assuming that a 70 kg individual could consume the entire contents of a pineapple clamshell container, they could conceivably consume 5.2 µg/kg bw/day isopropanol, which is considerably lower than the EPA-derived chronic reference dose (RfD) for oral exposure of 2 mg isopropanol/kg bw/day (EPA, 2014).

Therefore, exposure to 1-CMP, 3-CMP, methylene cyclopropane or isopropanol from use of 1-MCP as indicated in this GRAS dossier would not pose a toxicological concern.

PART 4. SELF-LIMITING LEVELS OF USE

Appropriate sticker sizes will be supplied to fit specific size packages, and guidance will be supplied for use to limit use of one sticker per package. It is likely that this guidance would be adhered to because of cost.

[7] Using 50th percentile weight of 24 month old male children from CDC at https://www.cdc.gov/growthcharts/data/who/GrChrt_Boys_24HdCirc-L4W_rev90910.pdf. Site visited August 18, 2023.
[8] https://toxtree.sourceforge.net/
[9] Using 50th percentile weight of 24 month old male children from CDC at https://www.cdc.gov/growthcharts/data/who/GrChrt_Boys_24HdCirc-L4W_rev90910.pdf. Site visited August 18, 2023.

PART 5. EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

1-MCP was not used in food prior to January 1, 1958, so a GRAS determination based on historical use in food would not apply. The statutory basis for the conclusion of GRAS status of 1-MCP in this document is based on scientific procedures in accordance with 21 CFR 170.30(a)(b).

PART 6. NARRATIVE

A. Introduction

The worldwide trade of plants and other fresh products has been a challenge for food producers as the products must always arrive fresh despite how they are produced and transported to the markets. As a result, producers have used a variety of pre- and post-harvests ways to prolong the ripening of the product to, in effect, extend the shelf life of the fresh product.

Optimal postharvest treatments for fresh produce seek to slow down physiological processes of senescence and maturation, reduce/inhibit development of physiological disorders and minimize the risk of microbial growth and contamination. Post-harvest treatments minimize post-harvest losses, maximize producer profits, and result in better appearance, more nutritious fruits and vegetables to the consumer. Examples of methods used to extend the post-harvest life of products are: physical treatments (heat, irradiation and edible coatings), chemical treatments (antimicrobials, antioxidants and anti-browning) and gaseous treatments. These treatments are sometimes used alone or in combination, depending on the type of product, all with the goal of maintaining the quality of the product and extending the shelf life of the product (Mahajan et al., 2014; Maiti et al., 2018).

One chemical that has a major role and impact on postharvest of fruits and vegetables is ethylene. Ethylene is a gaseous plant hormone that plays an important role in inducing the ripening process for many fruits, together with other hormones and signals. An unripe fruit generally has low levels of ethylene. As the fruit matures, ethylene is produced as a signal to induce fruit ripening. Ethylene production continues to increase after harvest, thus decreasing fruit shelf-life, storability capacity, and increasing its susceptibility to pathogen attacks. Thus, monitoring and managing ethylene production rates is of crucial importance so fruit does not become over-ripe on the tree or during postharvest storage, which will render it unmarketable and decrease profitability (Capino and Faruch, 2021).

Regarding postharvest ethylene management, 1-MCP is a chemical that has a very similar structure to ethylene. It blocks perception of ethylene by the fruit, thus delaying post-harvest ripening. The discovery of 1-MCP as an inhibitor of ethylene perception was a major breakthrough in controlling ethylene responses of horticultural products. Regulatory approval for use of 1-MCP has been obtained in more than 50 countries, and approval for use of the technology continues to occur around the world (Mahajan et al., 2014). The development of powdered products containing 1-MCP and α-cyclodextrin has stimulated commercialization because this combination permits release of 1-MCP in a safe and controlled manner.

Registration of 1-MCP for fresh fruits and vegetables has focused on major or specialty products important to specific countries. The apple has been an excellent crop for use of 1-MCP, and the technology is used extensively around the world to maintain quality through the whole marketing chain from storage to consumer. 1-MCP has been used for more than a decade to extend the life and usefulness of fresh cut flowers and potted flowering, bedding, nursery and foliage plants and harvested fruits and vegetables. Given the nature of 1-MCP, plants, fruits and vegetables are treated in enclosed areas such as rooms, coolers, greenhouses, truck trailers and shipping boxes/containers. In the US, its use is classified as indoor food and non-food crop application (EPA, 2008a). Apples, melons, tomatoes, pears, avocadoes, mangoes, papayas, kiwifruit, plums, apricots and persimmons have been exposed to 1-MCP in packing houses (EPA, 2002).

In the United States, commercialization of 1-MCP for ornamental crops was first undertaken by Floralife, Inc. The formulation contained α-cyclodextrin powder, which released 1-MCP when mixed with water. The product was approved by the EPA in 1999 for ornamentals and is sold under the name EthylBloc® (Blankenship and Dole, 2003). EthylBloc® contains 0.14% 1-MCP (EPA, 2008a). In 2002, commercial application of 1-MCP to edible crops was undertaken by AgroFresh, Inc. under the trade name SmartFresh® (Beaudry, 2021b; Blankenship and Dole, 2003). The content of 1-MCP in Smart Fresh® is 33 g/kg or 3.3%, with a range of 30 – 36 g/kg (EFSA, 2005). SmartFresh also contains α-cyclodextrin (Beaudry, 2021b). Since 2002, other products containing 3.3% 1-MCP and α-cyclodextrin have been developed (e.g., FirmConfirm® and easyfresh™). Like EthylBloc®, all of these products release 1-MCP when it is added to water (Beaudry, 2021b). FirmConfirm® is registered with EPA for postharvest use on apples (EPA, 2020) and easyfresh™ is used for the postharvest treatment of several different fruits and vegetables (Fine Americas, 2018).

FDA had no questions on the GRAS notification for the use of 1-MCP for use in fruits and vegetables in GRN 585 (see Part 6.A.1.a). Other regulatory groups that have approved the use of 1-MCP on fruits and vegetables include EPA, EFSA, and Health Canada (see Part 6.A.1.b, 6.A.2, and 6.A.3). Their analysis of the data available at the time of their approvals is applicable to Fresh Inset's use of 1-MCP as well.

1. United States

a. GRAS

1-MCP has been notified as Generally Recognized as Safe (GRAS) to FDA, under GRAS Notice GRN 585 (FDA, 2016). The intended use of the 1-MCP in GRN 585 is as a food ingredient in food packaging intended to inhibit maturation of packaged fruits and vegetables (not specified as to type).

GRN 585 contains the following information about use of 1-MCP:

- The technology involved incorporating 1-MCP into food packaging substrates including flexible films, lidding film, labels, inserts, and paper and paperboard.
- The authors stated that the uses in packaging materials would substitute for use in packing houses, so the use in packaging would not add to cumulative exposure. The authors also

mentioned that the EPA determined that the primary source for human exposure to 1-MCP from use in packing houses is from ingestion of the following raw food commodities and the processed food commodities derived from: apples, melons, tomatoes, pears, avocadoes, mangoes, papayas, kiwifruit, plums, apricots and persimmons (EPA, 2002). The only foods on this list that will be exposed to 1-MCP per the current GRAS dossier are apples and melons.

- The authors assumed that 4 ppb (0.004 ppm) residual 1-MCP residual would be present in the produce from the use of 1-MCP. They mentioned that EPA determined that the worst-case scenario (using the 0.004 ppm average residue concentration found in post-harvest treated apples and assuming that concentration is present in 100% of the diet regardless of crops treated) indicates that a daily diet of 1.5 kg/day could contain 0.006 mg 1-MCP. For the general population (assuming an average body weight of 60 kg), this would represent a daily intake of 0.0001 mg 1-MCP/kg bw, which is 90,000 to 150,000-fold less than the 9-15 mg/kg bw/day NOAEL indicated in the 90-day inhalation study in rats (EPA, 2002).
- The presence of 1-CMP and 3-CMP at a total of 0.017% of the 1-MCP was determined to be 8 x 10^{-5} ppb, based on the estimated dietary intake of 1-MCP that was calculated by the petitioner (0.5 ppb/day). The petitioner noted that 8 x 10^{-5} ppb (85 parts per quadrillion) is “well below the 50 parts per trillion level that FDA commonly applies as a risk assessment target for carcinogenic impurities in food contact materials”.
- The ADI for 1-MCP determined by EFSA is 0.0009 mg/kg bw/day (EFSA, 2005). This was calculated using the NOAEL of 9 mg/kg bw/day from the 90-day inhalation study in rats (see Part 6.C.2) and application of a 10,000x safety factor. An additional safety factor of 100 was applied to the default 100x safety factor to account for extrapolation from an inhalation study to a dietary study and from a “short term” study to lifetime exposure.
- 1-MCP was reviewed for classification by the European Chemicals Bureau (ECB) in November 2006 and January 2007. ECB has concluded that no health classification is needed for 1-MCP up to a maximum concentration of 5.0% in alpha-cyclodextrin (FAO, 2010).

b. EPA

In 2002, EPA issued a final rule (67 FR 48976) for 1-MCP, codified at 40 CFR 180.1220, when used as a post-harvest plant growth regulator for the purpose of inhibiting the effects of ethylene (EPA, 2002). The petition was submitted by AgroFresh, Inc. The rule established an exemption from the requirement of a tolerance for residues of 1-MCP when used for the stated purpose. Exposure information from this rule was stated in GRN 585 (see above).

Based on its evaluation of the submitted data, EPA made the following conclusions in the Federal Register:

> “*1. Acute toxicity (MRIDs 444647-04 to 08). 1-MCP exhibits low acute toxicity. It is a category IV biopesticide. The rat oral LD_{50} is greater than 5,000 milligrams/kilograms (mg/kg), the rabbit dermal LD_{50} is greater than 2,000 mg/kg and the rat inhalation LC_{50} is greater than 2.5 milligram/liter (mg/L) (or greater than 1,126 parts per million (ppm) v/v active ingredient in air). No deaths or clinical*

signs of systemic toxicity were observed following these acute exposures. 1-MCP produces minimal irritation of skin and eyes in rabbits and 1-MCP is not a skin sensitizer. No hypersensitivity incidents were observed following exposure to 1-MCP.

2. Genotoxicity (MRID 444647-09). 1-MCP was not mutagenic when tested as a gas in several short-term in vitro/in vivo assays, including a bacterial reverse mutation assay (Ames test), an in vitro mammalian point mutation assay in Chinese hamster ovary cells, an in vitro cytogenetics assay in human lymphocytes and an in vivo mouse micronucleus assay following inhalation exposure. In addition, 1-MCP is not mutagenic when tested as a suspension in cell media in the Ames test and in the in vitro mouse lymphoma forward mutation assay (MRID 444647-10) and is not mutagenic in the in vivo mouse micronucleus assay (MRID 444747-11) following oral exposure (gavage).

3. Developmental toxicity (MRID 454586-08). 1-MCP produces no developmental toxicity when tested in a standard developmental toxicity study in the rat via inhalation at concentrations up to and including 2.3 mg a.i./L (or 543 mg a.i.[10]/kg/day, 6 hr exposure/day). The no observed adverse effect level (NOAEL) for maternal toxicity was 0.24 mg a.i./L (56 mg a.i./kg/day, 6 hr exposure/day).

4. Subchronic toxicity (MRID 456090-01). 1-MCP was tested in a 90-day inhalation study at doses of 0.05, 0.24 and 2.3 mg a.i./kg in the rat. The NOAEL is 0.05 mg a.i./L (equivalent to 9 to 15 mg a.i./kg/day), based on minimal to mild effects on spleen and kidney histopathology at 0.24 mg a.i./L (equivalent to 39 to 66 mg a.i./kg/day). In this study there was no evidence of neurotoxicity, no effects on the respiratory tract and no effects on pathology of any endocrine or reproductive organs up to and including the highest dose tested of 2.3 mg a.i./L (or equivalent to 380 to 640 mg a.i./kg/day).”

EPA also stated the following in the rule: “based on all the available information, the Agency concludes that 1-MCP is practically non-toxic to mammals, including infants and children. Thus, there are no threshold effects of concern and as a result, the provision requiring an additional margin of safety does not apply. Further, based on the lack of observed developmental toxicity and extremely low exposure, there is reasonable certainty that no harm to infants, children, or adults will result from aggregate exposure to 1-MCP residues. Exemption of 1-MCP from the requirements of a tolerance should pose no significant risk to humans or the environment.” Further, “based on available data, no endocrine system-related effects have been identified with consumption of 1-MCP. In addition, 1-MCP does not share any structural similarity to any known endocrine disruptive chemical.” Additional information about the lack of endocrine effects is shown in Part 6.C.5.

An amendment to the final rule for 1-MCP was issued by EPA in 2008, to permit 1-MCP to be exempted from the requirement for residues on fruits and vegetables when applied or used outdoors for pre-harvest treatments (EPA, 2008b). In this rule, EPA stated that residues on apples, maize and

[10] Although not defined in the Federal Register document, it is assumed that this is active ingredient.

tomatoes treated in the field were extremely low. EPA did not calculate the anticipated exposure from this use, but reiterated the exposure previously stated for post-harvest use of 0.0001 mg 1-MCP/kg bw/day which was stated in EPA (2002).

It is apparent that FDA was aware of these documents and EPA's assessment as some of this information was included in the GRAS Notice (GRN 585) that was submitted to FDA in 2016. Thus, it is apparent that FDA agrees with EPA's assessment and determination.

It is also apparent that FDA was aware of safety determinations/evaluations of 1-MCP by other regulatory bodies and that FDA accepted these determinations as well.

Fresh Inset also agrees and accepts the conclusions rendered by EPA and the other regulatory authorities. Therefore, these conclusions will serve to support the GRAS determination that Fresh Inset has made for its use of 1-MCP.

2. Europe

In 2019, the European Commission approved use of 1-MCP for use as plant growth regulator for post-harvest storage in sealable warehouses (EC, 2019). No maximum usage rate or residuals were listed in the regulation; however, specifications for purity (≥ 980 g/kg for the technical concentrate) and impurities (maximum of 0.2 g/kg each of 1-CMP and 3-CMP) were established.

In 2018, EFSA evaluated the available data for SmartFresh, when used as a post-harvest plant growth regulator on apples in closed systems (EFSA, 2018). They increased the ADI of 1-MCP from 0.0009 mg/kg bw/day to 0.02 mg/kg bw/day based on results of an oral dog 90-day study (NOAEL 4.1 mg/kg bw/day), applying a safety factor of 100, plus an additional factor of 2 for subchronic to chronic/lifetime extrapolation (EFSA, 2018). The maximum residue level (MRL) for 1-MCP in apples was set as 0.01 mg/kg based on the presented data from a laboratory scale study with apples. EFSA concluded that that the presence of 1-CMP and 3-CMP at 0.2 g/kg in the technical specification results in margins of exposure of 150,000 and 3,200,000 for 1-CMP and 3-CMP respectively, which are unlikely to be of safety concern for consumers. EFSA also stated that under the stated conditions of use, long-range transport of 1-MCP in the atmosphere was highly unlikely (based on a half-life or 1.47 h) and the substance is of low risk to birds, mammals, aquatic organisms, soil-dwelling organisms and non-target plants.

3. Canada

In Canada, 1-MCP (SmartFresh) is currently registered for postharvest use on apples at a usage rate of 1 ppm and for use on apple trees (as Harvista 1.3 SC, which contains 1.3% 1-MCP) from 3 to 21 days prior to the anticipated harvest date. The petition for SmartFresh showed that maximum residues of 1-MCP in apples stored at 0–25.6°C, treated at 1.2 ppm (v/v) in a chamber for up to 7 days and sampled 0–336 hours following venting were 0.009 ppm (rounded up to 0.01 ppm) (Health Canada, 2004). The 0.01 ppm residual 1-MCP was also applied to use of Harvista (Health Canada, 2017a; Health Canada, 2017b).

The Proposed Registration Decision Document for 1-MCP issued by Health Canada states that 1-MCP is non-persistent in water-sediment systems when tested in a laboratory (Health Canada, 2017a). 1-MCP has high solubility in water, has a high vapor pressure and based on its Henry's Law Constant, will readily volatilize from moist soil or water surfaces. Volatilization is expected to be a major route of dissipation, and 1-MCP is not expected to be persistent in the environment. Using computer modelling, it is estimated that 1-MCP would undergo rapid photo-oxidation in the atmosphere through reactions with ozone and hydroxyl radicals, with a half-life of 0.123 days following 12 h of exposure to sunlight. Health Canada also stated that 1-MCP is not expected to bioaccumulate in biota based on a log Kow of 2.4. Therefore, although it is possible that 1-MCP could be discharged into air or water from use as described in this GRAS dossier, it would not pose an environmental concern. A Biopesticide Registration Action Document issued by the EPA for 1-MCP supports the conclusion that indoor use of 1-MCP on produce would not cause adverse effects on the environment (EPA, 2008a).

B. Absorption, Distribution, Metabolism & Excretion (ADME) Studies

A study report cited in the Renewal Assessment Report for Registration of 1-MCP for the European Commission conveys information for ADME of 1-MCP in rats after oral administration (EU, 2017). ^{14}C-1-MCP was administered by gavage in corn oil at 2 or 40 mg/kg bw for one day, or 2 mg/kg bw/day for 5 days. Absorption was > 86% regardless of dose. After a single dose of 2 or 40 mg/kg bw, a majority of the radioactivity was recovered in expired air (63 – 66%, CO_2 and organic volatiles); less was found in urine and cage rinse (28-30%) and feces (5-65). After multiple doses of 2 mg/kg bw/day, an equal amount was recovered in urine and expired air (42-44% in urine/cage rinse and 43-44% in expired air); 10-11% was recovered in feces. Excretion was rapid, with most of the radioactivity recovered within 48 h. Radioactivity was detected in blood and plasma up to 96 h post dose. The time to maximal concentration (T_{max}) ranged from 1-3 h and blood and the half-life ($t_{1/2}$) ranged from 40-73 h. Liver, kidneys, blood, plasma, adrenals, and thyroid contained more radioactivity than other tissues; however, accumulation was low. The organ with the highest amount of radioactivity 96 h after the low or high dose was the male liver, with <0.7 µg/Eq/g and <5 µg/Eq/g, respectively. There was no effect of gender on distribution or metabolism. Unchanged 1-MCP was not found in excreta. No significant difference was observed between metabolic profiles of low and high dose groups except that higher levels of M1 and M6 were observed in the high dose group. The metabolite profiles after a single or multiple doses of 2 mg/kg bw were similar and suggested no potential for bioaccumulation. All major metabolites were derived from glutathione conjugation and glucuronidation also was observed. Based on the results obtained, metabolism proceeds as in Figure 3.

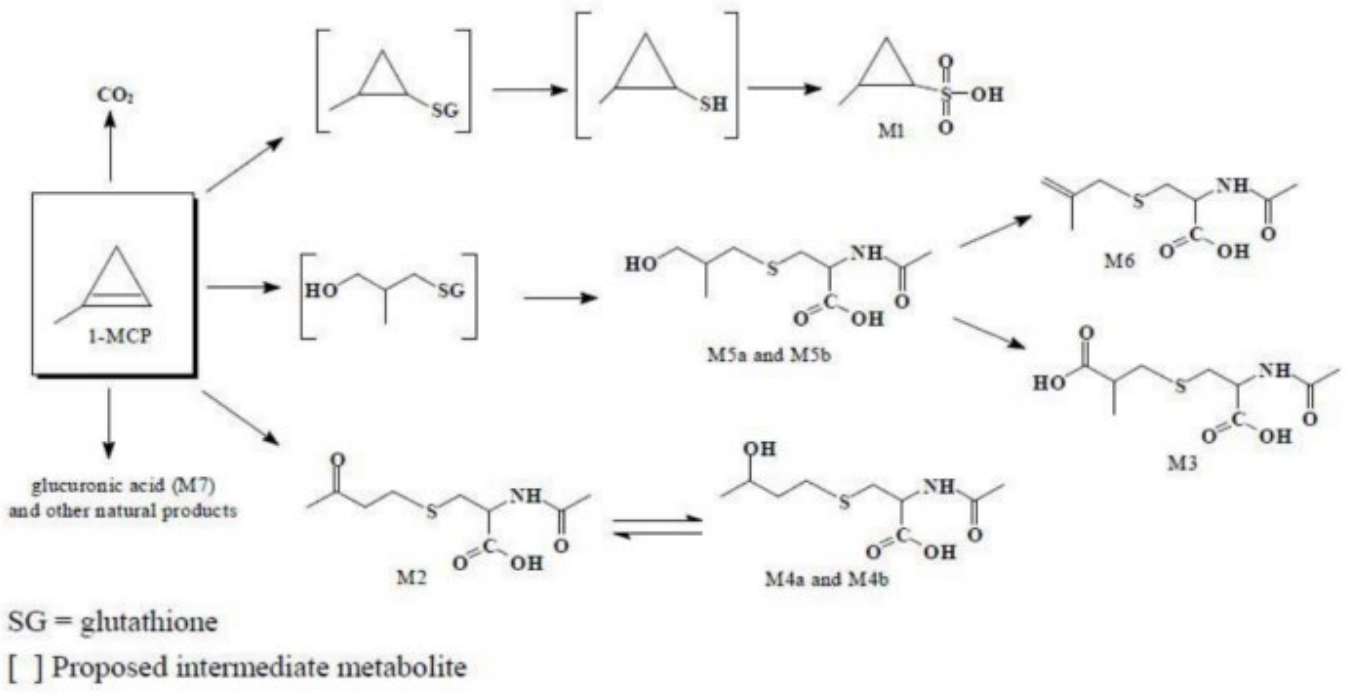

Figure 33. Metabolism of 1-Methylcyclopropene (1-MCP)[a]

[a] EU (2017)

C. Safety Studies

1. Acute Toxicity Studies

A study report cited in the Renewal Assessment Report for Registration of 1-MCP for the European Commission conveys information for acute oral toxicity of 1-MCP in rats (EU, 2017). The study was conducted according to OECD Guideline 425 and the product that was tested is described as "HAIP", which contained 4.5% 1-MCP. Three female rats were treated with 5000 mg/kg 1-MCP and were observed for 14 days. All animals survived, gained weight, appeared active and healthy, and exhibited no abnormalities at necropsy. The oral LD_{50} value of 1-MCP in rats is therefore > 5000 mg/kg bw, indicating that the substance has low potential for acute oral toxicity. EPA also sites an oral LD_{50} value of 1-MCP in rats of >5000 mg/kg bw (EPA, 2002).

2. Subchronic Toxicity Studies

a. Oral

Study reports cited in the Renewal Assessment Report for Registration of 1-MCP for the European Commission convey information for 14- and 91-day oral toxicity studies of 1-MCP in rats (EU, 2017). The product that was tested is described as High Active Ingredient Powder (HAIP), which contained 4.7% 1-MCP. For the 14-day study, the test substance was given to groups of male and female SD

rats at 5000, 10,000 or 20,000 ppm in the diet. These concentrations correspond to 235, 470 or 940 ppm 1-MCP and intakes of 22.5/23.9, 46.1/47.2 and 85.5/89.4 mg/kg bw/day 1-MCP in male/females, respectively. The study included two control groups – one on basal diet and another with 20,000 ppm α-cyclodextrin (carrier) in the diet. Endpoints measured include clinical condition, food consumption, body weight, hematology, coagulation, serum chemistry, organ weight and gross pathology. Reduced body weights and lower food consumption were noted throughout the study in the 20,000 ppm HAIP males and females compared to either control group. In females administered 10,000 or 20,000 ppm HAIP, lower mean absolute reticulocytes and lower red blood cell distribution width were observed compared to the carrier group. The carrier controls had higher alkaline phosphatase (ALP) activities than the basal diet control group; however, ALP was lower in the 10,000 ppm HAIP females and 20,000 ppm HAIP males and females. The changes in ALP were not considered toxicologically relevant.

For the 91-day study, the HAIP test substance that contained 4.7% 1-MCP was given to groups of male and female SD rats at 1500, 7500 or 20,000 ppm in the diet (EU, 2017). These concentrations correspond to 70.5, 352.5 or 940 ppm 1-MCP and intakes of 4.6/5.3, 22.4/26.5 and 60.6/71.1 mg/kg bw/day 1-MCP in male/females, respectively. The study was conducted according to OECD Guideline 408 and included two control groups – one on basal diet and another with 20,000 ppm α-cyclodextrin (carrier) in the diet. Endpoints measured include clinical condition, functional observational battery (FOB)/motor activity, ophthalmology, food consumption, body weight, hematology, coagulation, serum chemistry, organ weight, gross pathology and histopathology (control and high dose groups). The spleen and gross lesions from all animals were also examined at necropsy. There were no deaths and there was no effect of the test material on clinical observations, coagulation, clinical chemistry, FOB/motor activity, ophthalmology, or gross pathology. Reductions in body weight gains were noted early in the study in high dose males and females but there were no statistically significant changes in body weight gains of any treated group compared to basal diet controls at week 13. When compared to the carrier control group, reduced body weight gains in high dose animals were observed up to week 13. The authors stated that the high dose animals generally exhibited lower food intake early in the study but did not present the data. High dose males and females also exhibited lower red blood cell counts and higher reticulocyte counts, and high dose females had lower hemoglobin distribution width than controls. High dose animals also had higher relative liver weights than controls (both sexes) and spleen weights (females only). Microscopic changes were noted in the spleen consisting of a yellow-brown pigment, primarily in the red pulp of all male and female rats in the high dose groups. A higher incidence of extramedullary hematopoiesis was also found in several high dose females. The NOAEL assigned to the study was 7500 ppm, equivalent to 22.4/26.5 mg/kg bw/day 1-MCP in males/females.

EFSA (2018) stated that in a 90-day oral toxicity study (with MCP-HAIP) in dogs, the NOAEL of 1-MCP was 4.1 mg/kg bw per day, based on hematology and clinical chemistry changes, together with histopathological effects in the liver, testes and epididymides at higher doses. No further information about the oral toxicity study in dogs is publicly available. EFSA's approval for use of 1-MCP used the NOAEL from this study for the purpose of safety assessment.

b. Inhalation

In a 90-day rat inhalation toxicity study with 1-MCP gas, the no-observed-adverse-effect concentration (NOAEC) is 23.5 ppm (equivalent to an average systemic NOAEL of 6.5 mg/kg bw per day, assuming 100% absorption) based on histopathological effects in the kidney (hyaline droplets) and spleen (hemosiderosis) in males at higher doses (EFSA, 2018). Additional details for this study are provided in in the Renewal Assessment Report for Registration of 1-MCP for the European Commission (EU, 2017). The study was conducted according to OECD Guideline 413, exposure was whole body (6 h/day), and there were 4 male SD rats/group (target concentrations of 0, 20, 110, or 1000 ppm). Endpoints measured included clinical condition, ophthalmology, FOB/motor activity, food consumption, body weight, hematology, coagulation, serum chemistry, organ weight (spleen, liver and kidney), gross pathology and histopathology of the spleen, liver and kidney. At the highest dose there were hemolytic changes indicative of hemolytic anemia and there was a possible treatment-related increase in white blood cell count (WBC) of males. Bilirubin, total cholesterol and triglycerides, and weights and histopathology of the kidneys, liver and spleen were also affected at this dose. At the middle dose, there was an increase in hyaline droplets in the renal cortical tubular epithelium and splenic hemosiderosis in males (also described by EFSA). The NOAEC of 23.5 ppm was equivalent to a NOAEL of 9.0 mg/kg bw/day based on days of exposure and 6.5 mg/kg bw/day averaged for the whole study. The 9.0 mg/kg bw/day NOAEL was used to support safety of 1-MCP for GRN 585.

As stated in Part 6.A.1.b, EPA (2002) stated that 1-MCP was tested in a 90-day inhalation study at doses of 0.05, 0.24 and 2.3 mg a.i./kg in the rat (strain, age and numbers per group not stated), and that the NOAEL was 0.05 mg a.i./L (equivalent to 9 to 15 mg a.i./kg/day), based on minimal to mild effects on spleen and kidney histopathology at 0.24 mg a.i./L (equivalent to 39 to 66 mg a.i./kg/day). EPA stated that there was no evidence of neurotoxicity, no effects on the respiratory tract and no effects on pathology of any endocrine or reproductive organs up to and including the highest dose tested of 2.3 mg a.i./L (or equivalent to 380 to 640 mg a.i./kg/day). The NOAEL of 9.0 mg/kg bw/day is consistent with the previous study.

3. Reproductive and Developmental Toxicity Studies

The developmental toxicity of 1-MCP (96.42% pure) in “presumed pregnant” SD rats was assessed by exposing the rats to 0, 100, 300 or 1000 ppm by whole body inhalation on gestation days 6-19 (6 h/day) (EU, 2017). The average doses were calculated as 56, 174 and 543 mg/kg bw/day. This study also appears to have been evaluated by EPA (see Part 6.A.1.b) for the final rule on use of 1-MCP (EPA, 2002). Parameters measured included clinical signs, body weight, food consumption, gross pathology and uterus weight of dams, and numbers of corpora lutea, implantation sites and late resorptions, and fetuses/litter. All live fetuses were sexed, weighed, and examined for external alterations. Half of the fetuses from each litter were examined for soft tissue alterations, as well as fetuses that exhibited external abnormalities. The heads of these animals were also examined. Remaining fetuses were examined for skeletal abnormalities. The NOAEL was determined to be 56 mg/kg bw/day based on darkened and enlarged spleens at the middle dose. There was no effect of the test material on any indices related to fetal development with the exception that there was an

increase in preimplantation loss at the high dose, but values in all groups were low compared to historical values.

A multigeneration rat dietary study has been conducted with MCP-HAIP, however, no information about this study is available other than the fact that the parental and offspring NOAEL is 3.8 mg/kg bw (EFSA, 2018). EFSA (2018) stated "for the multigeneration rat dietary study (with MCP-HAIP), the parental and offspring NOAEL is 3.8 mg/kg bw per day based on effects on body weight (gain) and food consumption, whereas for the developmental rat inhalation study (with 1-methylcyclopropene gas), the maternal NOAEL is 56 mg/kg bw per day based on spleen effects (darkened and enlarged), while the developmental NOAEL is 543 mg/kg bw per day (top dose)." Therefore, EFSA did not consider the preimplantation loss in the inhalation study at the top dose (see above) to be toxicologically relevant.

4. Mutagenicity and Genotoxicity Studies

Study reports cited in the Renewal Assessment Report for Registration of 1-MCP for the European Commission convey information for genetic toxicity (EU, 2017). Results are summarized in Table 5. The FAO assessment states that results of an Ames test, mammalian point mutation test (HGPRT) in Chinese Hamster Ovary (CHO) cells, mammalian chromosome aberration tests in human lymphocytes and a micronucleus test in mice with 1-MCP vapor released from an α-cyclodextrin complex are negative (FAO, 2010). Tests were conducted up to 1000 ppm, which is the maximum safe concentration in air since higher concentrations could result in an explosion hazard (EU (2017) Volume 3CA B-6). It appears that FAO evaluated the same studies as the European Union. EPA (2002) also evaluated results of several unpublished genetic toxicity studies in their final rule for 1-MCP (see Part 6.A.1.b). EPA concluded that 1-MCP was not a genetic toxicant in any of the studies that have been conducted but they did not provide any details about the experiments, other than the types and exposure routes. From information available from the EPA rule, it appears that EPA evaluated the same studies as EU (2017). However, they also referenced three additional studies, the results of which have been added to Table 5.

Table 5. Genetic Toxicity Testing of 1-MCP

Endpoint	System	Concentration/Dose	Result	Reference
Reverse mutagenicity (Ames)	*S. typhimurium* TA 98, TA 100, TA 1535, TA 1537 and TA 102 Material tested was vapor released from 3.3 % 1-MCP in α-cyclodextrin	0, 100, 300 and 1000 ppm in the first assay and 0, 10, 100, 500 and 1000 ppm in the confirmatory assay, with and without rat liver S9	Negative	EU (2017)
Reverse mutagenicity (Ames)	*S. typhimurium* TA 98, TA 100, TA 1535, TA 1537 and *E. coli* WP2*uvr*A	0, 10, 30, 100, 300 and 1000 ppm, with and without rat liver S9	Negative	EU (2017)

Endpoint	System	Concentration/Dose	Result	Reference
	Material tested was 1-MCP vapor/gas (minimum purity 95%)			
Reverse mutagenicity (Ames)	Cells tested were not stated Material tested was a suspension in cell media	Not stated	Negative	EPA (2002)
Mammalian point mutation test	CHO cells, HGPRT locus Material tested was vapor released from 3.3 % 1-MCP in α-cyclodextrin	0, 100, 250, 500 and 1000 ppm in initial and confirmatory assays, with and without rat liver S9	Negative	EU (2017)
Mammalian point mutation test	CHO cells, HGPRT locus Material tested was 1-MCP vapor/gas (minimum purity 95%)	0, 10, 30, 100, 300 and 1000 ppm, with and without rat liver S9	Negative	EU (2017)
Mammalian cell forward mutation test	Mouse lymphoma cells Material tested was a suspension in cell media	Not stated	Negative	EPA (2002)
Mammalian chromosome aberration	Human peripheral blood lymphocytes Material tested was vapor released from 3.3 % 1-MCP in α-cyclodextrin	0, 100, 300 and 1000 ppm, with and without rat liver S9 in initial and confirmatory assays	Negative (positive response in initial assay was not confirmed)	EU (2017)
Mammalian chromosome aberration	Human peripheral blood lymphocytes Material tested was 1-MCP vapor/gas (minimum purity 95%)	0, 100, 300 and 1000 ppm, with and without rat liver S9	Negative but increased percentage of cells with gaps at all concentrations in the presence of S9 that was not dose dependent. Author concluded results were of questionable biological significance	EU (2017)
Mammalian micronucleus (mice bone marrow)	CD-1 mice (5/sex/group), 6-h inhalation exposure to 1-MCP gas	0, 100, 300 and 1000 ppm	Negative	EU (2017)
Mouse micronucleus*	Oral exposure, no further details	Not stated	Negative	EPA (2002)

1-MCP – 1-Methylcyclopropene; CHO – Chinese Hamster Ovary; h – Hours; HGPRT – Hypoxanthine-guanine phosphoribosyl transferase; ppm – Parts per million; S9 – Microsomal preparation containing metabolism enzymes
* Likely bone marrow based on date conducted

5. Potential for Endocrine Disruption

EFSA (2018) stated "1-Methylcyclopropene is not classified or proposed to be classified as toxic for reproduction category 2 or carcinogenic category 2, in accordance with the provisions of Regulation (EC) No 1272/2008, therefore the conditions of the interim provisions of Annex II, Point 3.6.5 of Regulation (EC) No 1107/2009 concerning human health for the consideration of endocrine disrupting properties are not met. On the basis of the available data and current knowledge (OECD Conceptual Framework, as analysed in the EFSA Scientific Opinion on the hazard assessment of endocrine disruptors, EFSA Scientific Committee, 2013), it is concluded that 1-methylcyclopropene is unlikely to have endocrine disrupting properties."

The Renewal Assessment Report for Registration of 1-MCP for the European Commission also states that there is no indication that 1-MCP acts as an endocrine disruptor (EU, 2017).

Results of structure-based modelling assays that have been conducted to determine the potential for 1-MCP to cause endocrine disruption are available from the Integrated Chemical Environment (ICE) program.[11] The results show that none of the 6 assays applied to 1-MCP (CoMPARA (androgen receptor binding, agonist and antagonist) and CERAPP (estrogen receptor binding, agonist and antagonist) were positive for endocrine disruption.

6. Potential for Toxicity From Inhalation or Dermal Exposure

It is possible that 1-MCP could be released from the containers into the breathing space of consumers when packages are opened or from holes in containers. It is also possible that dermal exposure to 1-MCP could occur from handling produce exposed to the 1-MCP. If no 1-MCP escaped from a package, it is possible that consumers could be exposed dermally to 1 ppm 1-MCP by handling the produce or by inhaling gas that escapes from the container. Inhalation exposure would be acute because 1-MPC gas would be released into the atmosphere upon opening the container and would not be present in the container the next time the container was opened. It is possible that dermal exposure could occur each time produce was removed from the container, but the highest amount of exposure would occur (1 ppm) the first time the produce was handled. Therefore, an acute exposure scenario also would be relevant for dermal exposure.

EPA cites a rabbit dermal LD_{50} value of > 2,000 mg/kg and a rat inhalation LC_{50}[12] value of > 2.5 mg/L (EPA, 2002). As mentioned in the 1-MCP Biopesticide Registration Document (EPA, 2008a), because acute inhalation toxicity studies for 1-MCP showed no toxicity (LC_{50} > 165 ppm, Toxicity Category IV), "the risks anticipated for this route of exposure are considered minimal." Further, results of an acute dermal study indicated low toxicity (LD_{50} >2000 mg/kg in albino rabbits, Toxicity Category III), and no significant dermal irritation (Toxicity Category IV). Based on these results, EPA concluded that the anticipated risks from dermal exposure are also considered minimal.

[11] Available at https://ice.ntp.niehs.nih.gov/Search (Accessed August 18, 2023). CAS No. 3100-04-7 as identifier.
[12] LC50 is the concentration of the chemical in the air that kills 50% of the test animals during the observation period.

7. Potential for Skin Reactions

1-MCP produces minimal irritation of skin and eyes in rabbits and is not a skin sensitizer (EPA, 2002), and no hypersensitivity incidents have been observed following exposure to 1-MCP (EPA, 2008b; EPA, 2002). Therefore, 1-MCP should not pose a hazard for allergenicity to consumers handling produce that may contain 1-MCP on the surface.

D. GRAS Criteria

FDA defines "safe" or "safety" as it applies to food ingredients as:

> "…reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use."[13]

Amplification is provided in that the conclusion of safety is to include probable consumption of the substance in question, the cumulative effect of the substance and appropriate safety factors. It is FDA's operational definition of safety that serves as the framework against which this evaluation is provided.

Furthermore, in discussing GRAS criteria, FDA notes that:[13]

> "…General recognition of safety requires common knowledge, throughout the expert scientific community knowledgeable about the safety of substances directly or indirectly added to food, that there is reasonable certainty that the substance is not harmful under the conditions of its intended use."
>
> "'Common knowledge' can be based on either "scientific procedures" or on experience based on common use of a substance in food prior to January 1, 1958."

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called "common knowledge element," in terms of the two following component elements:

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and
- There must be a basis to conclude that there is consensus (but not unanimity) among qualified scientists about the safety of the substance for its intended use, and this is established by relying upon secondary scientific literature such as published review articles, textbooks, or compendia, or by obtaining opinions of expert panels or opinions from authoritative bodies, such as JECFA and the National Academy of Sciences.

[13] See 21 CFR 170.3 (e)(i) and 81 FR 54959 Available at: https://www.federalregister.gov/documents/2016/08/17/2016-19164/substances-generally-recognized-as-safe (Accessed Aug. 23, 2023).

General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive. General recognition of safety through scientific procedures shall be based upon the application of generally available and accepted scientific data, information, or methods, which ordinarily are published, as well as the application of scientific principles, and may be corroborated by the application of unpublished scientific data, information, or methods.

The apparent imprecision of the terms “appreciable,” “at the time,” and “reasonable certainty” demonstrates that the FDA recognizes the impossibility of providing absolute safety in this or any other area (Lu, 1988; Renwick, 1990; Rulis and Levitt, 2009).

As noted below, this safety assessment to ascertain GRAS status for the specified food uses meets FDA criteria for reasonable certainty of no harm by considering both the technical and common knowledge elements.

E. Expert Panel Findings on Safety of 1-MCP

An evaluation of the safety and GRAS status of the intended use of Fresh Inset S.A.’s 1-MCP has been conducted by an Expert Panel convened by GRAS Associates; the Panel consisted of Marilyn Aardema, PhD, Robert Martin, PhD and Margitta Dziwenka, DVM, DABT, as Panel Chair. The Expert Panel reviewed Fresh Inset S.A.’s dossier as well as other publicly available information available to them. The individuals who served as Expert Panelists are qualified to evaluate the safety of foods and food ingredients by merit of scientific training and experience.

The GRAS Expert Panel report is provided in Appendix 4.

F. Common Knowledge Elements for GRAS Conclusions

The first common knowledge element for a GRAS conclusion requires that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing studies published in peer-reviewed scientific journals. The second common knowledge element for a GRAS conclusion requires that consensus exists within the broader scientific community.

1. Public Availability of Scientific Information

With regard to the safety documentation, the key data evaluated to establish safety is publicly available on websites of agencies that have approved 1-MCP for use on produce. These include FDA (GRN 585), EPA, The European Commission, and EFSA.

2. Scientific Consensus

The second common knowledge element for a GRAS conclusion requires that there be a basis to conclude that consensus exists among qualified scientists about the safety of the substance for its intended use.

1-MCP will be used at two different concentrations in the Vidre+ technology. Vidre+ contains 1000 g 1- Methylcyclopropene (1-MCP) Technical (3.3% 1-MCP + 96.7 % α-cyclodextrin) and 1000 g Polyvinylpyrrolidone (PVP), a 50:50 (w/w) mix. Vidre+ Low Concentration contains 100 g 1-MCP Technical, 1000 g PVP and an additional 900 g α-cyclodextrin. Isopropyl alcohol is mixed with either Vidre+ formulation to prepare a homogeneous mixture that is applied to the surface of a food grade paper or polypropylene sticker. Different printing techniques may be used to apply the mixture, such as flexography, flood coating systems, or screen printing. During the printing process, a thin layer of the mixture is placed on the sticker surface. Stickers of various sizes will be prepared for subsequent placement inside clamshell containers housing sliced or cut pineapple, mushrooms, or melon, or bags containing coleslaw or sliced apples. The mixture is applied to a 1 cm^2 sticker/liter volume of package to provide 1 ppm of 1-MCP gas, which will be released into the container.

For the purpose of estimating consumption of the substance, it is assumed that consumers of the produce in the containers will consume all of the 1-MCP that would be released onto the produce from the stickers. On a daily intake basis, teenage males are estimated to consume the most 1-MCP per day from the intended use (2.03 µg/day) while male children (2-5 years) are the highest consumer on a body weight basis (0.1 µg/kg bw/day). This estimate is conservative in that it assumes all produce consumed has been exposed to 1-MCP. Use of 1-MCP on stickers should likely be substitutive for other uses, as stated in GRN 585 (FDA, 2016). However, there is a possibility that there could be additional exposure to 1-MCP from post-harvest use on apples. Adding the intake of 0.27 µg 1-MCP/kg bw from post-harvest use on apples to the highest EDI intake a body weight basis (0.1067 µg/kg bw/day) from the intended use, highest cumulative exposure of 1-MCP on a body weight basis is estimated to be 0.38 µg/kg bw/day (the value for male children 2-5 years). On an absolute basis, highest cumulative 1-MCP exposure is estimated to be 8.03 µg/day (the value for male teenagers). Estimated intakes of contaminants of concern (1-CMP, 3-CMP and methylene cyclopropane) are lower than the TTC for these substances and are therefore not toxicologically relevant.

1-MCP has been notified as GRAS to FDA, under GRN 585 (FDA, 2016). For the general population (assuming an average bw of 60 kg), the daily intake of 1-MCP estimated in GRN 585 was 0.0001 mg 1-MCP/kg bw (0.1 µg/kg bw/day), approximately equal to the estimated 90th percentile intake of 1-MCP from the intended use of 1-MCP as described in the current GRAS dossier, if post-harvest use on apples is not taken into account. The ADI for 1-MCP determined by EFSA is 0.02 mg/kg bw/day (20 µg/kg bw/day) (EFSA, 2018), higher than the cumulative exposure of 1-MCP on a body weight basis estimated per the intended use.

1-MCP is not expected to accumulate in the blood or tissues of humans with the proposed use, based on results of a pharmacokinetic study in rats. 1-MCP has low potential for acute oral, dermal or inhalation toxicity and is not genotoxic or allergenic. Based on results of a developmental toxicity study in rats with inhalation exposure and a two-generation dietary study in rats, EFSA (2018) concluded that 1-MCP is not classified or proposed to be classified as toxic for reproduction category 2. EFSA also concluded that 1-MCP is unlikely to be carcinogenic or have endocrine disrupting properties. The Renewal Assessment Report for Registration of 1-MCP for the European Commission

also states that there is no indication that 1-MCP acts as an endocrine disruptor (EU, 2017). Further, none of the 6 assays applied to 1-MCP (CoMPARA (androgen receptor binding, agonist and antagonist) and CERAPP (estrogen receptor binding, agonist and antagonist)) as part of an ICE assessment were positive for endocrine disruption.

Study reports cited in the Renewal Assessment Report for Registration of 1-MCP for the European Commission convey information for a 91-day oral toxicity study of 1-MCP in rats (EU, 2017). The product that was tested is described as “HAIP”, which contained 4.7% 1-MCP. The NOAEL assigned to the study was 7500 ppm, equivalent to 22.4/26.5 mg/kg bw/day 1-MCP in males/females. Dogs are more sensitive to the repeated dose oral toxicity of HAIP (90-day oral NOAEL of 1-MCP was 4.1 mg/kg bw per day). EFSA’s approval for use of 0.02 mg/kg bw/day 1-MCP of used the NOAEL from the dog study for the purpose of safety assessment, applying an UF of 100, with an additional factor of 2 for subchronic to chronic/lifetime extrapolation.

In summary, a compelling case can be made that scientific consensus exists regarding the safety of 1-MCP at the anticipated exposure level from the intended use.

G. Discussion of Information Inconsistent with GRAS Conclusion

The authors of this GRAS dossier are not aware of information that would be inconsistent with a finding that the proposed use of 1-MCP in food is generally recognized as safe.

The regulatory framework for determining whether a substance is GRAS is in 21 CFR 170.30, which states that GRAS status through scientific procedures shall ordinarily be based upon published studies, which may be corroborated by unpublished studies and other data and information. These criteria have been applied to the existing data for 1-MCP.

H. Conclusion

In consideration of the aggregate safety information available, Fresh Inset S.A. concludes that 1-Methylcyclopropene (1-MCP) as defined in the subject notified dossier is safe for the intended use.

The weight of the publicly available evidence from studies with 1-Methylcyclopropene provide a basis upon which to conclude that the proposed uses of 1-Methylcyclopropene as described in this dossier satisfy the safety standard of Reasonable Certainty of No Harm and are safe. Based on the pivotal, published data and information that are generally available, one may conclude that the proposed use of 1-Methylcyclopropene which meets the food grade specifications presented in this dossier, are GRAS based on scientific procedures. Support for these conclusions by a consensus of qualified experts in the general scientific community is provided in Appendix 4 (Expert Panel Report).

Accordingly, 1-Methylcyclopropene meets FDA’s definition of safety in that there is “reasonable certainty of no harm under the intended conditions of use” as described herein and, therefore, is generally recognized as safe (GRAS).

PART 7. LIST OF SUPPORTING DATA AND INFORMATION IN THE GRAS DOSSIER

A. References

1. List of Acronyms

1-CMP	1-Chloro-2-methylpropene
1-MCP	1-Methylcyclopropene
3-CMP	3-Chloro-2-methylpropene
µg	Micrograms
µL	Microliters
ADI	Average daily intake
ADME	Absorption, Distribution, Metabolism and Excretion
ALP	Alkaline phosphatase
BMDL	Lowest reliable benchmark dose
bw	Body weight
C	Celsius
cm	Centimeters
ECB	European Chemicals Bureau
EDI	Estimated daily intake
EPA	Environmental Protection Agency
Eq	Equilibrium dissociation constant
F	Fahrenheit
FAO	Food and Agriculture Organization of the United Nations
FCC	Food Chemicals Codex
FD&C Act	Federal Food Drug and Cosmetics Act
FOB	Functional observational battery
g	Grams
GA	GRAS Associates
GRAS	Generally Recognized as Safe
GRN	GRAS Notification
h	Hours
HAIP	High active ingredient powder
ICE	Integrated Chemical Environment
kg	Kilograms
L	Liters
LD_{50}	Median lethal dose
m	Meters
mg	Milligrams
mL	Milliliters
ng	Nanograms
NHANES	National Health and Nutrition Examination Survey
NOAEC	No-observed-adverse-effect concentration
NOAEL	No observed adverse effect level
pg	Picograms
ppb	Parts per billion
ppm	Parts per million
PVP	Polyvinylpyrrolidone
rpm	Revolutions per minute
T_{max}	Time to maximum concentration
TTC	Threshold for toxicological concern
w/w	Concentration

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B. Appendices

Appendix 1 Methods of Analysis

CIPAC/4667/m

1-Methylcyclopropene Impurities

GC method

CIPAC Collaborative Trial
according to
CIPAC Information Sheet Number 282

D. Verona
AgroFresh, Inc.
727 Norristown Road
Spring House, Pa. 19477
United States

April 2009

1-Methylcyclopropene Impurities

OUTLINE OF METHOD

1-chloro-2-methylpropene (1-CMP) and 3-chloro-2-methylpropene (3-CMP) are impurities in the active ingredient 1-methylcyclopropene (1-MCP). 1-MCP is a gas at room temperature and is stabilized for commercial use by encapsulation in SmartFresh™ 3.3% Technology. 1-MCP, 1-CMP and 3-CMP are released for analysis by dissolving a formulation sample in water in an airtight container. The container headspace is sampled and analyzed by capillary gas chromatography with flame ionization detection and external standard calibration. 1-CMP and 3-CMP content is reported as percent relative to 1-MCP concentration which is determined by a separate method [1].

REAGENTS

Water purified to remove traces of heavy metals
1-chloro-2-methylpropene (1-CMP) purity 98.0% minimum
3-chloro-2-methylpropene (3-CMP) purity 98.0% minimum
1-CMP and 3-CMP Stock solution. Measure the 1-CMP (10 uL) into a 10 µL syringe, inject the 1-CMP into a 1 liter glass bottle equipped with a VICI gas tight valve. Repeat this procedure to introduce 3-CMP into the same bottle. Shake the bottle vigorously on a mechanical shaker for at least 60 minutes. Calculate the concentration (mg/mL) of the 1-CMP ($c_{s\ 1\text{-}CMP}$) and 3-CMP ($c_{s\ 3\text{-}CMP}$) (Note 1).
1-CMP and 3-CMP Calibration solution. Measure the stock solution (200 uL) into a 250 µL gas tight syringe, inject the contents of the syringe into a 250 mL glass bottle equipped with a VICI gas tight valve. Shake the bottle vigorously on a mechanical shaker for at least 60 minutes. Calculate the concentration (mg/mL) of 1-CMP ($c_{c\ 1\text{-}CMP}$) and 3-CMP ($c_{c\ 3\text{-}CMP}$) based on volumetric dilution of the stock solution. Prepare duplicate calibration solutions (solutions C_A and C_B) (Note 1).

APPARATUS

Gas chromatograph equipped with split/splitless inlet and a flame ionization detector
Capillary column DB-624: 30 m x 0.25 mm (i.d.) and 1.4 µm film thickness (Agilent, Inc.)
Electronic integrator or data system
Analytical balance with 0.1 mg sensitivity
Mechanical shaker rotary or reciprocating type
Mininert gas tight valves with 24 mm cap size for 250 mL bottles; with 1/8 inch NPT thread for 1 L bottles (Preceision Sampling , Inc.)

Gas tight glass syringes 0.25 mL and 0.50 mL, equipped with ~5 cm side port needles (Note 2)
Liquid syringe 10 µL
Glass or plastic syringe to measure 3 mL, equipped with a 22 to 25 gauge needle
Glass bottles 250 mL (nominal) Boston Round, 24 mm threaded neck; 1 liter (nominal) glass Media/solution bottle with polypropylene plug seal cap and pouring ring (Note 3)

PROCEDURE

(a) Gas chromatographic conditions (typical):

Column	DB-624 30 m length x 0.25 mm i.d. x 1.4 µm film thickness (Note 4)
Injection system	
Injector mode	spilt injection
Injector insert	4 mm i.d., straight through glass (no glass wool) (Note 5)
Injection volume	0.50 mL
Split flow	20 mL/min
Detector	flame ionization
Temperatures	
Injection port	75 °C
Detector	185 °C
Oven program	temp 1 40 °C, hold 0 min, ramp rate 25 °C/min
	temp 2 165 °C, hold 0 min
Gas flow rates	
Carrier:	
Helium	2 mL/min; approximately 40 cm/sec (run at constant flow)
Detector:	
Air	400 mL/min
Hydrogen	45 mL/min
Nitrogen (make up)	carrier flow + make up flow = 30 mL/min
Retention times	
1-CMP	2.6 min to 2.8 min
3-CMP	2.7 min to 2.9 min

(b) System equilibration. Inject 0.5 mL aliquots of the calibration solution C_A until the response factors obtained for two consecutive injections differ by less than 5%. Then inject 0.5 mL aliquots of calibration solution C_B. The response factor for solution C_B should not deviate by more than 5% from that of solution C_A, otherwise prepare new calibration solutions (Note 6). If the peak retention times are not within the listed time windows, adjust the carrier flow rate accordingly.

(c) Linearity check. Check the linearity of the method by injecting 0.50 mL aliquots of solutions with 1-CMP and 3-CMP concentrations of 0.5, 1 and 2 times that of the calibration solution before conducting analyses.

(d) Sample preparation. Prepare sample solutions in duplicate (solutions S_A and S_B). Weigh (to the nearest 0.1 mg) 90 mg to 110 mg (w mg) of SmartFresh™ 3.3% Technology into a 250 mL glass bottle. Cap the bottle with a gas tight valve immediately. Inject water (3 mL) through the valve into the bottle. Close the valve. Place the bottle on a mechanical shaker and mix vigorously for a minimum of 60 minutes (Note 7).

(e) Determination. Inject in duplicate 0.50 mL aliquots from the headspace of each sample solution bracketing them by injections of the calibration solutions as follows: calibration solution C_A, sample solution S_A, sample solution S_A, calibration solution C_B, sample solution S_B, sample solution S_B, calibration solution C_A, and so on. Measure the relevant peak areas.

(e) Calculations.
Calculate the concentration (mg/mL) of 1-CMP and 3-CMP in the stock solution.

$$c_{s\,1\text{-CMP}} = \frac{d_{1\text{-CMP}} \times V_{1\text{-CMP}} \times P_{1\text{-CMP}}}{V_{sb}} \qquad c_{s\,3\text{-CMP}} = \frac{d_{3\text{-CMP}} \times V_{3\text{-CMP}} \times P_{3\text{-CMP}}}{V_{sb}}$$

where:

$c_{s\,1\text{-CMP}}$ = concentration of the 1-CMP stock solution (mg/mL)
$c_{s\,3\text{-CMP}}$ = concentration of the 3-CMP stock solution (mg/mL)
$d_{1\text{-CMP}}$ = density 1-CMP (mg/mL)
$d_{3\text{-CMP}}$ = density of 3-CMP (mg/mL)
$P_{1\text{-CMP}}$ = purity of 1-CMP expressed as a decimal fraction
$P_{3\text{-CMP}}$ = purity of 3-CMP expressed as a decimal fraction
$V_{1\text{-CMP}}$ = volume of the 1-CMP standard material (mL)
$V_{3\text{-CMP}}$ = volume of the 3-CMP standard material (mL)
V_{sb} = volume of the stock solution preparation bottle (mL)

Calculate the concentration of 1-CMP and 3-CMP in the calibration solution.

$$c_{c\,1\text{-CMP}} = \frac{c_{s\,1\text{-CMP}} \times V_s}{V_{cb}} \qquad c_{c\,3\text{-CMP}} = \frac{c_{s\,3\text{-CMP}} \times V_s}{V_{cb}}$$

where:

$c_{c\,1\text{-CMP}}$ = concentration of the 1-CMP in the calibration solution (mg/mL)
$c_{c\,3\text{-CMP}}$ = concentration of the 3-CMP in the calibration solution (mg/mL)
V_s = volume of the stock solution aliquot diluted (mL)
V_{cb} = volume of the calibration standard preparation bottle (mL)

Calculate the response factors for 1-CMP and 3-CMP.

$$f_{i\,1\text{-CMP}} = \frac{c_{c\,1\text{-CMP}}}{H_{s\,1\text{-CMP}}} \qquad f_{i\,3\text{-CMP}} = \frac{c_{c\,3\text{-CMP}}}{H_{s\,3\text{-CMP}}}$$

where:

$fi_{\,1\text{-CMP}}$ = individual 1-CMP response factor
$fi_{\,3\text{-CMP}}$ = individual 3-CMP response factor
$c_{c\,1\text{-CMP}}$ = concentration of the 1-CMP in the calibration solution (mg/mL)
$c_{c\,3\text{-CMP}}$ = concentration of the 3-CMP in the calibration solution (mg/mL)
$H_{s\,1\text{-CMP}}$ = peak area of 1-CMP in the calibration solution
$H_{s\,3\text{-CMP}}$ = peak area of 3-CMP in the calibration solution

Calculate the mean value of each pair of response factors bracketing the two injections of a sample and use this value for calculating the 1-CMP and 3-CMP contents of the bracketed sample injections.

$$\text{1-CMP content} = \frac{f_{\text{1-CMP}} \times H_{\text{w 1-CMP}} \times HS \times 1000}{w} \text{ g/kg}$$

$$\text{3-CMP content} = \frac{f_{\text{3-CMP}} \times H_{w\,\text{3-CMP}} \times HS \times 1000}{w} \text{ g/kg}$$

where:

$f_{\text{1-CMP}}$ = mean 1-CMP response factor
$f_{\text{3-CMP}}$ = mean 3-CMP response factor
$H_{w\,\text{1-CMP}}$ = peak area of 1-CMP in the sample preparation
$H_{w\,\text{3-CMP}}$ = peak area of 3-CMP in the sample preparation
HS = volume of the headspace in the sample preparation bottle (mL)
(= bottle volume – water volume)
w = mass of sample taken (mg)

1-CMP and 3-CMP specifications are based on the percent relative concentration versus 1-MCP. 1-MCP content must be determined by a separate method [1]

$$\%\ \text{Relative content 1-CMP} = \frac{\text{1-CMP content g/kg}}{\text{1-MCP content g/kg}} \times 100$$

$$\%\ \text{Relative content 3-CMP} = \frac{\text{3-CMP content g/kg}}{\text{1-MCP content g/kg}} \times 100$$

REFERENCES

[1] 767. 1-Methylcyclopropene, GC Method

Note 1 Segregate the syringe used to prepare the CMP calibration solution; do not use for injection of sample preparations.

Note 2 Side port needles are required; standard taper needles will partially plug and cause irreproducible injection volumes.

Note 3 Accurately measure the total volume of each type of bottle using water i.e. vendor volume specifications are approximate and usually do not include the volume in the neck of the bottle. The plastic cap of the media bottle must be taped to make a hole with threads to accommodate a gas tight valve with a male 1/8 inch NPT thread.

Note 4 DB-624 is manufactured by J&W and distributed by Agilent. It is a proprietary phase; the performance of similar phases from other suppliers is uncertain. Condition the column before use at 225 °C following the manufactures instructions.

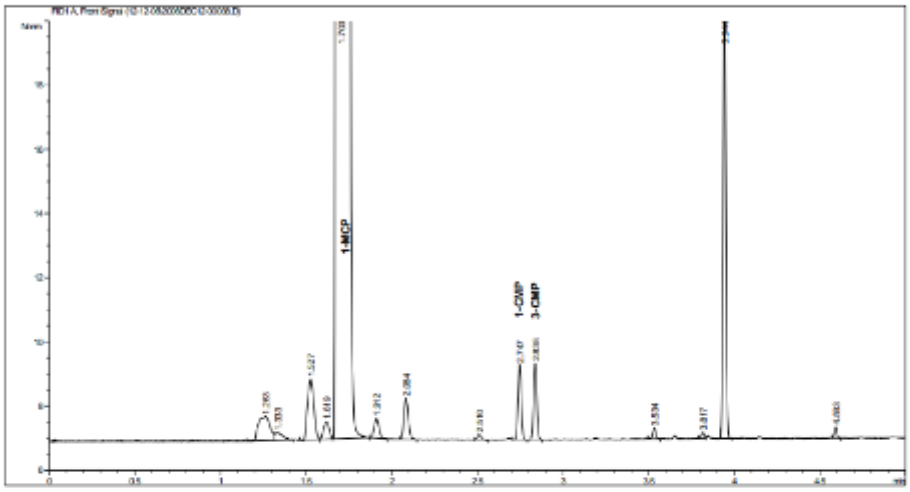
Note 5 An insert with an internal volume of approximately 1 mL is necessary to completely contain the large sample volume injected.

Note 6 Peak areas will vary with injection speed. During standard and sample injection depress the syringe plunger with a constant and consistent speed. Fast injection speeds can be reproduced most easily.

Note 7 Insure that all of the sample powder drops onto the bottom of the bottle and that none adheres to the iside wall. The sample must be in contact with the water during mixing. Wrist action shakers are not recommended. Static charge on the glass bottle can compromise weighing accuracy and precision, if static is a problem weigh the sample onto a piece of weighing paper and transfer into the bottle.

CIPAC/4667/m

Representative Chromatogram
SmartFresh™ 3.3 % Technology



REAGENT

cis-2-Butene surrogate reference standard. Analyze new lots for purity by injecting 0.25 mL of the neat gas through the valve into an empty sample preparation bottle. Mix the dilution for 30 min. Analyze under the method conditions using area percent calibration. Must have minimum purity of 99 % and must not contain significant impurities that co-elute with 1-MCP.

Working volume of cis-2-butene. Transfer neat *cis*-2-butene from the cylinder into a Tedlar bag for use while being careful to avoid entrainment of air during the transfer. Attach appropriately sized fittings and plastic connecting tubing to the pressurized cylinder or cylinder regulator. Flush the connecting line with gas to remove air and allow a slow flow of gas to continue. Open the valve of a Tedlar bag and push the connecting tubing over the valve stem while gas is flowing slowly. Increase the gas flow and partially fill the bag. Remove the connecting tubing, and then compress the bag by hand to remove most of the gas. Flush the bag in this way a total of 2 times. After flushing, fill the bag to about 80 % capacity and then close the bag valve. Prepare a fresh bag of *cis*-2-butene weekly as needed.

Water purified through a Milli Q water purification system or equivalent purity (Note 1).

APPARATUS

Capillary gas chromatograph equipped with split/splitless inlet and flame ionization detector

Electronic integrator or data system suitable for capillary GC peak integration

Capillary column PoraBOND Q: 25m x 0.25 mm (i.d.) x 3µm film thickness conditioned according to the manufacturers recommendations (Note 2)

Inlet liner 4 mm i.d. glass liner (straight through); no glass wool (Note 3)

Gas tight syringes 0.25 mL

Syringe needles 2 inch length with side port point (Note 4)

Analytical balance with +/- 0.01 mg sensitivity

Weighing paper waxed

Boston round sample bottle 250 mL nominal volume with 24mm-400 thread mouth

Gas tight valves Miniert 24 mm-400 thread (Precision Sampling, Inc.)

Glass or plastic syringe to deliver 2 mL with 22 or 25 gauge needle

Mechanical shaker reciprocating or orbital (rotating)

Thermometer range bracketing 20 °C

Barometer accuracy better than 1 % error

Tedlar air sampling bags 0.5 or 1 liter with septum/hose valve

1-Methylcyclopropene

767/TC/M/-

Chemical name:	1-Methylcyclopropene
Abbreviation:	1-MCP
CAS Number:	3100-04-7
Empirical formula:	C_4H_6
Molecular mass:	54.09
Boiling point:	4.7°C (calculated)
Activity:	plant growth regulator

1 **Sampling**. Take at least 25 g.

2 Identity test.

2.1 **GC/MS.** Use the capillary GC method below but with a mass selective detector. Obtain a spectrum of the peak in a sample solution that is within the retention time window specified for 1-MCP (Figure 1). The spectrum should not differ significantly from that in Figure 2.

3 1-Methylcyclopropene - SmartFresh™ 3.3 % Technology

767/VP/M/-

OUTLINE OF METHOD

This method determines the weight percent of 1-methylcyclopropene (1-MCP) in SmartFresh™ 3.3% Technology. 1-MCP, a gas at room temperature, is released from the formulation by sealing a sample in an airtight container and then introducing water to dissolve the sample. The 1-MCP/air mixture is drawn from the headspace of the container with a gas tight syringe for analysis. 1-MCP is determined by capillary GC using flame ionization detection. *cis*-2-Butene is used as a surrogate calibration standard for 1-MCP.

REAGENT

cis-2-Butene surrogate reference standard. Analyze new lots for purity by injecting 0.25 mL of the neat gas through the valve into an empty sample preparation bottle. Mix the dilution for 30 min. Analyze under the method conditions using area percent calibration. Must have minimum purity of 99 % and must not contain significant impurities that co-elute with 1-MCP.

Working volume of cis-2-butene. Transfer neat *cis*-2-butene from the cylinder into a Tedlar bag for use while being careful to avoid entrainment of air during the transfer. Attach appropriately sized fittings and plastic connecting tubing to the pressurized cylinder or cylinder regulator. Flush the connecting line with gas to remove air and allow a slow flow of gas to continue. Open the valve of a Tedlar bag and push the connecting tubing over the valve stem while gas is flowing slowly. Increase the gas flow and partially fill the bag. Remove the connecting tubing, and then compress the bag by hand to remove most of the gas. Flush the bag in this way a total of 2 times. After flushing, fill the bag to about 80 % capacity and then close the bag valve. Prepare a fresh bag of *cis*-2-butene weekly as needed.

Water purified through a Milli Q water purification system or equivalent purity (Note 1).

APPARATUS

Capillary gas chromatograph equipped with split/splitless inlet and flame ionization detector

Electronic integrator or data system suitable for capillary GC peak integration

Capillary column PoraBOND Q: 25m x 0.25 mm (i.d.) x 3µm film thickness conditioned according to the manufacturers recommendations (Note 2)

Inlet liner 4 mm i.d. glass liner (straight through); no glass wool (Note 3)

Gas tight syringes 0.25 mL

Syringe needles 2 inch length with side port point (Note 4)

Analytical balance with +/- 0.01 mg sensitivity

Weighing paper waxed

Boston round sample bottle 250 mL nominal volume with 24mm-400 thread mouth

Gas tight valves Miniert 24 mm-400 thread (Precision Sampling, Inc.)

Glass or plastic syringe to deliver 2 mL with 22 or 25 gauge needle

Mechanical shaker reciprocating or orbital (rotating)

Thermometer range bracketing 20 °C

Barometer accuracy better than 1 % error

Tedlar air sampling bags 0.5 or 1 liter with septum/hose valve

PROCEDURE

(a) Gas chromatographic conditions (typical):

Column	CP-PoraBOND Q, 25 m length x 0.25 mm i.d. x 3 µm film thickness (Note 5)
Injection system	
Injector	spilt injection
Injection volume	0.25 mL
Split flow	(Note 5)
Detector	flame ionization
Temperatures	
Injection port	75 °C
Detector	200 °C
Oven program	temp 1 75 °C, hold 1 min, ramp rate 5 °C/min temp 2 110 °C, hold 0 min
Carrier gas	helium
Gas flow rates	
Helium	approximately 50 cm/sec (Note 5)
Detector:	
Air	400 mL/min
Hydrogen	45 mL/min
Nitrogen (make up)	carrier flow + make up flow = 30 mL/min
Retention times	
1-MCP	6.0 to 6.2 min
cis-2-butene	6.7 to 6.9 min

(b) System equilibration. Set the carrier gas flow rate to about 3 mL/min and inject a sample preparation (section c). If the peak retention times are not within the specified time windows then adjust the flow rate accordingly. After setting the carrier flow rate, set the inlet split ratio to approximately 1:1, and inject a sample preparation. Reset the column split ratio to 20:1 and inject a sample preparation until the area ratio of the 1-MCP/*cis*-2-butene peaks differ by less than 1.0% in successive injections.

(c) Sample preparation. Prepare each sample in duplicate. Weigh (to the nearest 0.01 mg) 13 mg to 21 mg (*w* mg) of sample onto a weighing paper. Transfer the weighed sample into a 250 mL bottle and seal the bottle with a Mininert valve (Note 6). Measure 2 mL of water in a syringe. Open the valve on the sample bottle, inject the water through the valve into the bottle and then close the valve. Draw *cis*-2-butene into a gas tight syringe and measure 0.25 mL and while the

needle is still in the bag accurately record the ambient temperature and atmospheric pressure at the time the *cis*-2-butene volume is measured (Note 7). Remove the needle from the bag, open the bottle valve, insert the syringe needle through the bottle valve, inject the *cis*-2-butene into the sample bottle and then close the valve (Note 8). Gently swirl the bottle manually until all of the sample powder is wet and then place the bottle on a mechanical shaker and mix for a period of 60 min to 90 min. Analyze samples as soon as possible after mixing.

(d) Determination. Inject in duplicate 0.25 mL aliquots (Note 9) of each sample preparation headspace as follows: sample headspace S_{A1}, sample headspace S_{A2}, sample headspace S_{B1}, sample headspace S_{B2}, sample headspace S_{C1}, sample headspace S_{C2} (i.e. S_{A1} is sample A, preparation 1), and then repeat the injection sequence. Measure the relevant peak areas.

(e) Calculation. Calculate the moles of *cis*-2-butene in each sample preparation and use that value to calculate the corresponding 1-methylcyclopropene content in each sample preparation.

$$\text{mol}_c = \frac{p \times V \times P}{R \times T}$$

$$\text{Content of 1-MCP} = \frac{1000\,\text{g/kg} \times H_w \times \text{mol}_c \times f \times M}{I_q \times w}\ \text{g/kg}$$

Where:

f = relative response factor of *cis*-2-butene/1-MCP (1.034)
H_w = peak area of 1-MCP in the sample preparation
I_q = peak area of *cis*-2-butene standard in the sample preparation
T = temperature of *cis*-2-butene at time of sampling in degrees Kelvin (Kelvin = Celsius + 273.15)
mol_c = moles of *cis*-2-butene standard in the sample preparation
M = molar mass of 1-MCP (mg) = 54090 mg/mole
P = purity of the *cis*-2-butene expressed as a decimal fraction
p = atmospheric pressure of *cis*-2-butene at time of sampling (Note 10)
R = universal gas constant (Note 10)
V = volume (L) of *cis*-2-butene added to sample preparation (0.00025 L)
w = mass of sample taken (mg)

Note 1 Heavy metal ions particularly copper react with 1-MCP and cause low active ingredient results. Reagent water should be purified to reduce heavy metal ion concentrations to as low a level as practical.

Note 2 The specified column may not be substituted. Significant irreversible adsorption of 1-MCP may occur on similar columns from other vendors or on other type phases. In addition, the 1-MCP exo-isomer present as an impurity cannot be resolved on standard phases.

Note 3 The inlet liner should have an internal volume of about 1 mL to contain the large volume of gaseous sample injected.

Note 4 Side port needles are required; standard taper needles will partially plug and cause irreproducible injection volumes.

Note 5 PoraBOND Q columns are PLOT columns and particles often dislodge and partially plug the column. As a result column head pressure is not an accurate indicator of column flow rate. Column flow rate should be measured with a flow meter and adjusted to approximately 2.5 mL/min to 3 mL/min to provide a 1-MCP retention time of approximately 6.1 min. The split flow should be set to 20 times the measured column flow to provide a split ratio of 20:1. Follow the manufacturer's general care and use instructions as described in the document provided with the column. The column should be heat conditioned prior to its first use at a temperature of at least 140 °C for 2 hours. It is not necessary to condition the column at its upper operating temperature.

Note 6 Insure that all of the powder drops onto the bottom of the bottle with none adhering to the inside wall of the bottle. Weighing the powder directly into the bottle is not advised because static charge on the glass can compromise weighing accuracy and precision.

Note 7 The actual atmospheric pressure in the laboratory is required for accurate calculations. Atmospheric pressure measured with a barometer will provide the pressure at the elevation of the laboratory. Internet weather services may be able to provide data for you geographical area (i.e. http://weather.noaa.gov/), however, be aware that most weather services report pressures adjusted to sea level, and should be corrected to local elevation by subtracting 1 mbar for every 10 m above sea level.

Note 8 Segregate the syringe used to measure pure *cis*-2-butene; use a different syringe for injection of sample preparations.

Note 9 During sample injection depress the syringe plunger with a constant and consistent speed. Peak areas will vary with injection speed.

Note 10: Atmospheric pressure may be measured in various units of pressure. Select the appropriate universal gas constant, *R*, from the table:

0.08205746 L·atm·K^{-1}·mol^{-1}
8.31447 L·kPa·K^{-1}·mol^{-1}
0.8314472 L·hPa·K^{-1}·mol^{-1}
62.3637 L·mmHg·K^{-1}·mol^{-1}
62.3637 L·Torr·K^{-1}·mol^{-1}
83.14472 L·mbar·K^{-1}·mol^{-1}

CIPAC/4669/m

Figure 1: Representative Chromatogram - SmartFresh™ 3.3% Technology

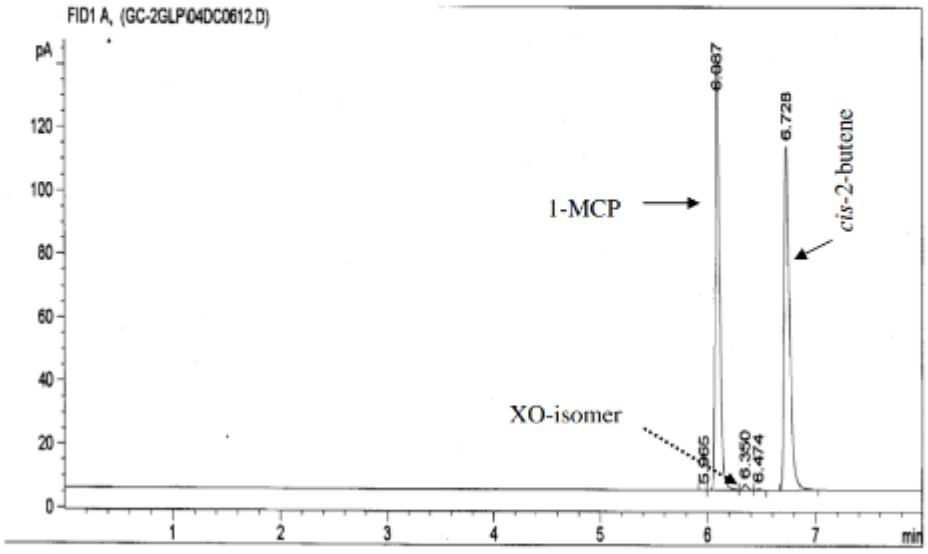
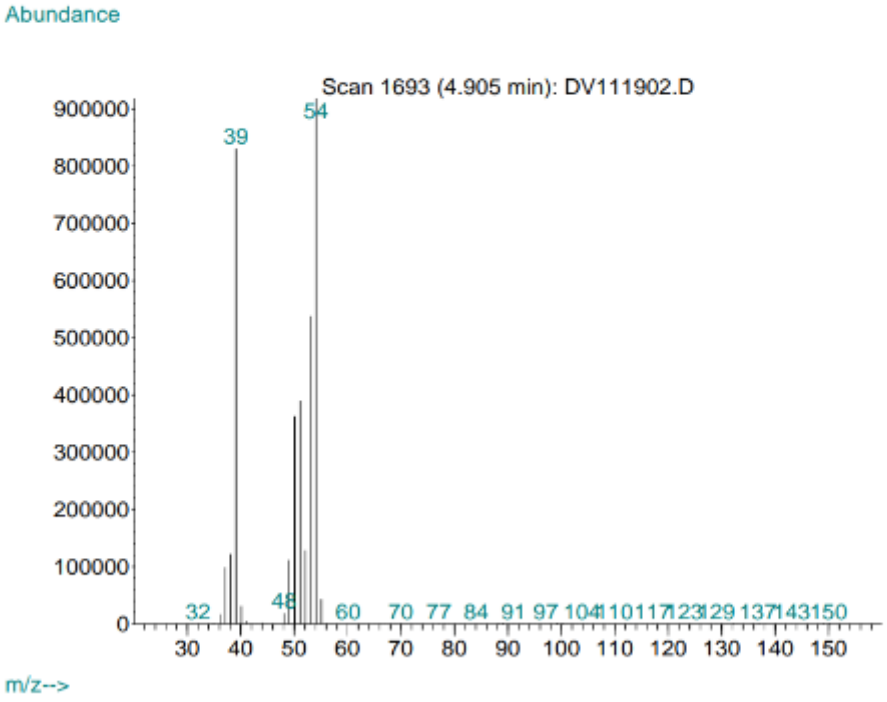


Figure 2: Mass Spectra of the 1-Methylcyclopropene Peak in a Sample Preparation of SmartFresh™ 3.3 % Technology



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Appendix 2 Specification and Certificates of Analysis for Multiple Lots

Appendix 2.1 Specification Sheet

Appendix 2.2. 1-Methylcyclopropene (1-MCP) Batch 01

Appendix 2.3 1-Methylcyclopropene (1-MCP) Batch 02

Appendix 2.4 1-Methylcyclopropene (1-MCP) Batch 03

Appendix 2.5 1-Methylcyclopropene (1-MCP) Batch 04

Appendix 2.6 1-Methylcyclopropene (1-MCP) Batch 05

Appendix 2.1 Specification Sheet

Address for correspondence:
Wileńska 4/A017
87-100 Toruń, Poland
tel.: + 48 512 038 628
andrzej.wolan@freshinset.pl

Freshinset

CONFIDENTIAL

Date: October 17, 2022

INFORMATION TO BE USED EXCLUSIVELY
FOR PRODUCT REGISTRATION PURPOSES ONLY

CERTIFICATE OF COMPOSITION

1-Methylcyclopropene (1-MCP)
3.330 % w/w (33.30 g/kg)
The certificate is made according to the analysis of 5 batches of 1-MCP technical material
(study number: DNA5387)

Ingredient	IUPAC Name	Function/ Chemical group	CAS N°	Content % w/w
1-MCP	1-methylcyclopropene	Active ingredient/cyclopropenes	3100-04-7	3.330 ± 0.0155

Ingredient	IUPAC Name	Function/ Chemical group	CAS N°	Concentration	
				Content % w/w	% w/w ** maximum
1-CMP	1-chloro-2-methylpropene	Impurities/vinyl chlorides	513-37-1	0.00142 ± 0.00041 *	≤0.02*
3-CMP	3-chloro-2-methylpropene	Impurities/allyl chlorides	563-47-3	0.00524 ± 0.00082 *	≤0.02*
Exo isomer	Methylidene Cyclopropane	Impurities/cyclo propanes	6142-73-0	0.0465 ± 0.00050	≤1.96*

* – Provided content of impurity is relative to the 1-MCP
** – The overall purity of the product according to EU regulations should be 980g/kg.
The upper limits for the content of impurities: 1-CMP and 3-CMP are defined by the regulation and their content should not exceed 0.2 g/kg.

Appendix 2.2 1-Methylcyclopropene (1-MCP) Batch 01

Address for correspondence:
ul. Gagarina 7/134B
87-100 Toruń, tel.: +48 512 038 628
wolan@synthex.com.pl
www.synthex.com.pl

Synthex TECHNOLOGIES

CERTIFICATE OF ANALYSIS

re-issued based on 5B analysis (study number: DNA5387)

Issue date: 17 March 2023

PRODUCT INFORMATION

PRODUCT NAME:	1-methylcyclopropene
	
PRODUCT CODE:	1-MCP 3.3% VP
CAS RN:	3100-04-7
FORMULA:	C_4H_6
MOLECULAR WEIGHT:	54,09
BATCH:	01/07052019
DNAL Sample Number:	DNA5387/1
Production date:	07.05.2019
Expiration date:	07.05.2021

Batch size: 2315 g

TECHNICAL INFORMATION

Appearance form:	1-MCP complex with α-cyclodextrin (α-CD)
Aspect:	White solid (powder)
1-MCP content on α-CD:	3.331 %
Purity of 1-MCP:	> 98 %

Impurity content	%(w/w)
Methylenecyclopropane	0.0466 %
1-CMP	0.00138 % *
3-CMP	0.00427 % *

* – provided content of impurity is relative to the 1-MCP

Andrzej Wolan, Ph.D.
Chief executive officer

Synthex Technologies Sp. z o.o.
ul. Gagarina 7/134B
87-100 Toruń
NIP: 956-229-96-41
REGON: 341218769
office@synthex.com.pl

17 March 2023

Appendix 2.3 1-Methylcyclopropene (1-MCP) Batch 02

Address for correspondence:
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87-100 Toruń, tel.: +48 512 038 628
wolan@synthex.com.pl
www.synthex.com.pl

Synthex TECHNOLOGIES

CERTIFICATE OF ANALYSIS

re-issued based on 5B analysis (study number: DNA5387)

Issue date: 17 March 2023

PRODUCT INFORMATION

PRODUCT NAME:	1-methylcyclopropene
	
PRODUCT CODE:	1-MCP 3.3% VP
CAS RN:	3100-04-7
FORMULA:	C_4H_6
MOLECULAR WEIGHT:	54,09
BATCH:	02/24052019
DNAL Sample Number:	DNA5387/2
Production date:	24.05.2019
Expiration date:	24.05.2021

Batch size: 2223 g

TECHNICAL INFORMATION

Appearance form:	1-MCP complex with α-cyclodextrin (α-CD)
Aspect:	White solid (powder)
1-MCP content on α-CD:	3.307 %
Purity of 1-MCP:	> 98 %

Impurity content	%(w/w)
Methylenecyclopropane	0.0457 %
1-CMP	0.00199 % *
3-CMP	0.00526 % *

* – provided content of impurity is relative to the 1-MCP

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17 March 2023

Appendix 2.4 1-Methylcyclopropene (1-MCP) Batch 03

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Synthex TECHNOLOGIES

CERTIFICATE OF ANALYSIS

re-issued based on SB analysis (study number: DNA5387)

Issue date: 17 March 2023

PRODUCT INFORMATION

PRODUCT NAME:	1-methylcyclopropene
	
PRODUCT CODE:	1-MCP 3.3% VP
CAS RN:	3100-04-7
FORMULA:	C_4H_6
MOLECULAR WEIGHT:	54,09
BATCH:	03/1006019
DNAL Sample Number:	DNA5387/3
Production date:	10.06.2019
Expiration date:	10.06.2021

Batch size: 2278 g

TECHNICAL INFORMATION

Appearance form:	1-MCP complex with α-cyclodextrin (α-CD)
Aspect:	White solid (powder)
1-MCP content on α-CD:	3.338 %
Purity of 1-MCP:	> 98 %

Impurity content	%(w/w)
Methylenecyclopropane	0.0467 %
1-CMP	0.00121 % *
3-CMP	0.00479 % *

* – provided content of impurity is relative to the 1-MCP

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17 March 2023

Appendix 2.5 1-Methylcyclopropene (1-MCP) Batch 04

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Synthex TECHNOLOGIES

CERTIFICATE OF ANALYSIS

re-issued based on 5B analysis (study number: DNA5387)

Issue date: 17 March 2023

PRODUCT INFORMATION

PRODUCT NAME:	1-methylcyclopropene
	
PRODUCT CODE:	1-MCP 3.3% VP
CAS RN:	3100-04-7
FORMULA:	C_4H_6
MOLECULAR WEIGHT:	54,09
BATCH:	04/2507019
DNAL Sample Number:	DNA5387/4
Production date:	25.07.2019
Expiration date:	25.07.2021

Batch size: 2150 g

TECHNICAL INFORMATION

Appearance form:	1-MCP complex with α-cyclodextrin (α-CD)
Aspect:	White solid (powder)
1-MCP content on α-CD:	3.350 %
Purity of 1-MCP:	> 98 %

Impurity content	%(w/w)
Methylenecyclopropane	0.0468 %
1-CMP	0.00127 % *
3-CMP	0.00587 % *

* – provided content of impurity is relative to the 1-MCP

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17 March 2023

Appendix 2.6 1-Methylcyclopropene (1-MCP) Batch 05

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Synthex TECHNOLOGIES

CERTIFICATE OF ANALYSIS

re-issued based on 5B analysis (study number: DNA5387)

Issue date: 17 March 2023

PRODUCT INFORMATION

PRODUCT NAME:	1-methylcyclopropene
	
PRODUCT CODE:	1-MCP 3.3% VP
CAS RN:	3100-04-7
FORMULA:	C_4H_6
MOLECULAR WEIGHT:	54,09
BATCH:	05/1208019
DNAL Sample Number:	DNA5387/5
Production date:	12.08.2019
Expiration date:	12.08.2021

Batch size: 2431 g

TECHNICAL INFORMATION

Appearance form:	1-MCP complex with α-cyclodextrin (α-CD)
Aspect:	White solid (powder)
1-MCP content on α-CD:	3.326 %
Purity of 1-MCP:	> 98 %

Impurity content	%(w/w)
Methylenecyclopropane	0.0466 %
1-CMP	0.00124 % *
3-CMP	0.00600 % *

* – provided content of impurity is relative to the 1-MCP

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[redacted]

17 March 2023

Appendix 3 Intake Analysis

Proposed Use and Food Codes Utilized for Intake Calculation

The dietary exposure distributions were calculated using the Creme Food Safety® model[i], a scientific cloud-based software service designed and developed to calculate dietary intakes of foods, chemicals, and nutrients in populations of consumers. This is achieved by linking food consumption data from the What We Eat In America (WWEIA) portion of the National Health and Nutrition Examination Survey (NHANES) to the appropriate food composition and chemical occurrence data using a number of validated and published models, available upon request from Crème Global (https://www.cremeglobal.com/). Calculations for this intake analysis were completed using deterministic (single points) input data. Output calculation types include daily average intakes, acute exposures, as well as population statistics such as mean, percentiles, standard errors, and confidence intervals. Results are output for “Consumers Only” (i.e., consumers of the food / substance of interest), and Total Population (consumers and non-consumers). Results of the exposure assessment are given in absolute terms (µg/day) as well as relative to the consumer’s body weight (µg/kg bw/day). The per unit of bodyweight exposure is calculated on a subject level using the bodyweight recorded by the NHANES data.

The proposed uses of 1-Methylcyclopropene (1-MCP) are provided below. 1-MCP is intended for use as a gas that surrounds cut produce and provides additional shelf-life. The cut produce food categories included in the intended use are melons, pineapples, apples, mushrooms and cabbage/coleslaw. All intended foods would be raw and shredded/sliced. The maximum estimated amount per serving of finished food product is noted in Table 1 below and for intake calculation purposes it is assumed that the maximum estimated use level is consumed. The food codes utilized to calculate the estimated daily intake were selected from the NHANES 2017 – 2020 pre-pandemic survey data. Food codes that most appropriately match the intended use were selected. The final food code selections are found in Table 2.

Table 1. Decision Criteria for Food Codes

Aggregate Category	Maximum Estimated Use Level (µg/kg)	Decision Criteria
Melons	9.086	Included all “Melons” category food codes.
Pineapples	7.273	Included “Pineapple, raw” food code. Excluded all others in “Pineapple” category as they would not be applicable to the target intended use.
Apples	4.125	Included “Apple, raw” food code. Excluded all others in “Apples” as they would not be applicable to the target intended use.
Mushrooms	9.086	Included “Mushrooms, raw” food code from “other vegetables and combinations” category.

Aggregate Category	Maximum Estimated Use Level (µg/kg)	Decision Criteria
Cabbage & Coleslaw	8.25	Included all cabbage-based or non-specific coleslaw food codes from "Coleslaw, non-lettuce salads" category. Included raw cabbage food codes from "Cabbage" category. Included all coleslaw dressing food codes from "Salad dressings and vegetable oils" category.

kg – Kilograms; µg - Micrograms

The food codes utilized in this analysis are shown below.

Table 2. 2017-2020 Pre-pandemic NHANES Food Codes Used for Intake Analysis

Food Code	Main Food Description
Melons	
63109010	Cantaloupe, raw
63110010	Cassaba melon, raw
63127010	Honeydew melon, raw
Pineapples	
63141010	Pineapple, raw
Apples	
63101000	Apple, raw
Mushrooms	
75115000	Mushrooms, raw
Cabbage & Coleslaw	
75103000	Cabbage, green, raw
75104000	Cabbage, Chinese, raw
75105000	Cabbage, red, raw
75140990	Coleslaw, fast food / restaurant
75141000	Coleslaw
75141005	Cabbage salad or coleslaw, made with light coleslaw dressing
75141020	Cabbage salad or coleslaw, made with Italian dressing
75141025	Cabbage salad or coleslaw, made with light Italian dressing
75141030	Cabbage salad or coleslaw, made with creamy dressing
75141035	Cabbage salad or coleslaw, made with light creamy dressing
75141100	Coleslaw, with fruit
75141200	Cabbage salad or coleslaw with pineapple, with dressing
75141040	Cabbage salad, NFS
83103000	Coleslaw dressing
83201400	Coleslaw dressing, light

Estimated Daily Intake of 1-Methylcyclopropene (1-MCP) from the Proposed Uses

The tables below provide Estimated Daily Intake from use in conventional foods for ages 2+ years.

Table 3. Estimated Daily Intake of Ingredient (µg/day)

Population Group	N	Percentage (%)	Ingredient Intake Per Capita (µg/day)				Ingredient Intake – All Consumers (µg/day)			
			Mean	±SE	90th Percentile	±SE	Mean	±SE	90th Percentile	±SE
Children, female (2-5 yr)	176	43.9%	0.28	0.04	0.74	0.19	0.58	0.06	1.46	0.20
Children, male (2-5 yr)	166	41.5%	0.37	0.07	0.75	0.21	0.71	0.08	1.66	0.40
Children, female (6-11 yr)	206	34.6%	0.20	0.03	0.55	0.08	0.54	0.05	1.02	0.08
Children, male (6-11 yr)	211	35.0%	0.31	0.05	0.84	0.17	0.84	0.08	1.85	0.12
Teenage, female (12-18 yr)	144	22.1%	0.16	0.03	0.50	0.14	0.60	0.05	1.50	0.16
Teenage, male (12-18 yr)	154	23.1%	0.20	0.05	0.49	0.09	0.81	0.09	2.03	0.66
Adults, female (19+ yr)	1064	30.0%	0.19	0.01	0.62	0.06	0.63	0.02	1.41	0.08
Adults, male (19+ yr)	806	24.8%	0.21	0.02	0.68	0.09	0.84	0.03	1.60	0.13
All Ages	3053	28.2%	0.21	0.01	0.62	0.04	0.71	0.02	1.48	0.03

kg – Kilograms; N – Number of users; SE – Standard error; yr – Year; µg – Micrograms

Table 4. Estimated Daily Intake of Ingredient (µg/kg bw/day)

Population Group	N	Percentage (%)	Ingredient Intake Per Capita (µg/kg bw/day)				Ingredient Intake – All Consumers (µg/kg bw/day)			
			Mean	±SE	90th Percentile	±SE	Mean	±SE	90th Percentile	±SE
Children, female (2-5 yr)	176	43.9%	0.0167	0.0027	0.0436	0.0098	0.0341	0.0031	0.0767	0.0087
Children, male (2-5 yr)	166	41.5%	0.0213	0.0039	0.0487	0.0105	0.0412	0.0040	0.1067	0.0225
Children, female (6-11 yr)	206	34.6%	0.0067	0.0013	0.0203	0.0026	0.0177	0.0019	0.0334	0.0023
Children, male (6-11 yr)	211	35.0%	0.0090	0.0015	0.0298	0.0044	0.0248	0.0022	0.0678	0.0084
Teenage, female (12-18 yr)	144	22.1%	0.0029	0.0006	0.0100	0.0022	0.0104	0.0010	0.0215	0.0031
Teenage, male (12-18 yr)	154	23.1%	0.0033	0.0008	0.0082	0.0016	0.0132	0.0015	0.0432	0.0105
Adults, female (19+ yr)	1064	30.0%	0.0026	0.0002	0.0084	0.0007	0.0088	0.0003	0.0197	0.0012
Adults, male (19+ yr)	806	24.8%	0.0023	0.0002	0.0073	0.0008	0.0092	0.0004	0.0170	0.0009
All Ages	3053	28.2%	0.0039	0.0003	0.0105	0.0006	0.0133	0.0004	0.0286	0.0010

bw – Body weight; kg – Kilograms; N – Number of users; SE – Standard error; yr – Year; µg – Micrograms

Food Intakes

Food Intake Reports of the food categories are provided below. All intakes are in grams and N equals the number of individuals reporting eating the foods and the “Percentage” is the percent of the population the “N” represents. “Per capita” intake refers to the estimated intake averaged over all individuals surveyed, regardless of whether or not they consumed food products to which the ingredient is intended to be added. Individuals were considered “consumers” if they reported

consumption of one or more food products from the selected food codes on either Day 1 or Day 2 of the survey. Sample sizes of n<30 for estimates of the mean and n<80 for estimates of the 90th percentile, respectively, may not be considered statistically reliable due to the limited sampling size.

Table 5. Summary of Consumption of All Foods g/Day (2017-2020)

Age Group	Per Capita Intake		Consumer-Only Intake			
	Mean	90th	N	Percentage	Mean	90th
Children, female (2-5 yr)	51.11	151.97	176	43.9%	104.26	224.93
Children, male (2-5 yr)	60.37	160.58	166	41.5%	117.08	219.73
Children, female (6-11 yr)	35.19	100.37	206	34.6%	93.38	165.18
Children, male (6-11 yr)	53.30	162.50	211	35.0%	147.05	330.34
Teenage, female (12-18 yr)	30.92	100.00	144	22.1%	112.56	239.98
Teenage, male (12-18 yr)	33.80	100.00	154	23.1%	134.67	323.14
Adults, female (19+ yr)	29.61	100.00	1064	30.0%	99.43	211.30
Adults, male (19+ yr)	33.36	111.50	806	24.8%	132.68	261.92
All Ages	33.33	108.86	3053	28.2%	114.47	232.97

g – Grams; N – Number of users; yr - Years

Table 6. Summary of Consumption of All Foods Grams/Kg (Body Weight)/Day (2017-2020)

Age Group	Per Capita Intake		Consumer-Only Intake			
	Mean	90th	N	Percentage	Mean	90th
Children, female (2-5 yr)	3.036	8.921	176	43.9%	6.194	12.633
Children, male (2-5 yr)	3.464	9.145	166	41.5%	6.719	14.102
Children, female (6-11 yr)	1.123	3.525	206	34.6%	2.981	5.205
Children, male (6-11 yr)	1.620	5.102	211	35.0%	4.468	9.802
Teenage, female (12-18 yr)	0.541	2.065	144	22.1%	1.968	3.786
Teenage, male (12-18 yr)	0.554	1.773	154	23.1%	2.208	5.248
Adults, female (19+ yr)	0.418	1.465	1064	30.0%	1.405	3.075
Adults, male (19+ yr)	0.370	1.341	806	24.8%	1.472	3.142
All Ages	0.645	1.903	3053	28.2%	2.216	4.744

kg – Kilograms; N – Number of users; yr - Years

Table 7. Summary of Consumption of Melons g/Day (2017-2020)

Age Group	Per Capita Intake		Consumer-Only Intake			
	Mean	90th	N	Percentage	Mean	90th
Children, female (2-5 yr)	13.43	37.43	38	9.5%	109.23	215.15
Children, male (2-5 yr)	22.58	58.81	38	9.5%	141.72	326.37
Children, female (6-11 yr)	10.61	24.10	51	8.6%	94.44	208.68
Children, male (6-11 yr)	15.78	0	45	7.5%	159.34	389.52
Teenage, female (12-18 yr)	5.55	0	31	4.8%	131.95	301.77

Age Group	Per Capita Intake		Consumer-Only Intake			
	Mean	90th	N	Percentage	Mean	90th
Teenage, male (12-18 yr)	11.79	0	28	4.2%	185.38	350.00
Adults, female (19+ yr)	9.11	0	260	7.3%	108.48	232.50
Adults, male (19+ yr)	11.53	0	223	6.9%	153.58	340.30
All Ages	10.59	0	750	6.9%	129.10	290.53

g – Grams; N – Number of users; yr - Years

Table 8. Summary of Consumption of Melons Grams/Kg (Body Weight)/Day (2017-2020)

Age Group	Per Capita Intake		Consumer-Only Intake			
	Mean	90th	N	Percentage	Mean	90th
Children, female (2-5 yr)	0.782	2.222	38	9.5%	6.359	11.676
Children, male (2-5 yr)	1.349	4.346	38	9.5%	8.466	18.554
Children, female (6-11 yr)	0.375	0.686	51	8.6%	3.342	5.141
Children, male (6-11 yr)	0.418	0	45	7.5%	4.221	7.936
Teenage, female (12-18 yr)	0.097	0	31	4.8%	2.312	4.679
Teenage, male (12-18 yr)	0.193	0	28	4.2%	3.028	5.649
Adults, female (19+ yr)	0.126	0	260	7.3%	1.505	3.291
Adults, male (19+ yr)	0.121	0	223	6.9%	1.610	4.442
All Ages	0.198	0	750	6.9%	2.416	5.225

kg – Kilograms; N – Number of users; yr - Years

Table 9. Summary of Consumption of Pineapples g/Day (2017-2020)

Age Group	Per Capita Intake		Consumer-Only Intake			
	Mean	90th	N	Percentage	Mean	90th
Children, female (2-5 yr)	1.327	0	13	3.2%	44.48	94.55
Children, male (2-5 yr)	1.130	0	11	2.8%	34.00	44.60
Children, female (6-11 yr)	1.365	0	15	2.5%	59.32	85.65
Children, male (6-11 yr)	1.476	0	16	2.7%	58.86	90.77
Teenage, female (12-18 yr)	1.675	0	15	2.3%	34.23	83.09
Teenage, male (12-18 yr)	1.070	0	13	2.0%	67.19	133.06
Adults, female (19+ yr)	1.880	0	154	4.3%	40.63	82.50
Adults, male (19+ yr)	1.608	0	104	3.2%	45.21	108.28
All Ages	1.644	0	357	3.3%	43.00	85.26

Table 10. Summary of Consumption of Pineapples Grams/Kg (Body Weight)/Day (2017-2020)

Age Group	Per Capita Intake		Consumer-Only Intake			
	Mean	90th	N	Percentage	Mean	90th
Children, female (2-5 yr)	0.089	0	13	3.2%	2.995	6.438
Children, male (2-5 yr)	0.076	0	11	2.8%	2.283	3.444

Age Group	Per Capita Intake		Consumer-Only Intake			
	Mean	90th	N	Percentage	Mean	90th
Children, female (6-11 yr)	0.040	0	15	2.5%	1.752	2.518
Children, male (6-11 yr)	0.052	0	16	2.7%	2.092	3.930
Teenage, female (12-18 yr)	0.031	0	15	2.3%	0.642	1.580
Teenage, male (12-18 yr)	0.017	0	13	2.0%	1.079	2.090
Adults, female (19+ yr)	0.027	0	154	4.3%	0.580	1.497
Adults, male (19+ yr)	0.017	0	104	3.2%	0.491	1.110
All Ages	0.029	0	357	3.3%	0.755	1.986

kg – Kilograms; N – Number of users; yr - Years

Table 11. Summary of Consumption of Apples g/Day (2017-2020)

Age Group	Per Capita Intake		Consumer-Only Intake			
	Mean	90th	N	Percentage	Mean	90th
Children, female (2-5 yr)	36.25	100.00	145	36.2%	89.80	176.12
Children, male (2-5 yr)	36.51	100.00	132	33.0%	96.44	200.00
Children, female (6-11 yr)	22.85	100.00	156	26.2%	86.07	150.00
Children, male (6-11 yr)	35.23	100.00	162	26.9%	129.33	279.43
Teenage, female (12-18 yr)	22.83	100.00	98	15.0%	122.63	221.00
Teenage, male (12-18 yr)	20.65	100.00	115	17.3%	118.59	200.00
Adults, female (19+ yr)	15.17	82.50	566	15.9%	109.05	200.00
Adults, male (19+ yr)	17.50	82.50	420	12.9%	133.73	242.00
All Ages	18.64	100.00	1879	17.3%	114.94	200.00

g – Grams; N – Number of users; yr - Years

Table 12. Summary of Consumption of Apples Grams/Kg (Body Weight)/Day (2017-2020)

Age Group	Per Capita Intake		Consumer-only Intake			
	Mean	90th	N	Percentage	Mean	90th
Children, female (2-5 yr)	2.158	6.445	145	36.2%	5.346	9.332
Children, male (2-5 yr)	2.030	6.167	132	33.0%	5.362	11.460
Children, female (6-11 yr)	0.697	2.745	156	26.2%	2.625	4.744
Children, male (6-11 yr)	1.129	3.861	162	26.9%	4.145	9.755
Teenage, female (12-18 yr)	0.401	1.692	98	15.0%	2.153	4.010
Teenage, male (12-18 yr)	0.341	1.525	115	17.3%	1.956	3.660
Adults, female (19+ yr)	0.218	1.005	566	15.9%	1.566	2.925
Adults, male (19+ yr)	0.201	0.863	420	12.9%	1.534	2.948
All Ages	0.386	1.325	1879	17.3%	2.380	4.773

kg – Kilograms; N – Number of users; yr - Years

Table 13. Summary of Consumption of Mushrooms g/Day (2017-2020)

Age Group	Per Capita Intake		Consumer-Only Intake			
	Mean	90th	N	Percentage	Mean	90th
Children, female (2-5 yr)	0	0	0	0.0%	0	0
Children, male (2-5 yr)	0	0	0	0.0%	0	0
Children, female (6-11 yr)	0	0	0	0.0%	0	0
Children, male (6-11 yr)	0.05	0	2	0.3%	9.99	10.73
Teenage, female (12-18 yr)	0.01	0	1	0.2%	17.50	17.50
Teenage, male (12-18 yr)	0.07	0	1	0.2%	8.75	8.75
Adults, female (19+ yr)	0.14	0	38	1.1%	10.21	28.21
Adults, male (19+ yr)	0.11	0	27	0.8%	9.09	20.24
All Ages	0.10	0	69	0.6%	9.70	25.14

g – Grams; N – Number of users; yr - Years

Table 14. Summary of Consumption of Mushrooms Grams/Kg (Body Weight)/Day (2017-2020)

Age Group	Per Capita Intake		Consumer-Only Intake			
	Mean	90th	N	Percentage	Mean	90th
Children, female (2-5 yr)	0	0	0	0.0%	0	0
Children, male (2-5 yr)	0	0	0	0.0%	0	0
Children, female (6-11 yr)	0	0	0	0.0%	0	0
Children, male (6-11 yr)	0.0019	0	2	0.3%	0.394	0.426
Teenage, female (12-18 yr)	0.0001	0	1	0.2%	0.163	0.163
Teenage, male (12-18 yr)	0.0006	0	1	0.2%	0.078	0.078
Adults, female (19+ yr)	0.0021	0	38	1.1%	0.147	0.491
Adults, male (19+ yr)	0.0013	0	27	0.8%	0.102	0.244
All Ages	0.0014	0	69	0.6%	0.130	0.317

kg – Kilograms; N – Number of users; yr - Years

Table 15. Summary of Consumption of Cabbage & Coleslaw g/Day (2017-2020)

Age Group	Per Capita Intake		Consumer-Only Intake			
	Mean	90th	N	Percentage	Mean	90th
Children, female (2-5 yr)	0.11	0	4	1.0%	11.35	41.25
Children, male (2-5 yr)	0.14	0	9	2.3%	7.79	25.34
Children, female (6-11 yr)	0.37	0	12	2.0%	18.25	41.16
Children, male (6-11 yr)	0.76	0	9	1.5%	54.26	137.50
Teenage, female (12-18 yr)	0.84	0	14	2.1%	28.97	72.52
Teenage, male (12-18 yr)	0.22	0	8	1.2%	29.44	81.47
Adults, female (19+ yr)	3.31	0	249	7.0%	46.59	109.50
Adults, male (19+ yr)	2.60	0	177	5.4%	53.81	112.50
All Ages	2.35	0	484	4.5%	47.65	110.00

g – Grams; N – Number of users; yr - Years

Table 16. Summary of Consumption of Cabbage & Coleslaw Grams/Kg (Body Weight)/Day (2017-2020)

Age Group	Per Capita Intake		Consumer-Only Intake			
	Mean	90th	N	Percentage	Mean	90th
Children, female (2-5 yr)	0.008	0	4	1.0%	0.834	3.078
Children, male (2-5 yr)	0.009	0	9	2.3%	0.511	1.882
Children, female (6-11 yr)	0.011	0	12	2.0%	0.533	1.470
Children, male (6-11 yr)	0.018	0	9	1.5%	1.268	3.132
Teenage, female (12-18 yr)	0.011	0	14	2.1%	0.379	1.039
Teenage, male (12-18 yr)	0.003	0	8	1.2%	0.401	0.879
Adults, female (19+ yr)	0.045	0	249	7.0%	0.636	1.547
Adults, male (19+ yr)	0.030	0	177	5.4%	0.617	1.602
All Ages	0.031	0	484	4.5%	0.626	1.602

kg – Kilograms; N – Number of users; yr - Years

Appendix 4 GRAS Associates Expert Panel Report

The Generally Recognized as Safe (GRAS) Status of the Proposed Uses of 1-Methylcyclopropene (1-MCP)

August 23, 2023

Foreword

An independent panel of experts ("Expert Panel") was convened by GRAS Associates, LLC on behalf of their client, Fresh Inset S.A., to evaluate the safety and Generally Recognized as Safe (GRAS) status of Fresh Inset S.A.'s proposed uses of 1-Methylcyclopropene (1-MCP) in conventional foods. The members of this Expert Panel† are qualified to serve in this capacity by qualification of scientific training and experience in the safety of food and food ingredients.

GRAS Associates and Fresh Inset S.A. ensured that all reasonable efforts were made to identify and select a balanced Expert Panel with expertise in food safety, toxicology, and nutrition. The Expert Panel was selected and convened in accordance with the Food and Drug Administration (FDA)'s guidance for industry on "Best Practices for Convening a GRAS Panel"[1]. Efforts were placed on identifying conflicts of interest or relevant "appearance issues" that could potentially bias the outcome of the deliberations of the Expert Panel and no such conflicts of interest or "appearance issues" were identified. The Expert Panel members received a reasonable honorarium as compensation for their time; the honoraria provided to the Expert Panel members were not contingent upon the outcome of their deliberations.

Discussion

1-MCP is used at two different concentrations in the Vidre+ technology. Vidre+ contains 1000 g 1-Methylcyclopropene (1-MCP) Technical (which is 3.3% 1-MCP + 96.7 % α-cyclodextrin) and 1000 g Polyvinylpyrrolidone (PVP), a 50:50 (w/w) mix. Vidre+ Low Concentration contains 100 g 1-MCP Technical, 1000 g PVP and an additional 900 g α-cyclodextrin. The concentrations of 1-MCP in Vidre+ and Vidre+ Low Concentration are therefore 1.65% and 0.165%, respectively. The α-cyclodextrin has been notified as GRAS to FDA (FDA, 2004) and the PVP meets Food Chemicals Codex (FCC) specifications. PVP is approved as a secondary direct food additive per 21 CFR 173.55. Compared to The Food and Agriculture Organization of the United Nations (FAO) specifications for 1-MCP (FAO, 2010), specifications for the 1-MCP that is used for the Vidre+ technology are more

† Dr. Robert Martin holds a Ph.D. in Chemistry with over 38 years of experience evaluating safety of food ingredients within FDA. He is a former deputy director of FDA's Division of Biotechnology and GRAS Notice Review. Dr. Marilyn Aardema is President of Marilyn Aardema Consulting, LLC. She is a globally recognized leader with over 30 years' experience in the area of genetic toxicology and human safety assessment with over 100 publications. Dr. Margitta Dziwenka, the Expert Panel Chair, holds a Doctor of Veterinary Medicine (DVM) and is a Diplomate with the American Board of Toxicology. She has over 24 years' experience as a practicing veterinarian as well as over 20 years of experience in research, preclinical regulatory toxicology, and safety evaluation of food and animal feed additives and GRAS dossier preparation. All three panelists have extensive technical backgrounds in the evaluation of food ingredient safety and in participating in deliberations of GRAS Expert Panels

[1] Available at: https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm583856.htm (Accessed Aug. 23, 2023).

stringent. FCC grade isopropyl alcohol is mixed with either Vidre+ formulation to prepare a homogeneous mixture that is applied in a thin layer to the surface of a food grade paper or polypropylene sticker. Samples from the printed material are taken and analyzed for 1-MCP content according to a published analytical method. If the product does not meet the quality control standard, it is not utilized.

In the dossier, Fresh Inset S.A. has stated the following conditions of use. The Vidre+ mixture is applied to the sticker at a rate of 1 g of 1-MCP Technical[2] per square meter of paper, which corresponds to 33 mg 1-MCP/m^2. A sticker size of 1 cm^2 would be used for a 1 liter package to provide 1 µL 1-MCP gas, or 1 ppm and a 1 cm^2 sticker would contain 3.3 µg of 1-MCP. Vidre+ Low Concentration will be applied at the same rate as Vidre+, which corresponds to 0.33 µg 1-MCP/cm^2 sticker given that the concentration of 1-MCP in Vidre+ Low Concentration is 1/10^{th} of Vidre+. Vidre+ Low Concentration will be used for small packages < 2 L in size due to the 1/10^{th} concentration allowing the use of a bigger sticker. These levels are below the threshold accepted in FDA's threshold of Regulation at 21 CFR 170.39. Instructions for use with the sticker will give specific guidelines to use one sticker of the proper size for each package. Stickers will be placed/adhered to the inside of lids of clamshell containers housing sliced or cut pineapple, mushrooms, or melon or inside plastic produce bags containing coleslaw or sliced apples. Under typical storage conditions, the active ingredient, 1-MCP, would be released from the Vidre+ sticker as a gas into the air surrounding the packaged produce. The 1-MCP gas will be released into the closed container over 25-50 hours, depending on storage temperature and humidity. The intent of the Vidre+ products will be to mitigate the action and formation of ethylene in a controlled fashion, which should extend shelf life of the cut produce. The Expert Panel has reviewed the use information provided in the dossier and has no concerns.

The Expert Panel reviewed the estimated intake of 1-MCP information as provided by Fresh Inset S.A. in the dossier. For the purpose of estimating consumption of the substance, Fresh Inset S.A. assumed that consumers of the produce in the containers will consume all of the 1-MCP that would be released onto the produce from the stickers. On a daily intake basis, teenage males are estimated to consume the most 1-MCP per day from the intended use (2.03 µg/day) while male children (2-5 years) are the highest consumer on a body weight basis (0.1067 µg/kg bw/day). This estimate is conservative in that it assumes all produce consumed has been exposed to 1-MCP. Because 1-MCP has been approved for direct or post-harvest use on crops in the United States, there could be some background exposure to 1-MCP from use on crops, which was added to estimated exposure to 1-MCP from the intended use to determine cumulative exposure. However, as 1-MCP is volatile, it is expected that intake from direct use on crops would be negligible. Because apples used for processing may be stored for a considerable amount of time, it is possible that they may be exposed to 1-MCP during storage. Therefore, by adding the intake of 0.27 µg 1-MCP/kg bw from post-harvest use on apples to the highest EDI intake a body weight basis (0.1067 µg/kg bw/day) from the intended use, highest cumulative exposure of 1-MCP on a body weight basis is estimated to be 0.38 µg/kg

[2] Since the composite mix is 50/50 mix of "1-MCP Technical" and PVP, and the composite (50:50) mix is mixed with isopropanol and then coated on the paper, the paper is actually coated with 2 grams of the 50/50 mix (1 gram of 1-MCP Technical and 1 gram of PVP) per square meter.

bw/day (the value for male children 2-5 years). The Expert Panel concurs with Fresh Inset S.A.'s estimated daily intake assumptions and has not concerns.

Regulatory approval for use of 1-MCP has been obtained in more than 50 countries, and approval for use of the technology continues to occur around the world (Mahajan et al., 2014). In 2002, commercial application of a 1-MCP/ α-cyclodextrin formulation to edible crops was undertaken by AgroFresh, Inc. under the trade name SmartFresh® (Beaudry, 2021a; Blankenship and Dole, 2003). The content of 1-MCP in Smart Fresh® is 33 g/kg or 3.3%, with a range of 30 – 36 g/kg (EFSA, 2005). This product became available after EPA issued a final rule (67 FR 48976) for 1-MCP, codified at 40 CFR 180.1220, when used as a post-harvest plant growth regulator for the purpose of inhibiting the effects of ethylene (EPA, 2002). FDA had no questions on the GRAS notification for the use of 1-MCP for packaging fruits and vegetables in GRN 585 (FDA, 2016). Other regulatory groups that have approved the use of 1-MCP on fruits and vegetables include EFSA and Health Canada.

The results of several toxicology studies were made available to EPA, FDA and EFSA as part of their evaluations for 1-MCP and reviewed by the Expert Panel. These include genetic toxicity, acute, subchronic, and reproductive/ developmental toxicity, as well as endocrine disruption, allergenicity, eye and skin irritation and allergenicity studies. Although most of the studies have been performed with 1-MCP vapor, some (including subchronic toxicity studies in rats and dogs with "HAIP", which contained 4.7% 1-MCP) have been performed with oral formulations. Results of these studies have been favorable and have been accepted by authoritative bodies as providing evidence of safety of 1-MCP. The Acceptable Daily Intake (ADI) for 1-MCP which was determined by EFSA is 0.02 mg/kg bw/day (20 µg/kg bw/day) (EFSA, 2018), higher than the cumulative exposure of 1-MCP on a body weight basis estimated by Fresh Inset S.A. per the intended use outlined in the dossier. This ADI was determined by applying an uncertainty factor of 200 to the 90-day oral NOAEL in dogs of 4.1 mg 1-MCP/kg bw per day. The NOAEL used for the EFSA evaluation is lower than NOAELs reported for subchronic toxicity studies of 1-MCP in rats (by either oral or inhalation exposure). For GRN 585, the NOAEL used for the purpose of safety assessment was 9 mg 1-MCP/kg bw/day from a 90-day inhalation study in rats.

Possible contaminants of 1-MCP include 1-chloro-2-methylpropene (1-CMP), (3-CMP) and methylene cyclopropane, all of which have been found in 1-MCP. It is also possible that consumers of the produce could be exposed to isopropanol leaching from the sticker. Specifications that have been established for maximum concentrations of 1-CMP, 3-CMP and methylene cyclopropane ensure that exposure to these substances from use of 1-MCP as indicated above would not be a toxicological concern. It is also possible that consumers of the produce could be exposed to isopropanol from the substance leaching from the sticker; however, exposure would likely be less than the EPA-derived chronic reference dose (RfD) for oral exposure to isopropanol of 2 mg/kg bw/day (EPA, 2014). The Expert Panel has no concerns regarding the exposure of consumers to potential contaminants.

In summary, the Expert Panel agrees with Fresh Inset S.A. that there is sufficient qualitative and quantitative scientific evidence to support the safety-in-use of Fresh Inset S.A.'s 1-Methylcyclopropene (1-MCP) preparation given the following conditions:

- Fresh Inset S.A.'s 1-Methylcyclopropene (1-MCP) preparation continues to meet the designated specifications; and

- The proposed intended use and use levels do not change

Conclusion

We, the undersigned independent qualified members of the GRAS Panel, have individually and collectively critically evaluated the data provided by Fresh Inset S.A., as well as other publicly available information about the safety and use of 1- Methylcyclopropene, and conclude that 1-Methylcyclopropene is Generally Recognized as Safe (GRAS) based on scientific procedures, under the conditions of intended use specified herein.

It is our opinion that other qualified experts would concur with these conclusions.

Margitta Dziwenka, DVM, DABT
Chair

Robert Martin, PhD

Marilyn Aardema, PhD

References

Beaudry, R. (2021) Options for Sourcing 1-MCP for 2021: Michigan State University Department of Horticulture. Available at: https://www.canr.msu.edu/news/options-for-sourcing-1-mcp (Accessed: August 16, 2023).

Blankenship, S. M. and Dole, J. M. (2003) '1-Methylcyclopropene: a review', Postharvest Biology and Technology, 28(1), pp. 1-25.

EFSA (2005) 'Conclusion regarding the peer review of the pesticide risk assessment of the active substance 1-methylcyclopropene finalized 2 May 2005', EFSA Scientific Report, 30, pp. 1-46.

EFSA (2018) 'Peer review of the pesticide risk assessment of the active substance 1-methylcyclopropene', EFSA Journal, 16(7), pp. 5308.

EPA (2002) Final Rule: 1-Methylcyclopropene; Exemption from the Requirement of a Tolerance. 67 FR 48796, July 26, 2002. Available at: https://www.govinfo.gov/content/pkg/FR-2002-07-26/pdf/02-18868.pdf.

FDA (2016) *GRAS Notice (GRN) 585, 1-methylcyclopropene*. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=585.

FAO (2010) FAO Specifications and Evaluations for Agricultural Pesticides: 1-Methylcyclopropene. Available at: https://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/1-MCP10.pdf.

Mahajan, P. V., Caleb, O. J., Singh, Z., Watkins, C. B. and Geyer, M. (2014) 'Postharvest treatments of fresh produce', Philosophical Transactions of The Royal Society, A 372, pp. 20130309.

END

Form Approved: OMB No. 0910-0342; Expiration Date: 07/31/2022
(See last page for OMB Statement)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
001155	Sep 6, 2023
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: _____

4 For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Tim Malefyt PhD	Fresh Inset Chief Technology Officer
Organization *(if applicable)*	
Fresh Inset S.A.	
Mailing Address *(number and street)*	
Wilenska 4/A017	

City	State or Province	Zip Code/Postal Code	Country
Torun	Kujawsko-Pomorskie	87-100	Poland

Telephone Number	Fax Number	E-Mail Address
+1 267-808-0540		Tim.malefyt@freshinset.pl

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position or Title
William Rowe	President and CEO
Organization *(if applicable)*	
GRAS Associates, LLC	
Mailing Address *(number and street)*	
11810 Grand Park Avenue, Suite 500	

City	State or Province	Zip Code/Postal Code	Country
North Bethesda	Maryland	20852	United States of America

Telephone Number	Fax Number	E-Mail Address
772-532-3454		amozingo@gras-associates.com

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

1-Methylcyclopropene (1-MCP)

2. Submission Format: *(Check appropriate box(es))*

☐ Electronic Submission Gateway

☒ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

1 CD

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☐ Yes *(Proceed to Item 5)* ☒ No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☐ e) Other or Additional *(describe or enter information as above)* _____

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8) and 170.250(d) and (e))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

1-MCP is placed on the surface of a food grade sticker and placed inside the container of cut produce (melon, pineapple, apple, mushrooms, shredded cabbage/coleslaw) to extend the shelf life of the produce. 1-MCP is released from the sticker as a gas into the air surrounding the packaged produce at max. estimated exposure of 4.125-9.086 µg 1-MCP/kg produce.

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☒ Yes ☐ No

Did you include this other information in the list of attachments?

☒ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Fresh Inset S.A.
(name of notifier)

has concluded that the intended use*(s)* of 1-Methylcyclopropene (1-MCP)
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Fresh Inset S.A. *(name of notifier)* agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.

Wilenska 4/A017, 87-100 Torun, Poland
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
Amy Mozingo Digitally signed by Amy Mozingo Date: 2023.08.28 09:00:31 -04'00'	Amy Mozingo on behalf of William J. Rowe, President	08/28/2023

SECTION G – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	G10736_Transmittal Letter.pdf	Not Applicable
	G10736_FDA 3667_1-MCP.pdf	Not Applicable
	FreshInset_GRAS_1-MCP_Final_23Aug2023.pdf	Not Applicable

GRAS ASSOCIATES
Food Safety Regulatory Services,
A Nutrasource Company

GRAS Associates, LLC
11810 Grand Park Ave
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February 12, 2024

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety
Division of Petition Review
5001 Campus Drive
College Park, MD 20740-3835

Attention: Marissa Santos, MS
Re: GRN 1155 – Response to Questions Posed in the FDA Letter Dated January 26, 2024

Dear Ms. Santos:

Thank you for the opportunity to provide responses to the questions posed for GRN 1155. Per your request, GRAS Associates, LLC, acting as the agent for Fresh Inset S.A., is providing a response to FDA's questions and comments per the letter dated January 26, 2024.

FDA's Question for the Notifier and Responses

Question 1: *Please confirm if 1-methylcyclopropene (1-MCP) is manufactured in accordance with good manufacturing practices.*

Response:
The facility that produces the1-MCP Technical manufactures the material to meet the highest standards and specifications as required and is registered in the EU and with the U.S. EPA (US Reg. number 93837-2). The composite mix, which is the raw mix of 1-MCP Technical and PVP (Vidre+) for use on food, will be manufactured in a GMP facility.

The requirements regarding the purity of the product on the European Market is 980 g/kg. The Sponsor is maintaining this level of purity. Synthex is the manufacturer for the 1-MCP products in Europe. Their source of 1-methylcyclopropene (under Article 38 of Regulation (EC) No. 1107/2009 and in line with Guidance Document SANCO/10597/2003) is technically equivalent to the current reference specification for the active substance.

Question 2: *On page 7, the notifier states that toluene is used for the manufacture of 1-MCP. Please discuss residual levels of toluene in the final 1-MCP product and why there will be no safety concern with these toluene levels.*

• • •

Response:
As shown in Appendix 1 of the GRAS notice, 1-MCP Technical samples were assayed by GC-FID to determine the content of any peaks larger than 0.05% (500 ppm) w/w relative to the 1-MCP and none were found. Based on a detection limit of 0.05%, up to 500 ppm toluene could be present in the 1-MCP Technical. As stated in the GRAS Dossier, the Vidre+ mixture is applied to the sticker at a rate of 1 g of 1-MCP Technical per square meter of paper, which corresponds to 33 mg 1-MCP/square meter. We also stated in the dossier that a 1 cm^2 sticker would contain 1/10,000th of the amount of 1-MCP coated on a square meter surface, or 3.3 µg. At room temperature, toluene is not expected to volatize with 1-MCP and would remain on the sticker. The worst-case scenario exposure to toluene would be from a person inadvertently consuming a sticker. Because 500 ppm is equivalent to 500 picograms/microgram, a sticker containing 3.3 micrograms 1-MCP Technical could contain 1.65 ng toluene if the 1-MCP contained 500 ppm toluene. This is a negligible amount of toluene and is not expected to be a safety concern.

Question 3: *In Table 1 (page 9), the notifier provides the analytical methods used to test the batches for conformance with the specifications. Please confirm that the analytical methods used to test for the specification parameters have been validated for their intended purpose.*

Response:
The CIPAC 767 method (CIPAC/4669/m) is used for determining the content of 1-MCP and the X-O isomer. The CIPAC/4667/m method is used for determining the levels of 1-CMP and 3-CMP. Both of these methods are included in Appendix 1 of the Dossier. The methods are adopted by Collaborative International Pesticides Analytical Council Limited and additional validation was not performed.

Question/Comment 4: *We note that the specification range for the 1-MCP content (Tables 1-2, pages 9-10) is narrow (3.330 ± 0.0155 %). Based on the result from the analysis of batch #04/2507019 in Table 2, the 1-MCP content (3.350 %) in this batch is above the specification range. We recommend that the notifier establish a wider specification range for the 1-MCP content that is reflective of the results from the batch analyses or address how batches that do not meet specifications will be handled.*

Response:
The specification range for the 1-MCP content is revised to 3.3 ± 0.165%. This change to the specification range is still within the FAO range.

Question 5: *In Table 4 (page 13), the notifier provides the estimates of dietary exposure to 1-MCP for different population groups. Please clarify what age range the "All Ages" population group represents.*

Response:
The "All Ages" group included 0 years and above.

Question 6: *On page 15, the notifier cites the CDC growth chart for boys from birth to 24 months of age. We note that footnotes 7 and 9 provide citations to the same chart. For the administrative record, please clarify whether you intended both footnotes to cite the same information.*

• • •

Response: Yes, the source information is the same for both footnote 7 and 9. Footnote 9 should have been numbered as 7.

Question/Comment 7: O*n page 8, the notifier states that polyvinylpyrrolidone (PVP) "is approved as a secondary direct food additive per 21 CFR 173.55." We note that this regulation allows for the use of PVP as a secondary food additive in certain food categories as either a tableting adjuvant, stabilizer, bodying agent, dispersant, or clarifying agent and would not be applicable to your use of PVP. We are aware that PVP is authorized for use in multiple food contact applications. 21 CFR 175.105 permits the use of PVP as a component in adhesives used for packaging, transporting, or holding food. Please provide a statement correcting the citation on page 8 from 21 CFR 173.55 to 21 CFR 175.105.*

Response: On page 8 we cited 21 CFR 173.55 for the approval of polyvinylpyrrolidone (PVP) as a secondary direct food additive. We are correcting this citation to:
21 CFR 175.105 permits the use of PVP as a component in adhesives used for packaging, transporting, or holding food.

Sincerely,

Amy Mozingo, MS
VP US Nutra Regulatory Sciences
GRAS Associates, LLC
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March 12, 2024

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety
Division of Petition Review
5001 Campus Drive
College Park, MD 20740-3835

Attention: Marissa Santos, MS
Re: GRN 1155 – Response to Questions Posed in the FDA Letter Dated February 28, 2024

Dear Ms. Santos:

Thank you for the opportunity to provide responses to the follow up questions to the amendment dated February 12, 2024 for GRN 1155. Per your request, GRAS Associates, LLC, acting as the agent for Fresh Inset S.A. is providing a response to FDA's questions and requests per the letter dated February 28, 2024.

FDA's Question for the Notifier and Responses

Question 1: *In response to Question 1 (amendment dated February 12, 2024), you stated that 1-methylcyclopropene (1-MCP) Technical is manufactured to meet the highest standards and specifications and it is manufactured in a GMP facility. We note that your response does not directly address our question. Please confirm that 1-MCP is manufactured in accordance with good manufacturing practices.*

Response: Fresh Inset S.A. confirms that 1-MCP is manufactured in accordance with good manufacturing practices.

Question 2: *In response to Question 2 (amendment dated February 12, 2024), you stated that residual toluene was not detected in 1-MCP Technical at the limit of detection (LOD) of 0.05%. We recommend that you establish a specification limit for residual toluene based on the results from the batch analyses. In addition, please provide a revised Table 1 of GRN 001155 that includes a limit for residual toluene and the analytical method used to determine the residual toluene levels. Likewise, please provide a revised Table 2 of GRN 001155 that includes the results for toluene*.

Response: Thank you for the opportunity to update these Tables. A specification for toluene is set at <0.05%, which is the limit of detection for the method used. Revised Table 1 including the limit for

residual toluene and the analytical method used to determine the residual toluene, and a revised Table 2 including the results for toluene are provided below.

Table 1. Specifications for Fresh Inset's 1-MCP Compared to FAO Specifications

Physical and Chemical Parameters	FAO 1-MCP Specifications		Fresh Inset 1-MCP Technical Specifications	
	Specification	Method	Specification	Method
Description	Homogeneous powder mixture of 1-MCP at a concentration of 3.3% together with related manufacturing impurities, in the form of a complex with alpha-cyclodextrin, together with any other necessary co-formulants. It shall be in the form of a powder free from visible extraneous matter and added modifying agents except for the diluents.	None mentioned	Powder mixture of 1-MCP at a concentration of 3.3% together with related manufacturing impurities, in the form of a complex with alpha-cyclodextrin	None mentioned
Identification	The active ingredient shall comply with an identity test and, where the identity remains in doubt, shall comply with at least one additional test.	Note 1	Retention time matches that of 1-MCP in GC/MS	Note 1
1-MCP Content	Up to 33 g/kg ± 10% of the declared content (equivalent to up to 3.3 ± 0.3 %)	Note 1	3.3 ± 0.165 %	Note 1
3-CMP	≤ 0.05% of the 1-MCP content	Note 2	≤ 0.02% of the 1-MCP content	Note 2
1-CMP	≤ 0.05% of the 1-MCP content	Note 2	≤ 0.02% of the 1-MCP content	Note 2
Methylidine Cyclopropane (X-O isomer)*	None	None	≤ 1.96% of the 1-MCP content	Note 1
Toluene	None	None	<0.05% of the 1-MCP content	GC/FID (CIPAC)

CIPAC – Collaborative International Pesticides Analytical Council; g – Grams; GC/FID – Gas chromatography/flame ionization detector (per CIPAC method 4667; Appendix 1); GC/MS – Gas chromatography/mass spectroscopy; kg – Kilograms; 1-MCP – 1-Methylcyclopropene; 1-CMP – 1-Chloro-2-methylpropene; 3-CMP – 3-Chloro-2-methylpropene

* IUPAC name for methylene cyclopropane

Note 1: Methods for the identification and determination of 1-MCP content were presented at the CIPAC Meeting in 2009 and provisionally adopted as CIPAC methods. See Appendix 1 for method

Note 2: The independent laboratory validated capillary GC-FID method (CIPAC/4667) for the determination of the relevant impurities 3-chloro-2-methylpropene and 1-chloro-2- methylpropene in 1-MCP was adopted by CIPAC in 2009. See Appendix 1 for method.

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Table 2. Lot Conformance With Specifications for 1-MCP

Physical & Chemical Parameters	Fresh Inset S.A.s Specifications for 1-MCP	1-MCP Representative Lots				
		Batch #01/07052019	Batch #02/24052019	Batch #03/1006019	Batch #04/2507019	Batch #05/1208019
1-MCP Content	3.3 ± 0.165 %	3.331	3.307	3.338	3.350	3.326
3-CMP	≤ 0.02% of the 1-MCP content	0.00427	0.00526	0.00479	0.00587	0.00600
1-CMP	≤ 0.02% of the 1-MCP content	0.00138	0.00199	0.00121	0.00127	0.00124
Methylidine Cyclopropane (X-O isomer)*	≤ 1.96% of the 1-MCP content	0.0466	0.0457	0.0467	0.0468	0.0466
Toluene	<0.05% of the 1-MCP content**	<0.05%	<0.05%	<0.05%	<0.05%	<0.05%

1-MCP – 1- Methylcyclopropene; 1-CMP – 1-Chloro-2-methylpropene; 3-CMP – 3-Chloro-2-methylpropene
* IUPAC name for methylene cyclopropane; **0.05% is the limit of detection.

Question 3: *In response to Question 3 (amendment dated February 12, 2024), you stated that the CIPAC methods listed in Table 1 (page 9 of GRN 001155) are included in Appendix 1 of GRN 001155, were adopted by the Collaborative International Pesticides Analytical Council (CIPAC), and that additional validation was not performed. We note that your response does not directly address our question. We also note that Appendix 1 (pages 42 and 50) describes the CIPAC methods as draft methods. Please confirm that all analytical methods used to test for the specification parameters have been validated for their intended purpose.*

Response: Fresh Inset S.A. confirms that all analytical methods used to test for the specification parameters have been validated for their intended purpose.

Question/Comment 4: *For the record, please provide a statement that 1-MCP is not intended for use in any products under the jurisdiction of the U.S. Department of Agriculture or in infant formula.*

Response: 1-MCP is not intended for use in any products under the jurisdiction of the U.S. Department of Agriculture or in infant formula.

Sincerely,

[redacted]

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