ORIGINAL SUBMISSON

SRS INTERNATIONAL® CORPORATION

1901 L Street, NW • Suite 250 • Washington, DC 20036-3503
Telephone (202) 223-0157/0298 • Fax (202) 835-8970
E-Mail: mainsrs@srsinternational.com http://www.srsinternational.com

Hand Delivered by Courier June 27, 2003

Office of Premarket Approval
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, NW
Washington, DC 20204

Attention: Dr. Negash Belay

- Office Address -
  Office of Premarket Approval (HFS-255)
  1110 Vermont Avenue, Room 1295
  Washington, DC 20006

- Courier Delivery Address -
  Office of Food Additive Safety
  U.S. FDA
  5100 Paint Branch Parkway
  College Park, MD 20740

Subject: Notice of Claim of GRAS Exemption from Premarket Approval Requirements

Dear Dr. Belay:

Pursuant to the procedures and guidance provided in 21 CFR 170.36 [*Proposed Rule: 62 FR 18937 - 18964, April 17, 1997*] and additional guidance provided by the Agency in a meeting on May 16, 2003, we are submitting a Claim of GRAS Exemption from Premarket Approval Requirements as specified below -

> CAREX, Inc. (as Notifier) through its agent SRS International Corporation (as Notifier's Agent) make a claim that Volatile Oil of Mustard (*Brassica spp.*), when used in or on certain foods (meats, poultry, fish and shellfish, baked goods and food products derived therefrom and either in bulk, in holding and transport containers, in display cases, or in consumer packaged forms) at levels below the level which would impart noticeable flavor to a specific subject food but at a level sufficient to provide for shelf life extension and/or an anti-spoilage effect in the specific subject food, is exempt from the premarket approval requirements

of the federal Food, Drug, and Cosmetic Act because the Notifier has determined such use is GRAS.

The Notifier provides below the information required under 21 CFR 170.36 [*Proposed Rule: 62 FR 18937 - 18964, April 17, 1997*] to be included in a claim of GRAS exemption from premarket approval requirements:

170.36(c)(1) [*Proposed*]: Claim of GRAS exemption, dated and signed by Notifier or Notifier's agent

This is provided by the present letter making such claim as above stated and is signed and dated by the Notifier's Agent.

170.36(c)(1)(i) [*Proposed*]: Name and address of the Notifier

Notifier - CAREX, Inc.
1-1-47, Chuo, Joto-ku
Osaka, 536-0005, JAPAN

Contact: Mr. Yashushi Sekiyama
Director, Marketing and Business Development

Agent - SRS International Corporation
1901 L Street NW
Suite 250
Washington, DC 20036

Contact: Dr. John A. Todhunter
Tel. (202) 223 - 0157
Fax (202) 835 - 8970
e-mail: todhunter@srsinternational.com

170.36(c)(1)(ii) [*Proposed*]: Common or Usual Name of the Substance

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(1)(iii) [*Proposed*]: Applicable Conditions of Use [including foods in which the notified substance is to be used, levels of use in such foods, purpose for which the substance is used, and (if appropriate), description of the population expected to consume the substance.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(1)(iv) [*Proposed*]: Basis for the GRAS Determination

The Notifier has relied on scientific procedures for its determination of the GRAS status of the use which is claimed as exempt.

170.36(c)(1)(v) [*Proposed*]: Statement as to availability for FDA review and copying of data and information that are the basis for the Notifier's GRAS determination

The data and information that are the basis of the Notifier's GRAS determination are available for the Food and Drug Administration's (FDA) review and copying at reasonable times at the address specified below

SRS International Corporation
1901 L Street, NW / Suite 250 Tel. (202) 223 - 0157
Washington, DC 20036 Fax (202) 835-8970

or will be sent to FDA upon request.

170.36(c)(2) [*Proposed*]: Detailed information about the identity of the notified substance.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(3) [*Proposed*]: Information on any self-limiting levels of use.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(4) [*Proposed*]: Detailed summary of the basis for the Notifier's determination that the notified substance is GRAS.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(4)(i)(A) [*Proposed*]: Comprehensive discussion of, and citations to, generally available and accepted scientific data, information, methods, or principles the Notifier relies on to establish safety, including a discussion of the probable consumption of the substance and the effects thereof.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(4)(i)(B) [*Proposed*]: Comprehensive discussion of reports of investigations or other information that may appear to be inconsistent with the GRAS determination.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(4)(i)(C) [*Proposed*]: The basis for concluding, in light of the data and information under paragraphs (c)(1), (c)(2), (c)(3), (c)(4)(i)(A), and (c)(4)(i)(B) that there is consensus among experts qualified by scientific training and experience to evaluate the safety of substances added to food that there is reasonable certainty that the substance is not harmful under the intended conditions of use.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

Respectfully submitted on behalf of CAREX, Inc.

_____

John A. Todhunter, Ph.D.
President,
SRS International Corporation
Agents for CAREX, Inc.

_____

Date

INFORMATION ATTACHMENT TO NOTICE OF GRAS EXEMPTION CLAIM FOR:

VOLATILE OIL OF MUSTARD (VOM) AS A SHELF LIFE EXTENSION AND ANTI-SPOILAGE AGENT FOR CERTAIN FOODS

NOTIFIER:

CAREX, INC.
1-1-47, Chuo, Joto-ku
Osaka, 536-0005, Japan

NOTIFIER'S AGENT:

SRS INTERNATIONAL CORPORATION
1901 L Street, NW / Suite 250
Washington, DC 20036

June 27, 2003

TABLE OF CONTENTS

REFERENCED GRAS EXEMPTION CLAIM

This Information Attachment is incorporated by reference into a GRAS Exemption Claim dated June 27, 2003 and submitted on behalf of the Notifier (CAREX, Inc.) by the Notifier's Agent (SRS International Corporation) to the Office of Premarket Approval (HFS-200), Center for Food Safety and Applied Nutrition. Food and Drug Administration, 200C Street, SW Washington, DC 20204.

The referenced GRAS Exemption Claim makes a claim that Volatile Oil of Mustard (*Brassica spp.*), when used in or on certain foods (meats, poultry, fish and shellfish, baked goods and food products derived therefrom and either in bulk, in holding and transport containers, in display cases, or in consumer packaged forms) at levels below the level which would impart noticeable flavor to a specific subject food but at a level sufficient to provide for shelf life extension and/or an anti-spoilage effect in the specific subject food, is exempt from the premarket approval requirements of the federal Food, Drug, and Cosmetic Act because the Notifier has determined such use is GRAS.

This Information Attachment provides certain information, specified in the referenced GRAS Exemption Claim, which is required to be provided as part of such a GRAS Exemption Claim by 21 CFR 170.36 [*Proposed Rule: 62 FR 18937 - 18964, April 17, 1997*].

The information provided herein consists of the following:

170.36(c)(1)(ii) [*Proposed*]: Common or Usual Name of the Substance

170.36(c)(1)(iii) [*Proposed*]: Applicable Conditions of Use [including foods in which the notified substance is to be used, levels of use in such foods, purpose for which the substance is used, and (if appropriate), description of the population expected to consume the substance.

170.36(c)(2) [*Proposed*]: Detailed information about the identity of the notified substance.

170.36(c)(3) [*Proposed*]: Information on any self-limiting levels of use.

170.36(c)(4) [*Proposed*]: Detailed summary of the basis for the Notifier's determination that the notified substance is GRAS.

170.36(c)(4)(i)(A) [*Proposed*]: Comprehensive discussion of, and citations to, generally available and accepted scientific data, information, methods, or principles the Notifier relies on to establish safety, including a discussion of the probable consumption of the substance and the effects thereof.

170.36(c)(4)(i)(B) [*Proposed*]: Comprehensive discussion of reports of investigations or other information that may appear to be inconsistent with the GRAS determination.

170.36(c)(4)(i)(C) [*Proposed*]: The basis for concluding, in light of the data and information under paragraphs (c)(1), (c)(2), (c)(3), (c)(4)(i)(A), and (c)(4)(i)(B) that there is consensus among experts qualified by scientific training and experience to evaluate the safety of substances added to food that there is reasonable certainty that the substance is not harmful under the intended conditions of use.

170.36(c)(1)(ii) [*Proposed*]: Common or Usual Name of the Substance

The common or usual name of the notified substance is -

Volatile Oil of Mustard

and it is also commonly known as -

Volatile mustard oil
Mustard oil
Oil of black mustard
Oleum sinapis
Senfoel
Allyl mustard oil
Allyl Isothiocyanate (natural)
Allyl thioisocyanate (natural)

This substance is referred to in the balance of this Information Attachment as "VOM".

170.36(c)(1)(iii) [*Proposed*]: Applicable Conditions of Use [including foods in which the notified substance is to be used, levels of use in such foods, purpose for which the substance is used, and (if appropriate), description of the population expected to consume the substance.

The specific use of VOM which is claimed as exempt from premarket approval requirements on the basis that the Notifier has determined that such use is GRAS is the use of VOM in or on certain foods (meats, poultry, fish and shellfish, baked goods and food products derived therefrom and either in bulk, in holding and transport containers, in display cases, or in consumer packaged forms) at levels below the level which would impart noticeable flavor to a specific subject food but at a level sufficient to provide for shelf life extension and/or an anti-spoilage effect in the specific subject food.

Foods in Which the Substance is to be Used:

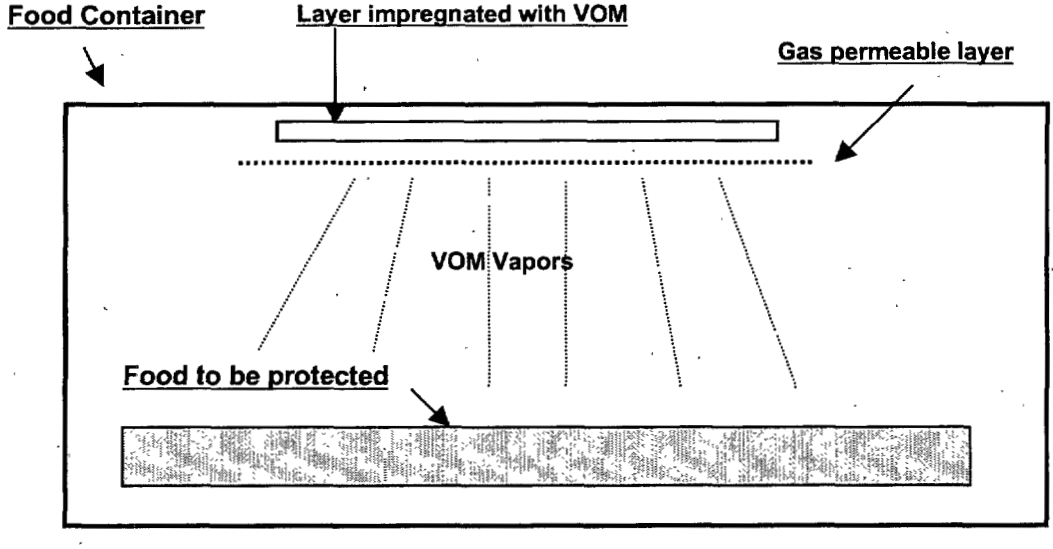
The foods in which the Notified Substance is to be used include meats, poultry, fish and shellfish, baked goods and food products derived therefrom and either in

bulk, in holding and transport containers, in display cases, or in consumer packaged forms.

VOM will be used in such foods by being incorporated into one of the layers (either the adhesive layer or a structural layer) of either a plastic film, paper tape, or labeling film which will serve as a delivery vehicle and reservoir for the VOM and which are sufficiently gas permeable as to allow the contained VOM to volatilize over time. For use, the VOM containing film, tape, or labeling is affixed to the inside of a food container or display case or used as film wrap for food. The volatilization of the VOM from the VOM impregnated material provides for exposure of the food to VOM vapors for the purpose of protection from spoilage. An schematic illustration of this concept is provided below.

As an alternative mode of use, VOM containing "packets" are prepared by impregnation of cellulose beads with VOM and enclosing these within a gas permeable, multilayer film container.

Figure: VOM gaseous delivery schematic representation



000037

Levels of Use for the Notified Substance

The notified substance, VOM, will be used at levels below those which produce a noticeable flavor effect in the subject food but sufficient to provide the intended technical effect of shelf life extension and/or spoilage retardation ("anti-spoilage")

Usual VOM levels in some foods (selected from FASEB 1975, Table 1) which result from the flavoring uses of mustard oil are:

	Usual	Maximum
Meat products	35 ppm	61 ppm
Baked goods	14 ppm	29 ppm
Gravies, sauces	46 ppm	201 ppm

Therefore, the VOM levels in foods would be less than the above-cite "usual" flavoring levels.

The shelf life extension / anti-spoilage use has only the intended technical effect of retarding the spoilage of food with the primary intention not being to affect the organoleptic properties of the food. Published literature (see the Bibliography[1]) establishes that the levels needed to inhibit the growth of microorganisms in or on foods are from:

2 - 6 ppm for fungi/molds;
10 ppm for bacteria; and,
20 - 35 ppm for yeast

000038

in the growth zone for these organisms.

Therefore, while VOM concentrations which inhibit the growth of yeast can approach the "usual" level for flavoring: (a) inhibition of fungi/molds and bacteria is attained at much less usual typical flavoring levels; and, (b) adequate inhibitory action against yeast can be obtained at less then the "usual" flavoring levels for VOM.

Also, since the inhibitory concentration needs only to be achieved in the potential growth zone for the microorganisms, VOM concentration is not distributed throughout the whole food being protected. This is readily conceptualized when one considers the example of a piece of meat in a VOM atmosphere. Transfer of VOM across the meat / air interface will be efficient as there is no diffusion barrier. Further diffusion of the VOM into the meat from the interface zone will be slower due to the need for the VOM to diffuse into a semi-solid matrix. Therefore, VOM concentrations near the surface of the meat (i.e., in the potential

[1] Foter and Golick, 1938; Tokuoka *et al.*, 1992; Isshiki *et al.*, 1992; Tokuoka and Isshiki, 1994; Holley, 1997; Delaquis and Sholberg, 1997; Ward *et al.*, 1998; Park *et al.*, 2000

microbial growing zone) will be higher than they will be deeper into the meat itself. Accordingly, flavoring of the entire food is not intended nor expected and this is borne out by the notification sponsor's experience with it's brand of VOM (Wasa Ouro) in use outside the U.S.

Purpose for which the Notified Substance is to be Used

The purpose for which VOM will be used under the claimed GRAS exemption from premarket approval requirements is for shelf life extentions and/or spoilage retardation in or on certain foods (meats, poultry, fish and shellfish, baked goods and food products derived therefrom and either in bulk, in holding and transport containers, in display cases, or in consumer packaged forms).

Description of the Population Expected to Consume the Substance

Due to the broad nature of the foods in which the Notified Substance is to be used the population expected to consume the substance is the entire U.S. population and all age groups within this population.

170.36(c)(2) [*Proposed*]: Detailed information about the identity of the notified substance.

(a) Common Names

Volatile Oil of Mustard

Also known as -
Mustard oil
Oil of black mustard
Oleum sinapis
Senfoel
Volatile mustard oil
Allyl mustard oil

(b) Chemical Name (of main consituent)

1-Propene, 3-isothiocyanato- (9CI) (CA INDEX NAME)

Also known as -
Isothiocyanic acid, allyl ester (6CI, 8CI)
2-Propenyl isothiocyanate
3-Isothiocyanato-1-propene
Allyl isothiocyanate
Allyl thioisocyanate

(c) Chemical Abstract Service (CAS) Registry Number

57-06-7

(d) Empirical Formula

$C_4 H_5 N S$

(e) Molecular Weight

99

(f) Structural Formula

$S = C = N - CH_2 - CH = CH_2$

(g) Specifications for Food Grade Material

Per the Food Chemicals Codex (FCC), Food Grade volatile oil of mustard shall meet the specifications below –

Assay	≥ 93% allyl isothiocyanate
Arsenic (as As)	≤ 3 ppm (0.0003%)
Heavy Metals (as Pb)	≤ 40 ppm (0.004%)
Phenols	Passes FCC Specified Test

(h) Quantitative Composition

Allyl isothiocyanate	≥ 95%
Allyl cyanide (AC)	< 2%
Carbon disulfide (CS)	< 2%
AC + CS Total	2% - 3%
Water	≤ 1%

(i) Manufacturing Process (excluding trade secrets)

The VOM which is the subject of this Notification is produced in Japan. This is done by the Notifier:

CAREX, Inc.
Office:
1-1-47 Chuo, Joto-ku
Osaka, 536-0005 Japan

000040

Contact: Mr. Yasushi Sekiyama
Telephone: 81-6-6937-9255

The production process is conducted in accordance with current international (ISO) hygienic standards and good manufacturing practices for the manufacture of food chemicals and is as follows:

(1) The seeds of brown mustard (*Brassica juncea*) are pressed to remove non-volatile oils.

(2) The residue of pressed seeds is macerated with warm, deionized water and allowed to stand for one hour. This allows the endogenous myrosinase activity to liberate AIT from the endogenous sinigrin (a glucoside also known as potassium myronate).

(3) The macerate is distilled (i.e., steam distillation) to yield a volatile fraction which is typically $\geq$ 95% AIT

(4) Quality control inspections are conducted to assure that the product meets the following release specifications:

AIT Content	Minimum 94% (by GLC)
Specific Gravity, 15 ^{O}C	1.014 - 1.022
Refractive Index, 20 ^{O}C	1.523 - 1.529
Optical Rotation	$\pm 0^{O}$
Solubility in Water	Soluble in 160 to 300 parts of water
Solubility in 90% EtOH	1 : 0.5
Boiling Range	148 ^{O}C - 154 ^{O}C
Arsenic (as As)	$\leq$ 3 ppm (0.0003%)
Heavy Metals (as Pb)	$\leq$ 40 ppm (0.004%)
Phenols	Passes FCC Specified Test

000041

170.36(c)(3) [*Proposed*]: Information on any self-limiting levels of use.

The notified substance, VOM, will have self-limiting use levels. This limitation is created by that fact that use of VOM, for the herein claimed GRAS use, at levels which produce a noticeable flavor effect in the subject food will lead in most cases to food which is rejected by the consumer on the basis or taste and/or odor (of VOM).

Usual VOM levels in some foods (selected from FASEB 1975, Table 1) which result from the flavoring uses of mustard oil are:

	Usual	Maximum
Meat products	35 ppm	61 ppm
Baked goods	14 ppm	29 ppm
Gravies, sauces	46 ppm	201 ppm

Therefore, the VOM levels in foods would be less than the above-cite "usual" flavoring levels.

The shelf life extension / anti-spoilage use has only the intended technical effect of retarding the spoilage of food with the primary intention not being to affect the organoleptic properties of the food. Published literature (see the Bibliography[2]) establishes that the levels needed to inhibit the growth of microorganisms in or on foods are from:

2 - 6 ppm for fungi/molds;
10 ppm for bacteria; and,
20 - 35 ppm for yeast

in the growth zone for these organisms.

Therefore, while VOM concentrations which inhibit the growth of yeast can approach the "usual" level for flavoring: (a) inhibition of fungi/molds and bacteria is attained at much less usual typical flavoring levels; and, (b) adequate inhibitory action against yeast can be obtained at less then the "usual" flavoring levels for VOM.

170.36(c)(4) [*Proposed*]: Detailed summary of the basis for the Notifier's determination that the notified substance is GRAS.

The following is a detailed summary of the basis for the Notifier's determination that the notified substance is GRAS.

[2] Foter and Golick, 1938; Tokuoka *et al.*, 1992; Isshiki *et al.*, 1992; Tokuoka and Isshiki, 1994; Holley, 1997; Delaquis and Sholberg, 1997; Ward *et al.*, 1998; Park *et al.*, 2000

170.36(c)(4)(i)(A) [*Proposed*]: Comprehensive discussion of, and citations to, generally available and accepted scientific data, information, methods, or principles the Notifier relies on to establish safety, including a discussion of the probable consumption of the substance and the effects thereof.

and

170.36(c)(4)(i)(B) [*Proposed*]: Comprehensive discussion of reports of investigations or other information that may appear to be inconsistent with the GRAS determination.

Note: These two information items are provided in an integrated manner as they are inter-related for Volatile Oil of Mustard

Mustard, mustard oil, and other spices and spice essential oils have been used since the dawn of recorded history for their food preserving properties as well as their flavoring effects. Indeed, one is hard pressed to know whether the now-desired flavor effect of spices originated from human acculturation to consuming food preserved with mustard / mustard oil and other spices / spice oils or whether spices / spice oils where first sought for their flavoring properties and then – once in common use – their preservative properties became understood.

Various anti-spoilage uses of VOM have been reported in the technical / scientific literature since at least the 1920's. These include uses in preserving fruit juices, bread, and wine on a regular or regionally popular basis, as well as other foods on a more limited scale. (For references, see the Bibliography)

The use of VOM vapor for its anti-bacterial effects has been known since at least 1938 (Foter and Golick, 1938). Since the early 1990's a considerable amount of new research has been published on vapor-phase transfer delivery of VOM so as to achieve its known anti-spoilage effect (e.g.: Tokuoka *et al.*, 1992; Isshiki *et al.*, 1992; Tokuoka and Isshiki, 1994; Holley, 1997; Delaquis and Sholberg, 1997; Ward *et al.*, 1998; Park *et al.*, 2000).

This vapor phase method of using VOM for its anti-spoilage effect is identical to that used in the Wasa Ouro technology above-described. It consists of exposing the food to be protected to an atmosphere containing VOM vapor, during which exposure a small amount of VOM adsorbs onto the surface of the food and provides a surface protection against the growth of bacteria and fungi (both pathogens and spoilage agents). Effective control of microbial growth has been reported at VOM air concentrations ranging from 1 μg VOM / L air to 720 μg VOM / L air (see the above references). Technical studies with Wasa Ouro brand VOM establish the following vapor MIC's for VOM's effects on various microorganisms:

		(ppm in Air)	µg / L air
MOLDS	*Alternaria alternata*	≤ 20 ppm	≤ 81
	Mucor racemosus	≤ 20 ppm	≤ 81
	Penicillium chrysogenum	20 - 40 ppm	81 - 162
	other *Penicillium* spp.	≤ 20 ppm	≤ 81
	Aspergillus spp.	20 - 60 ppm	81 - 242
	Cladosporidium spp.	≤ 20 ppm	≤ 81
	Rhizopus javanicus	40 - 90 ppm	162 - 364
	Fusarium spp.	≤ 20 ppm	≤ 81
YEASTS	*Candida albicans*	≤ 20 ppm	≤ 81
	Candida tropicalis	≤ 20 ppm	≤ 81
	Torulopsis versatilis	≤ 20 ppm	≤ 81
	Debarymyces kloeckeri	21 - 60 ppm	81 - 242
GRAM (+) BACTERIA	*Staphylococcus aureus*	40 - 90 ppm	162 - 364
	Staphylococcus epidermidis	40 - 90 ppm	162 - 364
	Bacillus spp.	21 - 90 ppm	81 - 364
	Streptococcus faecalis	61 - 120 ppm	242 - 485
	Pediococcus spp.	≥ 361 ppm	≥ 1460
	Lactobacillus spp.	≥ 361 ppm	≥ 1460
GRAM (-) BACTERIA	*Escherichia coli*	≤ 20 ppm	≤ 81
	Salmonella typhimurium	20 - 40 ppm	81 - 162
	Salmonella paratyphi B	21 - 60 ppm	81 - 242
	Other *Salmonella* spp.	20 - 40 ppm	81 - 162
	Shigella fexneri 1b	21 - 60 ppm	81 - 262
	Vibrio cholerae	≤ 20 ppm	≤ 81
	Vibrio parahaemolyticus	20 - 40 ppm	81 - 162
	Pseudomonas aeruginosa	≤ 20 ppm	≤ 81

Note: 1 ppm VOM in air = 4.04 µg AIT / L air

The above air-borne MIC's can be compared to solid and liquid culture media inhibitory concentrations of VOM reported in various scientific publications and summarized in Delaquis and Mazza's review (P.J. Delaquis and G. Mazza, "Antimicrobial properties of isothiocyanates in food preservation", Food Technology, Nov. 1995: 73 - 78):

Yeasts	20 - 35 µg/ml	(viable cells)
Fungi	2 - 6 µg/ml	(mycellar growth)
	150 - 600 µg/ml	(conidial germination)
Bacteria	*ca.* 10 µg/ml	(growth)

000044

The actual concentration of VOM in a protected food will be a function of the food type, the duration of exposure to VOM, and the air concentration of VOM. With this in mind, the levels of VOM which are required within the effective microbial growing zone on a food need to be in the above-stated range of 2 - 10 μg/ml (or 2 - 10 ppm) to achieve effective inhibition of bacterial and fungal / mold growth and 20 - 35 μg/ml for yeasts.

The use which is here determined by the Notifier to be GRAS is for shelf life extension / anti-spoilage protection of foods. In the U.S. such foods will primarily be meats (i.e., beef, pork, lamb, veal, chicken, turkey, etc.), fish and shellfish, and baked goods (primarily pies). Meats to be protected can be protected both in typical pre-package forms or when displayed in meat counter cases. For pre-packaged meats, the packaging would include an VOM-containing matrix (not in direct contact with the meat) which would generate and sustain the desired VOM air concentration inside the packaging. For display case use, the VOM-containing matrix would be placed inside the display and would generate an VOM atmosphere inside the display case. Baked goods (pies) would have a VOM in-package delivery system essentially like that for pre-packaged meats.

For most meat, it is expected that the absorbed VOM would be driven off during cooking of the meat prior to consumption. Possible exceptions would be ham, bologna, salami, or other processed meats eaten without being first cooked or heated. For baked goods (pies), VOM loss due to cooking / heating is not expected to be significant since these baked goods are pre-baked. VOM loss, therefore, is expected only when the consumer pre-heated the item prior to consumption.

Beyond this, and of importance, is that only adherence to current good practice in the manufacture, processing, handling, and storage of food is necessary to assure low levels of VOM in foods as consumed. This is because foods which acquire a flavor from VOM or an odor of a more than very transient nature will be rejected by consumers. Experience with the use of Wasa Ouro brand VOM-containing packaging in Japan has shown that the packaging / holding container delivery system for AIT must be tailored to the type and amount of food in the package / holding container as well as to the package / holding container materials and volume so as to provide the desired anti-spoilage effect without imparting undesired flavor or odor. This integration of delivery system design with specific food packaging / holding systems assures that foods treated with airborne VOM as an anti-spoilage agent will not contain excessive levels of VOM (i.e., sufficient to impart noticeable flavor or odor to the food). In this way, the proposed uses is VOM is self-limiting.

The estimated dietary intake associated with shelf-life extension / anti-spoilage uses of VOM on relevant foods is provided in Tables D-1A through D-10B (following the Bibliography). Tables D-1A, D-1B, D-2A, and D-2B provide the estimated daily dietary VOM intake associated with all VOM treated foods and for all age groups. The other tables provide the daily intake of VOM associated with specific food types.

The basic food consumption data are from USDA's Home Economics Research Repot No. 44, "Foods Commonly Eaten by Individuals". Age-specific average daily consumption of a given food type is obtained by multiplying the average amount consumed per eating occasion (for individuals consuming the food at least one in any given three day period) by the percent of individuals consuming the food at least once during any given three day period. The average daily intake of VOM for a given food type is then estimated by multiplying the average daily consumption (by age) for the food type by the concentration of VOM in the food. This type of assessment approach is one normally relied upon by experts in the evaluation of exposures to substances in foods.

For purposes of the estimation of daily dietary intake of VOM the following assumptions were made:

(a) 100% of any given food type was assumed to be treated with VOM. This is a conservative assumption because not all foods of any given type will be exposed to VOM for anti-spoilage purposes.

(b) Flavoring uses of VOM were assumed to produce an average 35 ppm VOM in food. This is a reasonable, fact-based assumption.

(c) Anti-Spoilage uses of VOM were assumed to produce 10 ppm in the entire food item. This is a conservative assumption because the 10 ppm will generally be in the surface VOM diffusion zone for a food (as opposed to being uniformly distributed throughout the food) and because this assumption ignores the effect of cooking of foods in reducing pre-cooking VOM levels in or on the surface of the food.

As can be seen from Tables D-1A through D-2B, the total VOM dietary load (as parts per billion [ppb] in the total daily diet) for the U.S. population which is associated with both flavoring and shelf life extension / anti-spoilage uses is:

Age Group	Flavoring		Anti-Spoilage	
	Male (D-1A)	*Female (D-2A)*	*Male (D-1B)*	*Female (D-2B)*
0 - 1 yr.	1.06 ppb	1.06 ppb	0.27 ppb	0.27 ppb
1 - 2 yr.	1.69 ppb	1.69 ppb	0.44 ppb	0.44 ppb
3 - 5 yr.	1.47 ppb	1.47 ppb	0.38 ppb	0.38 ppb
6 - 8 yr.	1.37 ppb	1.37 ppb	0.35 ppb	0.35 ppb
9 - 14 yr.	1.48 ppb	1.26 ppb	0.38 ppb	0.32 ppb
15 - 18 yr.	1.64 ppb	1.17 ppb	0.42 ppb	0.30 ppb
19 - 34 yr.	1.85 ppb	1.21 ppb	0.48 ppb	0.31 ppb
35 - 64 yr.	1.90 ppb	1.32 ppb	0.49 ppb	0.34 ppb
65 - 74 yr.	1.59 ppb	1.15 ppb	0.41 ppb	0.30 ppb

The flavor uses of VOM are, therefore, reasonably estimated to produce to dietary loads of > 1 ppb for all age and sex groups and between 1.4 - 1.9 ppb for all age groups other than infants (0 - 1 yr.).

In contrast, the shelf life extension / anti-spoilage uses is reasonably expected to lead to produce dietary loads of < 0.5 ppb for all age and sex groups.

The flavor uses of VOM are already listed as GRAS at 21 CFR 182.20. The shelf life extension / anti-spoilage uses, would of necessity be safe (due to an even lower dietary exposure) and this is generally recognized to be the case by experts qualified in the evaluation of the safety of substances added to food (see information for 170.36(c)(4)(i)(C), below).

Further to the safety of VOM, the dietary safety of VOM (including, also, that of synthetic ally isothiocyanate) was extensively reviewed as part of the Agency's review of GRAS substances in the 1970's (Food & Drug Research Labs, 1972; FASEB, 1975). As noted in the conclusion of this extensive review report:

> ***There is no evidence in the available information on allyl isothiocyanate, p-hydroxybenzyl isothiocyanate, and brown and yellow mustard that demonstrates or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current of that might reasonably be expected in the future.*** **(FASEB, 1975 at p. 15)**

The above, quite clear cut, safety picture was complicated in 1982 by the results of carcinogencity testing carried out by the National Toxicology Program (NTP, 1982). In this study, conducted with synthetic allyl isothiocyanate ("AIT") as opposed to the natural product obtained from mustard (*Brassica* spp.), the result for AIT was negative in male and female mice and equivocal in female rats. In male rats, the result was positive on the basis of transitional cell papillomas of the urinary bladder.

The literature review provided in the NTP study report serves to update the mutagenicity / gene toxicity data base for AIT at the time. Since the FASEB review in 1975 (FASEB, 1975), bacterial mutagenicity tests in *B. subtillis* H17 and M45 were negative as were Ames tests in *S. typhimurium* TA98, TA100, TA 1535 and TA 1537 and the *rec* A test in *E. coli* WP2. These negative mutagenicity and gene toxicity tests, together with the above-reported negative uniformity of earlier mutagenicity and gene toxicity tests for AIT (FASEB, 1975) strongly suggest that AIT will not act, *in vivo*, through any direct, genotoxic carcinogenic mechanism.

In this context, it must also be taken into account that there was a corresponding and marked dose- related increase in hyperplasia of the urinary bladder epithelium in these male rats (0/49, 1/49, & 6/49 for control, low, and high dose groups respectively). In these male rats, the incidences of transitional cell papillomas were: 0/49, 2/49, and 4/49 for controls, low dose, and high dose groups, respectively. In female rats the

incidence of transitional cell papillomas of the bladder was 0/49, 0/49, & 1/50 for controls, low dose, and high dose and the incidence of epithelial hyperplasia of the bladder was 0/49, 0/49, & 1/50 for control, low, and high dose groups, respectively. In both male and female mice, no epithelial hyperplasia of the urinary bladder was reported in any of the study animals and no transitional cell papillomas of the bladder are observed.

These data suggest a connection between the occurrence of epithelial hyperplasia and transitional papillomas in these test species, in fact the incidences of the two types of lesions appear to mirror each other. The fact that a transitional cell papilloma was observed in a female of the high dose group precludes any possible argument that perhaps female rats do not form transitional cell papillomas.

An earlier study by Major (1970), using the mouse two-stage skin carcinogenesis model, showed that synthetic AIT, when applied to mouse skin at an irritant dose level, induced a transient hyperplasia and an elevated mitotic index in the skin.

IARC (1985) reviewed a mouse skin carcinogenesis study which included natural VOM (as opposed to synthetic AIT). Volatile Oil of Mustard, which contained > 90% allyl isothiocyanate, was tested in a two stage mouse skin assay. Three control groups of 12-16 'S' strain mice (actual strain, age and sex unspecified) received 0.3 ml of 0.1-0.15% 7,12-dimethylbenz(a)anthracene (DMBA) on the skin of the back followed by no treatment (two groups) or 21 days later by twice weekly applications for 12 weeks and weekly applications for 15 weeks of acetone. A fourth group of 16 mice received an initial skin application of 0.3 ml of 0.1% DMBA followed 39 days later by applications of a 3-4% solution of mustard oil in acetone weekly for 20 weeks. The incidence of skin papillomas among animals surviving to the end of the experiment was 4/21, 1/12 and 1/16 in the combined DMBA control groups with no secondary treatment, in controls receiving DMBA followed by acetone, and in treated mice receiving DMBA and mustard oil, respectively. In this study, **natural** VOM was neither carcinogenic nor co-carcinogenic.

The suggestion from both the data reported above is that: (a) the transitional cell papillomas which were observed were secondary to the effect of hyperplasia on promotion of pre-existing tumor precursor cells and/or increasing the susceptibility of normal bladder stem cells to transformation due to increased time spent cell cycle phases in which chromatin is un-condensed and more exposed; (b) that such effects would not be expected at lower, non-irritant doses of AIT; (c) that the result in the NTP biosassy was not mediated via a genotoxic mechanism of carcinogenesis and, therefore, will have a low dose threshold; and, (d) that there may be a difference in effect between natural Volatile Oil of Mustard and synthetic AIT.

Since the NTP Bioassay, no additional reliable *in vivo* evidence suggesting an AIT or VOM carcinogenic dietary risk to humans has been reported.

Kasamaki *et al.* (1987) reported than AIT (synthetic) was able to induce cell transformation of Chinese hamster B241 cells. This study, however, did not include adequate controls for the effects of the extensive passaging which was required to observe the low level of transformants reported.

Musk and Johnson (1993) reported that AIT (synthetic) did not produce chromosome aberrations *in vitro* under conditions in which benzyl-, phenethyl-, and phenyl-isothiocyanates produced chromosomal aberrations.

Bechtel, *et al.* (1998) reported that AIT (synthetic) did not induce unscheduled DNA synthesis in male rats when given at up to 125 mg/kg. This supports that AIT lacks the ability to directly induce cancer by a genotoxic mechanism *in vivo.*

Kassie and Knasmuller (2000) reported on an *in vitro* gene-toxicity battery study of AIT (synthetic) and phenethyl isothiocyanate (PEITC). The test battery included Ames tests in TA 98 and TA 100, the DNA repair test in E. coli, and a micronucleus test with human derive Hep G2 cells. AIT was reported to be more active in the bacterial test systems than in the mammalian cell test system. When tested *in vivo* at very high doses (90 and 270 mg/kg), using an E. coli marker, AIT's activity was significantly attenuated. This attenuation was suggested to be due to protein binding of AIT under *in vivo* conditions. Further investigations *in vitro* suggested that the formation of reactive oxygen species could be mediated by AIT and PEITC. The suggestion that AIT might mediate the formation of reactive oxygen species has also been investigated by Murata at al. (2000) and Yonezawa et al., (1997) in *in vitro* studies.

Since the time of the NTP bioassay the metabolism of AIT has, also, been shown to differ between rat and the mouse and human (with the human metabolizing AIT like the mouse - which was negative in the NTP bioassay - and not like the rat).

Bollard et al. (1997) report that urinary metabolites account for the bulk of administered AIT (synthetic) doses in mice and rats (80% and 75% respectively). In mice, there are three major urinary species: inorganic thiocyanate ion, the glutathione conjugate[3], and the cysteine conjugate[4]. In contrast, rats do not form the cysteine conjugate and, also, show a greater retention of ^{14}C-AIT than do mice at 4 days (18-24% in rats *versus* 2-5% in mice). The results of this study suggest that in mice, AIT is primarily metabolized via hydrolysis whereas in rats glutathione conjugation is the predominant metabolic route. Of marked interest to the urinary papilloma picture for rats, a sex difference was reported for AIT in bladder tissue of rats, with higher levels of AIT / AIT-metabolites seen in the bladder tissue of male rats than in female rats. This finding would correlate with the observed differences in both bladder hyperplasia and papilloma incidence seen between male and female rats. In humans, as in mice, the major urinary metabolite is the cysteine conjugate and glutathione conjugation appears to be at most a minor pathway for the human metabolism (Jiao *et al.*, 1994).

---

[3] Allylthiocarbamoylmercapturic acid

[4] Allylthiocarbamoylcysteine

The International Agency for Research on Cancer (IARC), has reviewed the overall weight of the evidence for carcinogencity of AIT and concluded that the evidence in animals is "limited" and in humans is "not classifiable" (IARC, 1999).

Beyond this immediate safety issue, AIT is probably an anti-carcinogen in the human diet. Therefore, its incorporation in the human diet may present potential public health benefits. Some selected citations follow.

Musk and Johnson (1993) reported on the selective toxicity of AIT to the human colorectal cell line HT29. A later report by Smith *et al.*, (1996) extended this in vitro finding to in vivo suppression of aberrant crypt foci in the colonic mucosa of rats.

Hasegawa *et al.*, (1993) reported on AIT inhibition of the malignant human HeLa cell line.

Xu and Thornalley (2000) have reported on the inhibition of human leukemia cells by AIT. As these authors note, the anti-cancer effect of consumption of cruciferous vegatables – which are rich in AIT – is well known.

Therefore, the current general recognition among experts as to the safety of VOM can be summarized as follows:

- AIT and/or VOM is consistently reported as non-genotoxic *in vivo* and usually is reported to be non-genotoxic *in vitro*.
- AIT has irritant properties which have been reported to produce transient hyperplasia of mouse skin when applied to mouse skin.
- In dermal mouse skin carcinogenicity bioassays, VOM was not carcinogenic.
- In a corn oil gavage carcinogencity bioassay in mice and rats, AIT was not carcinogenic in male or female mice, was considered equivocal in female rats, and was considered to increase the incidence of transitional cell papillomas in male rats. In male rats, the incidence of transitional cell papillomas mirrored that of bladder epithelial hyperplasia. In female rats, a single animal with transitional cell papilloma of the bladder was observed in the high dose group and, again, the incidence of bladder epithelial hyperplasia mirrored that of transitional cell papillomas in female rats.
- The metabolism of AIT differs significantly between rats and mice. In rats, glutathione conjugation is the major pathway and there is a sex difference in the accumulation of AIT / AIT-metabolites in the rat bladder with males showing higher levels than females. In mice, hydrolysis with formation of thiocyanate ion and of the cysteine conjugate is the major pathway for AIT. Humans appear to metabolize AIT similarly to mice.

- The weight of the evidence strongly supports that the increased incidence of transitional cell papillomas seen in male rats in the NTP bioassay is specific to the male rat under these test conditions and is probably due to accumulation of levels of the AIT-glutathione conjugate which induce epithelial hyperplasia in the male rat under the NTP test conditions and that the papillomas are secondary to this hyperplasia. Female rats were observed, in the NTP bioassay, to be capable of forming transitional cell papillomas in the bladder but the incidence of epithelial hyperplasia was much lower than male rats and, accordingly, so was the incidence of papillomas (1/50 in high dose females *vs.* 0/49 in control females for both hyperplasia and papillomas). This is most likely a reflection of the fact that female rats do not accumulate AIT metabolites in the bladder to the same extent that male rats do. Given the known differences in AIT metabolism between rats and humans and the similarities between mouse and human AIT metabolism, the NTP bioassay actually suggests that AIT will be non-carcinogenic in humans, as was the case for the mice in the NTP bioassay.

- The NTP bioassay results in rats are not, therefore, an appropriate predictor, in this case, for AIT carcinogenic risk to humans at the levels presently consumed in the daily diet. The NTP bioassay results for mice, which metabolize AIT differently from rats but similarly to humans, indicate that AIT is not expected to be carcinogenic in the human diet and at the low levels associated with current food uses of AIT or of VOM both are generally considered as safe for human consumption.

170.36(c)(4)(i)(C) [*Proposed*]: The basis for concluding, in light of the data and information under paragraphs (c)(1), (c)(2), (c)(3), (c)(4)(i)(A), and (c)(4)(i)(B) that there is consensus among experts qualified by scientific training and experience to evaluate the safety of substances added to food that there is reasonable certainty that the substance is not harmful under the intended conditions of use.

A number of scientific studies which have been published in the open literature clearly establish that there is a current consensus, i.e., general recognition, of the safety of AIT (synthetic) and VOM (natural) as currently consumed in the human diet. This is that at current consumption levels, AIT is toxicologically safe for humans and, to a reasonable degree of scientific certainty, does not have any expected carcinogenic potential. The anti-spoilage use of AIT will not significantly alter human dietary consumption of AIT as the resultant levels in foods, of necessity, are lower than current levels for use in imparting flavor to relevant foods.

IARC – an internationally recognized body of experts – in 1999 re-stated its conclusions as to the dietary safety of AIT (and VOM) and lack of weight of evidence for a carcinogenic risk to humans.

The U.S. Food and Drug Administration currently lists the flavor uses (and other potential but unspecified uses) as GRAS uses (21 CFR 182.20). The above referenced

IARC expert review addresses the dietary safety of VOM at these low levels of use associated with flavoring and, of necessity, yet lower levels of VOM in the diet. As is discussed and supported above, the dietary VOM levels estimated, on a worst case basis, to be associated with the use for which the Notifier has made a GRAS determination and for which the Notifier is claiming a GRAS exemption from premarket approval requirements are much lower than those associated with flavoring uses and are, therefore, also generally recognized as safe for human consumption.

APPENDIX 1

Tables for Dietary Exposures to VOM Associated with both Flavoring and Anti-Spoilage Uses

000053

Table D-1A: Total Dietary Loading from Consumption of Foods Containing 35 ppm Volatile Oil of Mustard (i.e.: flavoring use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	**CONSUMPTION: g/day**								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	66.20	92.30	104.125	106.12	78.84
Poultry	5.64	13.93	16.19	19.39	24.81	29.78	30.324	30.60	27.56
Fish & Shellfish	0.35	3.81	5.51	7.20	8.80	9.71	15.56	16.68	17.05
Pies	0.11	1.74	3.12	3.65	5.92	8.43	8.84	9.42	13.07
TOTALS:	**15.15**	**48.41**	**62.91**	**78.46**	**105.72**	**140.22**	**158.85**	**162.82**	**136.52**
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Total Foods per Kg-day	1.87	4.28	3.77	3.38	2.83	2.34	2.18	2.09	1.75
Volatile Oil of Mustard Level in Food	**35 ppm**		**100% of Food Assumed to Contain Volatile Oil of Mustard**						
Dietary Load as ug/Kg/day	0.0653	0.1498	0.1318	0.1184	0.0992	0.0819	0.0762	0.0731	0.0613
Dietary Load as ug/day	0.530	1.694	2.202	2.746	3.700	4.908	5.560	5.699	4.778
Dietary Load As ppb in Total Diet	1.06	1.69	1.47	1.37	1.48	1.64	1.85	1.90	1.59
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT				**NOEL ≅ 8.6 mg/Kg/day**					
	131,740	57,412	65,229	72,656	86,693	104,968	112,918	117,713	140,389

Table D-1B: Total Dietary Loading from Consumption of Foods
Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	**CONSUMPTION: g/day**								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	66.20	92.30	104.125	106.12	78.84
Poultry	5.64	13.93	16.19	19.39	24.81	29.78	30.324	30.60	27.56
Fish & Shellfish	0.35	3.81	5.51	7.20	8.80	9.71	15.56	16.68	17.05
Pies	0.11	1.74	3.12	3.65	5.92	8.43	8.84	9.42	13.07
TOTALS:	**15.15**	**48.41**	**62.91**	**78.46**	**105.72**	**140.22**	**158.85**	**162.82**	**136.52**
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Total Foods per Kg-day	1.87	4.28	3.77	3.38	2.83	2.34	2.18	2.09	1.75
Volatile Oil of Mustard Level in Food	**9 ppm**		**100% of Food Assumed to Contain Volatile Oil of Mustard**						
Dietary Load as ug/Kg/day	0.0168	0.0385	0.0339	0.0304	0.0255	0.0211	0.0196	0.0188	0.0158
Dietary Load as ug/day	0.136	0.436	0.566	0.706	0.951	1.262	1.430	1.465	1.229
Dietary Load As ppb in Total Diet	0.27	0.44	0.38	0.35	0.38	0.42	0.48	0.49	0.41
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT				**NOEL = 8.6 mg/Kg/day**					
	512,322	223,269	253,868	282,550	337,138	408,209	439,126	457,771	545,956

Table D-2A: Dietary Loading from Consumption of Foods Containing 35 ppm Volatile Oil of Mustard (i.e.: flavoring use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	**CONSUMPTION: g/day**								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	56.41	62.78	64.038	68.88	52.46
Poultry	5.64	13.93	16.19	19.39	20.68	22.29	23.328	24.53	24.81
Fish & Shellfish	0.35	3.81	5.51	7.20	7.56	10.89	11.308	13.72	12.35
Pies	0.11	1.74	3.12	3.65	5.62	4.60	5.1	6.43	8.91
TOTALS:	**15.15**	**48.41**	**62.91**	**78.46**	**90.27**	**100.55**	**103.77**	**113.56**	**98.52**
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	**2**	2.5	3	3	3	3
g Total Foods per Kg-day	2.02	4.40	3.86	3.34	2.45	1.80	1.73	1.75	1.43
Volatile Oil of Mustard Level in Food	**35 ppm**		**100% of Food Assumed to Contain Volatile Oil of Mustard**						
Dietary Load as ug/Kg/day	0.0709	0.1540	0.1351	0.1169	0.0869	0.0630	0.0605	0.0611	0.0500
Dietary Load as ug/day	0.530	1.694	2.202	2.746	3.159	3.519	3.632	3.974	3.448
Dietray Load As ppb in Total Diet	1.06	1.69	1.47	1.37	1.26	1.17	1.21	1.32	1.15
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	121,356	55,838	63,667	73,595	**NOEL = 8.6 mg/Kg/day** 100,170	136,599	142,067	140,648	172,097

Table D-2B: Dietary Loading from Consumption of Foods
Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	56.41	62.78	64.038	68.88	52.46
Poultry	5.64	13.03	16.19	19.39	20.68	22.29	23.328	24.53	24.81
Fish & Shellfish	0.35	3.81	5.51	7.20	7.56	10.89	11.308	13.72	12.35
Pies	0.11	1.74	3.12	3.65	5.62	4.60	5.1	6.43	8.91
TOTALS:	**15.15**	**48.41**	**62.91**	**78.46**	**90.27**	**100.55**	**103.77**	**113.56**	**98.52**
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Total Foods per Kg-day	2.02	4.40	3.86	3.34	2.45	1.80	1.73	1.75	1.43
Volatile Oil of Mustard Level in Food	9 ppm			100% of Food Assumed to Contain Volatile Oil of Mustard					
Dietary Load as ug/Kg/day	0.0182	0.0396	0.0347	0.0300	0.0221	0.0162	0.0156	0.0157	0.0128
Dietary Load as ug/day	0.136	0.436	0.566	0.706	0.812	0.905	0.934	1.022	0.887
Dietray Load As ppb in Total Diet	0.27	0.44	0.38	0.35	0.32	0.30	0.31	0.34	0.30
Dietary Load Safety Factor to NOEL In Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	471,942	217,149	247,593	286,204	389,552	531,218	552,483	546,965	669,265

Table D-3A: Dietary Loading from Consumption of Beef, Pork, Lamb, and Veal (all forms) Containing 35 ppm Volatile Oil of Mustard (i.e.: flavoring use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	66.20	92.30	104.125	106.12	78.84
TOTALS:	**9.05**	**28.93**	**38.09**	**48.22**	**66.20**	**92.30**	**104.13**	**106.12**	**78.84**
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Meat Foods per Kg-day	1.11	2.56	2.28	2.08	1.77	1.54	1.43	1.36	1.01
Volatile Oil of Mustard Level in Food	35 ppm	100% of Food Assumed to Contain Volatile Oil of Mustard							
Dietary Load as ug/Kg/day	0.0390	0.0895	0.0798	0.0727	0.0621	0.0539	0.0499	0.0476	0.0354
Dietary Load as ug/day	0.317	1.013	1.333	1.688	2.317	3.230	3.644	3.714	2.760
Dietary Load As ppb in Total Diet	0.63	1.01	0.89	0.84	0.93	1.08	1.21	1.24	0.92
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	220,513	96,047	107,735	118,215	138,455	159,470	172,265	180,606	243,084

Table D-3B: Dietary Loading from Consumption of Beef, Pork, Lamb, and Veal (all forms) Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	66.20	92.30	104.125	106.12	78.84
TOTALS:	**9.05**	**28.93**	**38.09**	**48.22**	**66.20**	**92.30**	**104.13**	**106.12**	**78.84**
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Meat Foods per Kg-day	1.11	2.56	2.28	2.08	1.77	1.54	1.43	1.36	1.01
Volatile Oil of Mustard Level in Food	9 ppm	100% of Food Assumed to Contain Volatile Oil of Mustard							
Dietary Load as ug/Kg/day	0.0100	0.0230	0.0205	0.0187	0.0160	0.0139	0.0128	0.0122	0.0091
Dietary Load as ug/day	0.081	0.260	0.343	0.434	0.596	0.831	0.937	0.955	0.710
Dietary Load As ppb in Total Diet	0.16	0.26	0.23	0.22	0.24	0.28	0.31	0.32	0.24
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL ≡ 8.6 mg/Kg/day								
	857,550	373,517	418,971	459,726	538,435	620,161	669,921	702,356	945,327

Table D-4A: Dietary Loading from Consumption of Beef, Pork, Lamb, and Veal (all forms) Containing 35 ppm Volatile Oil of Mustard (i.e.: flavoring use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	56.41	62.78	64.038	68.88	52.46
TOTALS:	**9.05**	**28.93**	**38.09**	**48.22**	**56.41**	**62.78**	**64.04**	**68.88**	**52.46**
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Meat Foods per Kg-day	1.21	2.63	2.34	2.05	1.53	1.12	1.07	1.06	0.76
Volatile Oil of Mustard Level in Food	35 ppm		100% of Food Assumed to Contain Volatile Oil of Mustard						
Dietary Load as ug/Kg/day	0.0423	0.0921	0.0818	0.0718	0.0537	0.0393	0.0374	0.0371	0.0266
Dietary Load as ug/day	0.317	1.013	1.333	1.688	1.974	2.197	2.241	2.411	1.836
Dietary Load As ppb in Total Diet	0.63	1.01	0.89	0.84	0.79	0.73	0.75	0.80	0.61
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	203,132	93,415	105,155	119,744	160,284	218,801	230,220	231,873	323,216

Table D-4B: Dietary Loading from Consumption of Beef, Pork, Lamb, and Veal (all forms) Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	56.41	62.78	64.038	68.88	52.46
TOTALS:	9.05	28.93	38.09	48.22	56.41	62.78	64.04	68.88	52.46
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Meat Foods per Kg-day	1.21	2.63	2.34	2.05	1.53	1.12	1.07	1.06	0.76
Volatile Oil of Mustard Level in Food	9 ppm	100% of Food Assumed to Contain Volatile Oil of Mustard							
Dietary Load as ug/Kg/day	0.0109	0.0237	0.0210	0.0185	0.0138	0.0101	0.0096	0.0095	0.0068
Dietary Load as ug/day	0.081	0.260	0.343	0.434	0.508	0.565	0.576	0.620	0.472
Dietary Load As ppb in Total Diet	0.16	0.26	0.23	0.22	0.20	0.19	0.19	0.21	0.16
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	789,960	363,279	408,936	465,670	623,328	850,891	895,302	901,729	1,256,950

Table D-5A: Dietary Loading from Consumption of Poultry (all forms) Containing 35 ppm Volatile Oil of Mustard (i.e.: flavoring use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Poultry	5.64	13.93	16.19	19.39	24.81	29.78	30.324	30.60	27.56
TOTALS:	**5.64**	**13.93**	**16.19**	**19.39**	**24.81**	**29.78**	**30.32**	**30.60**	**27.56**
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Poultry Foods per Kg-day	0.69	1.23	0.97	0.84	0.67	0.50	0.42	0.39	0.35
Volatile Oil of Mustard Level In Food	35 ppm		100% of Food Assumed to Contain Volatile Oil of Mustard						
Dietary Load as ug/Kg/day	0.0243	0.0431	0.0339	0.0293	0.0233	0.0174	0.0145	0.0137	0.0124
Dietary Load as ug/day	0.197	0.487	0.567	0.679	0.868	1.042	1.061	1.071	0.965
Dietary Load As ppb in Total Diet	0.39	0.49	0.38	0.34	0.35	0.35	0.35	0.36	0.32
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	353,633	199,557	253,486	293,950	369,443	494,168	591,516	626,310	695,418

Table D-5B: Dietary Loading from Consumption of Poultry (all forms)
Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Poultry	5.64	13.93	16.19	19.39	24.81	29.78	30.324	30.60	27.56
TOTALS:	5.64	13.93	16.19	19.39	24.81	29.78	30.32	30.60	27.56
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Poultry Foods per Kg-day	0.69	1.23	0.97	0.84	0.67	0.50	0.42	0.39	0.35
Volatile Oil of Mustard Level in Food	9 ppm	100% of Food Assumed to Contain Volatile Oil of Mustard							
Dietary Load as ug/Kg/day	0.0063	0.0111	0.0087	0.0075	0.0060	0.0045	0.0037	0.0035	0.0032
Dietary Load as ug/day	0.051	0.125	0.146	0.175	0.223	0.268	0.273	0.275	0.248
Dietary Load As ppb in Total Diet	0.10	0.13	0.10	0.09	0.09	0.09	0.09	0.09	0.08
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL ≅ 8.6 mg/Kg/day								
	1,375,241	776,054	985,778	1,143,139	1,436,723	1,921,763	2,300,341	2,435,650	2,704,403

Table D-6A: Dietary Loading from Consumption of Poultry (all forms) Containing 35 ppm Volatile Oil of Mustard (i.e.: flavor use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Poultry	5.64	13.93	16.19	19.39	20.68	22.29	23.328	24.53	24.81
TOTALS:	5.64	13.93	16.19	19.39	20.68	22.29	23.33	24.53	24.81
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Poultry Foods per Kg-day	0.75	1.27	0.99	0.83	0.56	0.40	0.39	0.38	0.36
Volatile Oil of Mustard Level in Food	35 ppm	100% of Food Assumed to Contain Volatile Oil of Mustard							
Dietary Load as ug/Kg/day	0.0264	0.0443	0.0348	0.0289	0.0197	0.0140	0.0136	0.0132	0.0126
Dietary Load as ug/day	0.197	0.487	0.567	0.679	0.724	0.780	0.816	0.859	0.868
Dietary Load As ppb in Total Diet	0.39	0.49	0.38	0.34	0.29	0.26	0.27	0.29	0.29
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	325,761	194,087	247,414	297,751	437,248	616,298	631,981	651,098	683,503

Table D-6B: Dietary Loading from Consumption of Poultry (all forms)
Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Poultry	5.64	13.93	16.19	19.39	20.68	22.29	23.328	24.53	24.81
TOTALS:	**5.64**	**13.93**	**16.19**	**19.39**	**20.68**	**22.29**	**23.33**	**24.53**	**24.81**
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Poultry Foods per Kg-day	0.75	1.27	0.99	0.83	0.56	0.40	0.39	0.38	0.36
Volatile Oil of Mustard Level in Food	9 ppm	100% of Food Assumed to Contain Volatile Oil of Mustard							
Dietary Load as ug/Kg/day	0.0068	0.0114	0.0089	0.0074	0.0051	0.0036	0.0035	0.0034	0.0032
Dietary Load as ug/day	0.051	0.125	0.146	0.175	0.186	0.201	0.210	0.221	0.223
Dietary Load As ppb in Total Diet	0.10	0.13	0.10	0.09	0.07	0.07	0.07	0.07	0.07
Dietary Load Safety Factor to NOEL In Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	1,266,848	754,783	962,167	1,157,921	1,700,408	2,396,714	2,457,705	2,532,047	2,658,066

Table D-7A: Dietary Loading from Consumption of Fish and Shellfish (all forms) Containing 35 ppm Volatile Oil of Mustard (i.e.: flavor use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Fish & Shellfish	0.35	3.81	5.51	7.20	8.80	9.71	15.56	16.68	17.05
TOTALS:	**0.35**	**3.81**	**5.51**	**7.20**	**8.80**	**9.71**	**15.56**	**16.68**	**17.05**
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Sea Foods per Kg-day	0.04	0.34	0.33	0.31	0.24	0.16	0.21	0.21	0.22
Volatile Oil of Mustard Level in Food	35 ppm	100% of Food Assumed to Contain Volatile Oil of Mustard							
Dietary Load as ug/Kg/day	0.0015	0.0118	0.0115	0.0109	0.0083	0.0057	0.0075	0.0075	0.0077
Dietary Load as ug/day	0.012	0.133	0.193	0.252	0.308	0.340	0.545	0.584	0.597
Dietary Load As ppb in Total Diet	0.02	0.13	0.13	0.13	0.12	0.11	0.18	0.19	0.20
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	5,783,188	730,170	744,994	791,746	1,041,494	1,516,411	1,152,625	1,149,161	1,124,089

Table D-7B: Dietary Loading from Consumption of Fish and Shellfish (all forms) Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Fish & Shellfish	0.35	3.81	5.51	7.20	8.80	9.71	15.56	16.68	17.05
TOTALS:	**0.35**	**3.81**	**5.51**	**7.20**	**8.80**	**9.71**	**15.56**	**16.68**	**17.05**
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Sea Foods per Kg-day	0.04	0.34	0.33	0.31	0.24	0.16	0.21	0.21	0.22
Volatile Oil of Mustard Level in Food	9 ppm	100% of Food Assumed to Contain Volatile Oil of Mustard							
Dietary Load as ug/Kg/day	0.0004	0.0030	0.0030	0.0028	0.0021	0.0015	0.0019	0.0019	0.0020
Dietary Load as ug/day	0.003	0.034	0.050	0.065	0.079	0.087	0.140	0.150	0.153
Dietary Load As ppb in Total Diet	0.01	0.03	0.03	0.03	0.03	0.03	0.05	0.05	0.05
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	22,490,177	2,839,552	2,897,200	3,079,012	4,050,253	5,897,154	4,482,429	4,468,961	4,371,457

Table D-8A: Dietary Loading from Consumption of Fish and Shellfish (all forms) Containing 35 ppm Volatile Oil of Mustard (i.e.: flavoring use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Fish & Shellfish	0.35	3.81	5.51	7.20	7.56	10.89	11.308	13.72	12.35
TOTALS:	0.35	3.81	5.51	7.20	7.56	10.89	11.31	13.72	12.35
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Sea Foods per Kg-day	0.05	0.35	0.34	0.31	0.21	0.19	0.19	0.21	0.18
Volatile Oil of Mustard Level In Food	35 ppm	100% of Food Assumed to Contain Volatile Oil of Mustard							
Dietary Load as ug/Kg/day	0.0016	0.0121	0.0118	0.0107	0.0072	0.0068	0.0066	0.0074	0.0063
Dietary Load as ug/day	0.012	0.133	0.193	0.252	0.264	0.381	0.396	0.480	0.432
Dietary Load As ppb in Total Diet	0.02	0.13	0.13	0.13	0.11	0.13	0.13	0.16	0.14
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	5,327,371	710,157	727,150	801,984	1,196,544	1,261,288	1,303,755	1,164,098	1,372,817

Table D-8B: Dietary Loading from Consumption of Fish and Shellfish (all forms) Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Fish & Shellfish	0.35	3.81	5.51	7.20	7.56	10.89	11.308	13.72	12.35
TOTALS:	**0.35**	**3.81**	**5.51**	**7.20**	**7.56**	**10.89**	**11.31**	**13.72**	**12.35**
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Sea Foods per Kg-day	0.05	0.35	0.34	0.31	0.21	0.19	0.19	0.21	0.18
Volatile Oil of Mustard Level in Food	9 ppm	100% of Food Assumed to Contain Volatile Oil of Mustard							
Dietary Load as ug/Kg/day	0.0004	0.0031	0.0030	0.0028	0.0018	0.0018	0.0017	0.0019	0.0016
Dietary Load as ug/day	0.003	0.034	0.050	0.065	0.068	0.098	0.102	0.123	0.111
Dietary Load As ppb In Total Diet	0.01	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.04
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	20,717,552	2,761,721	2,827,806	3,118,827	4,653,228	4,905,010	5,070,157	4,527,049	5,338,731

Table D-9A: Dietary Loading from Consumption of Pies (all forms) Containing 35 ppm Volatile Oil of Mustard (i.e.: flavor use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Pies	0.11	1.74	3.12	3.65	5.92	8.43	8.84	9.42	13.07
TOTALS:	**0.11**	**1.74**	**3.12**	**3.65**	**5.92**	**8.43**	**8.84**	**9.42**	**13.07**
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Pies per Kg-day	0.01	0.15	0.19	0.16	0.16	0.14	0.12	0.12	0.17
Volatile Oil of Mustard Level in Food	35 ppm	100% of Food Assumed to Contain Volatile Oil of Mustard							
Dietary Load as ug/Kg/day	0.0005	0.0054	0.0065	0.0055	0.0056	0.0049	0.0042	0.0042	0.0059
Dietary Load as ug/day	0.004	0.061	0.109	0.128	0.207	0.295	0.309	0.330	0.457
Dietary Load As ppb in Total Diet	0.01	0.06	0.07	0.06	0.08	0.10	0.10	0.11	0.15
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	18,138,182	1,598,061	1,313,517	1,563,943	1,549,213	1,745,527	2,029,089	2,034,577	1,466,951

Table D-9B: Dietary Loading from Consumption of Pies (all forms)
Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Pies	0.11	1.74	3.12	3.65	5.92	8.43	8.84	9.42	13.07
TOTALS:	**0.11**	**1.74**	**3.12**	**3.65**	**5.92**	**8.43**	**8.84**	**9.42**	**13.07**
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Pies per Kg-day	0.01	0.15	0.19	0.16	0.16	0.14	0.12	0.12	0.17
Volatile Oil of Mustard Level in Food	9 ppm	100% of Food Assumed to Contain Volatile Oil of Mustard							
Dietary Load as ug/Kg/day	0.0001	0.0014	0.0017	0.0014	0.0014	0.0013	0.0011	0.0011	0.0015
Dietary Load as ug/day	0.0010	0.016	0.028	0.033	0.053	0.076	0.080	0.085	0.118
Dietary Load As ppb in Total Diet	0.00	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.04
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	70,537,374	6,214,683	5,108,123	6,082,000	6,024,716	6,788,162	7,890,900	7,912,243	5,704,809

Table D-10A: Dietary Loading from Consumption of Pies (all forms) Containing 35 ppm Volatile Oil of Mustard (i.e.: flavor use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Pies	0.11	1.74	3.12	3.65	5.62	4.60	5.1	6.43	8.91
TOTALS:	**0.11**	**1.74**	**3.12**	**3.65**	**5.62**	**4.60**	**5.10**	**6.43**	**8.91**
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Pies per Kg-day	0.01	0.16	0.19	0.16	0.15	0.08	0.09	0.10	0.13
Volatile Oil of Mustard Level in Food	35 ppm	100% of Food Assumed to Contain Volatile Oil of Mustard							
Dietary Load as ug/Kg/day	0.0005	0.0055	0.0067	0.0054	0.0053	0.0029	0.0030	0.0035	0.0045
Dietary Load as ug/day	0.004	0.061	0.109	0.128	0.197	0.161	0.179	0.225	0.312
Dietary Load As ppb in Total Diet	0.01	0.06	0.07	0.06	0.08	0.05	0.06	0.07	0.10
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	16,708,571	1,554,259	1,282,056	1,584,166	1,609,520	2,985,963	2,890,756	2,485,439	1,903,693

Table D-10B: Dietary Loading from Consumption of Pies (all forms)
Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Pies	0.11	1.74	3.12	3.65	5.62	4.60	5.1	6.43	8.91
TOTALS:	0.11	1.74	3.12	3.65	5.62	4.60	5.10	6.43	8.91
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Pies per Kg-day	0.01	0.16	0.19	0.16	0.15	0.08	0.09	0.10	0.13
Volatile Oil of Mustard Level in Food	9 ppm	100% of Food Assumed to Contain Volatile Oil of Mustard							
Dietary Load as ug/Kg/day	0.0001	0.0014	0.0017	0.0014	0.0014	0.0007	0.0008	0.0009	0.0012
Dietary Load as ug/day	0.001	0.016	0.028	0.033	0.051	0.041	0.046	0.058	0.080
Dietary Load As ppb in Total Diet	0.00	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.03
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	64,977,778	6,044,342	4,985,773	6,160,646	6,259,246	11,612,077	11,241,830	9,665,595	7,403,249

APPENDIX 2

Bibliography

000074

Prior to 1958

1. Anderson, E.E., et al. 1951. Pasteurized fresh whole pickles. II. Thermal resistance of microorganisms and peroxidase. Food Technol. 5(9): 364-368.

2. Anderson, E.E., et al. 1953. Effect of essential oils on the inhibition and thermal resistance of microorganisms in acid food products. Food Research. 18: 40-47.

3. Blum, H.B. and F.W. Fabian. 1943. Spice oils and their components for controlling microbiol surface growth. Fruit Products J. 22: 326-329, 347.

4. Delage, J.C. 1923. Use of mustard meal and of mustard oil in enology, Ann. Fals. 16: 483-491.

5. Ferre, L. 1925. Action of allyl mustard oil as a wine and must preservative. Ann. fals. 18: 34-37.

6. Kosker, O.; et al. 1949. Mustard as a preservative for fruit juices. The Glass Packer. 28: 818-820, 836, 838, 841.

7. Kosker, O.; et al. 1951. Effect of allyl isothiocyanate and related substances on the thermal resistance of Aspergillus niger, Saccharomyces ellipsoideus, and Bacillus thermoacidurans. Fd. Res. 16(6): 510-514.

8. Malvrzin and G. Bidart. 1923. The action of standard allyl mustard oil solutions in preventing secondary fermentations. Ann. fuls. 16: 534-542.

9. Perazzo, A. and L. de prado. 1937. The action of mustard essence on wines. Its use as antifermentative agent. Rev. assoc. bioquim. argentina. 5: 54-61.

10. Roos, L. and E. Hugues. 1924. Allyl mustard oil in enology. Ann. Fals. 17: 413-421.

11. Vaughan, J.G. and J.S. Hemingway. 1959. The utilization of mustards. Econ. Bot. 13(3): 196-204.

12. Vincens. 1924. Experiments on the use of mustard oil (allyl) in enology. Ann. fals. 17: 541-551.

13. Walker, J.C.; et al. 1937. Toxicity of mustard oils and related sulfur compounds to certain fungi. Am. J. Bot. 24(8); 536-541 (2 fig.).

Post 1958 References

Bechtel D; Henderson L; and Proudlock R (1998) "Lack of UDS activity in the livers of rats exposed to allylisothiocyanate." Teratogenesis, Carcinogenesis, And Mutagenesis, 18 (5): 209-217.

Bollard M; Stribbling S; Mitchell S; Caldwell J (1997) "The disposition of allyl isothiocyanate in the rat and mouse." Food And Chemical Toxicology, 35 (10-11): 933-943.

Delaquis P J and Sholberg P L (1997) "Antimicrobial activity of gaseous allyl isothiocyanate." Journal Of Food Protection, 60 (8): 943-947.

FASEB [Federation of American Societies for Experimental Biology] (1975) "Evaluation of the Health Aspects of Mustard and Oil of Mustard as Food Ingredients" National Technical Information Service, No. PB-254528

Food and Drug Research Labs., Inc. (1972) "GRAS (Generally Recognized as Safe) Food Ingredients – Oil of Mustard and Allyl Isothiocyanate", National Technical Information Service, No. PB-221215

Holley R A (1997) "Impact of slicing hygiene upon shelf life and distribution of spoilage bacteria in vacuum packaged cured meats" Food Microbiology (London), 14 (3): 201-211

IARC (1999) "Allyl isothiocyanate" IARC Monographs On The Evaluation Of Carcinogenic Risks To Humans, 73: 37-48.

IARC (1985) "Allyl isothiocyanate" IARC Monographs On The Evaluation Of Carcinogenic Risks To Humans, 36: pp. 60 ff.

Isshiki K; Tokuoka K; Mori R; and Chiba S (1992) "Preliminary examination of allyl isothiocyanate vapor for food preservation." Biosci Biotechnol Biochem, 56 (9): 1476-1477.

Jiao D; Ho C T; Foiles P; Chung F L (1994) "Identification and quantification of the N-acetylcysteine conjugate of allyl isothiocyanate in human urine after ingestion of mustard." Cancer Epidemiology, Biomarkers And Prevention, 3(6): 487-92.

Kasamaki A; Yasuhara T; Urasawa S, (1987) "Neoplastic transformation of Chinese hamster cells in vitro after treatment with flavoring agents." Journal Of Toxicological Sciences, 12(4): 383-96.

Kassie F; Knasmuller S (2000) "Genotoxic effects of allyl isothiocyanate (AITC) and phenethyl isothiocyanate (PEITC)." Chemico-Biological Interactions, 127(2): 163-80.

Murata M; Yamashita N; Inoue S; Kawanishi S (2000)
"Mechanism of oxidative DNA damage induced by carcinogenic allyl isothiocyanate." Free Radical Biol. Med., 28(5): pp. 797-805.

Musk S R; Johnson I T (1993) "The clastogenic effects of isothiocyanates." Mutation Research, 300(2): 111-117.

Musk SR R; Johnson IT (1993) "Allyl isothiocyanate is selectively toxic to transformed cells of the human colorectal tumor line HT29." Carcinogenesis, 14(10): pp. 2079-83.

Hasegawa T; Nishino H; Iwashima A (1993) "Isothiocyanates inhibit cell cycle progression of HeLa cells at G2/M phase." Anti-Cancer Drugs, 4(2):, pp. 273-9.

National Toxicology Program (1982) "Carcinogensis Bioassay of Allyl Isothiocyanate (CAS No. 57-06-7) in F344/N Rats and B6C3F1 Mice (Gavage Study)", NTP Technical Report No. 234 / National Technical Information Service, No. PB-83-144238.

Park C M; Taormina P J; and Beuchat L R (2000) "Efficacy of allyl isothiocyanate in killing enterohemorrhagic Escherichia coli O157:H7 on alfalfa seeds." International Journal Of Food Microbiology, 56 (1): 13-20.

Smith T; Musk S R; Johnson I T (1996) "Allyl isothiocyanate selectively kills undifferentiated HT29 cells in vitro and suppresses aberrant crypt foci in the colonic mucosa of rats." Biochemical Society Transactions, 24(3): 381S.

Tokuoka K; Mori R; and Isshiki K (1992) "Inhibitory effects of volatile mustard extract on the growth of yeasts." Journal Of The Japanese Society for Food Science and Technology, 39 (1): 68-71.

Tokuoka K and Isshiki K (1994) "Possibility of application of allylisothiocyanate vapor for food preservation." Journal Of The Japanese Society for Food Science and Technology, 41 (9): 595-599.

Ward S M; Delaquis P J; Holley R A; and Mazza G (1998) "Inhibition of spoilage and pathogenic bacteria on agar and pre-cooked roast beef by volatile horseradish distillates." Food Research International, 31(1): 19-26.

Yonezawa Y; Nishikawa K; Nishioka H (1997) "Implication of intracellular reactive oxygen species in DNA damage by allyl isothiocyanate." Sci. Eng. Rev. Doshisha Univ, 38(1): pp. 1-8.

Xu K; Thornalley PJ (2000) "Studies on the mechanism of the inhibition of human leukemia cell growth by dietary isothiocyanates and their cysteine adducts in vitro." Biochem. Pharmacol., 60(2): pp. 221-231.

SUBMISSION END

SRS INTERNATIONAL® CORPORATION

1901 L Street, NW • Suite 250 • Washington, DC 20036-3503
Telephone (202) 223-0157/0298 • Fax (202) 835-8970
E-Mail: mainsrs@srsinternational.com http://www.srsinternational.com

Hand Delivered by Courier

October 14, 2003

Office of Premarket Approval
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, NW
Washington, DC 20204

Attention: Dr. Negash Belay

- Office Address -
  Office of Premarket Approval (HFS-255)
  1110 Vermont Avenue, Room 1295
  Washington, DC 20006

- Courier Delivery Address -
  Office of Food Additive Safety
  U.S. FDA
  5100 Paint Branch Parkway
  College Park, MD 20740

Subject: Notice of Claim of GRAS Exemption from Premarket Approval Requirements
Revisions to June 27, 2003 Submission per Agency comments and discussion by telephone September 15, 2003

Dear Dr. Belay:

Pursuant to the procedures and guidance provided in 21 CFR 170.36 [*Proposed Rule: 62 FR 18937 - 18964, April 17, 1997*] and additional guidance provided by the Agency in a meeting on May 16, 2003, we are submitting certain revisions to a Claim of GRAS Exemption from Premarket Approval Requirements previously submitted dated June 27, 2003. The revisions are as follows:

The baked goods claim should be narrowed to pies and dry baked goods should not appear on the claim.

This has been addressed in the revised GRAS Claim which follows

"Real numbers' should be used for anticipated VOM levels in foods as a result of the proposed use.

This has now been done both at pages 13 – 14 of the revised Information Attachment as well as in Appendix 1 to same (Dietary Exposure Tables).

A brief discussion of why the use would not inadvertently elevate the risk of botulism growth should be included. This concern arose due to the use of a modified food packaging atmosphere or in the event of use with vacuum packed foods.

This has now been done at page 18 of the revised Information Attachment.

The dietary exposure tables should be reviewed in detail so as to assure consistent use of units of measure and to incorporate data-based VOM levels for the assessment of dietary exposure associated with the proposed use.

This has now been done both in Appendix 1 to the revised Information Attachment (Dietary Exposure Tables).

The REVISED GRAS claim follows below -

> CAREX, Inc. (as Notifier) through its agent SRS International Corporation (as Notifier's Agent) make a claim that Volatile Oil of Mustard (*Brassica spp.*), when used in or on certain foods (meats, poultry, fish and shellfish, pies and moisture containing baked goods) and food products derived therefrom and either in bulk, in holding and transport containers, in display cases, or in consumer packaged forms at levels below the level which would impart noticeable flavor to a specific subject food but at a level sufficient to provide for shelf life extension and/or an anti-spoilage effect in the specific subject food, is exempt from the premarket approval requirements of the federal Food, Drug, and Cosmetic Act because the Notifier has determined such use is GRAS.

The Notifier provides below the information required under 21 CFR 170.36 [*Proposed Rule: 62 FR 18937 - 18964, April 17, 1997*] to be included in a claim of GRAS exemption from premarket approval requirements:

170.36(c)(1) [*Proposed*]: Claim of GRAS exemption, dated and signed by Notifier or Notifier's agent

> This is provided by the present letter making such claim as above stated and is signed and dated by the Notifier's Agent.

170.36(c)(1)(i) [*Proposed*]: Name and address of the Notifier

Notifier - CAREX, Inc.
1-1-47, Chuo, Joto-ku
Osaka, 536-0005, JAPAN

Contact: Mr. Yashushi Sekiyama
Director, Marketing and Business Development

Agent - SRS International Corporation
1901 L Street NW
Suite 250
Washington, DC 20036

Contact: Dr. John A. Todhunter
Tel. (202) 223 - 0157
Fax (202) 835 - 8970
e-mail: todhunter@srsinternational.com

170.36(c)(1)(ii) [*Proposed*]: Common or Usual Name of the Substance

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(1)(iii) [*Proposed*]: Applicable Conditions of Use [including foods in which the notified substance is to be used, levels of use in such foods, purpose for which the substance is used, and (if appropriate), description of the population expected to consume the substance.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(1)(iv) [*Proposed*]: Basis for the GRAS Determination

The Notifier has relied on scientific procedures for its determination of the GRAS status of the use which is claimed as exempt.

170.36(c)(1)(v) [*Proposed*]: Statement as to availability for FDA review and copying of data and information that are the basis for the Notifier's GRAS determination

The data and information that are the basis of the Notifier's GRAS determination are available for the Food and Drug Administration's (FDA) review and copying at reasonable times at the address specified below

SRS International Corporation
1901 L Street, NW / Suite 250 Tel. (202) 223 - 0157
Washington, DC 20036 Fax (202) 835-8970

or will be sent to FDA upon request.

170.36(c)(2) [*Proposed*]: Detailed information about the identity of the notified substance.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(3) [*Proposed*]: Information on any self-limiting levels of use.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(4) [*Proposed*]: Detailed summary of the basis for the Notifier's determination that the notified substance is GRAS.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(4)(i)(A) [*Proposed*]: Comprehensive discussion of, and citations to, generally available and accepted scientific data, information, methods, or principles the Notifier relies on to establish safety, including a discussion of the probable consumption of the substance and the effects thereof.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(4)(i)(B) [*Proposed*]: Comprehensive discussion of reports of investigations or other information that may appear to be inconsistent with the GRAS determination.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(4)(i)(C) [*Proposed*]: The basis for concluding, in light of the data and information under paragraphs (c)(1), (c)(2), (c)(3), (c)(4)(i)(A), and (c)(4)(i)(B) that there is consensus among experts qualified by scientific training and experience to evaluate the safety of substances added to food that there is reasonable certainty that the substance is not harmful under the intended conditions of use.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

Respectfully submitted on behalf of CAREX, Inc.

John A. Todhunter, Ph.D.
President,
SRS International Corporation
Agents for CAREX, Inc.

10/14/03
Date

INFORMATION ATTACHMENT TO NOTICE OF GRAS EXEMPTION CLAIM FOR:

VOLATILE OIL OF MUSTARD (VOM) AS A SHELF LIFE EXTENSION AND ANTI-SPOILAGE AGENT FOR CERTAIN FOODS

NOTIFIER:

CAREX, INC.
1-1-47, Chuo, Joto-ku
Osaka, 536-0005, Japan

NOTIFIER'S AGENT:

SRS INTERNATIONAL CORPORATION
1901 L Street, NW / Suite 250
Washington, DC 20036

Original: June 27, 2003
Revised: October 14, 2003

TABLE OF CONTENTS

REFERENCED GRAS EXEMPTION CLAIM

This Information Attachment is incorporated by reference into a GRAS Exemption Claim dated June 27, 2003 and submitted on behalf of the Notifier (CAREX, Inc.) by the Notifier's Agent (SRS International Corporation) to the Office of Premarket Approval (HFS-200), Center for Food Safety and Applied Nutrition. Food and Drug Administration, 200C Street, SW Washington, DC 20204.

The referenced GRAS Exemption Claim makes a claim that Volatile Oil of Mustard (*Brassica spp.*), when used in or on certain foods (meats, poultry, fish and shellfish, baked goods and food products derived therefrom and either in bulk, in holding and transport containers, in display cases, or in consumer packaged forms) at levels below the level which would impart noticeable flavor to a specific subject food but at a level sufficient to provide for shelf life extension and/or an anti-spoilage effect in the specific subject food, is exempt from the premarket approval requirements of the federal Food, Drug, and Cosmetic Act because the Notifier has determined such use is GRAS.

This Information Attachment provides certain information, specified in the referenced GRAS Exemption Claim, which is required to be provided as part of such a GRAS Exemption Claim by 21 CFR 170.36 [*Proposed Rule: 62 FR 18937 - 18964, April 17, 1997*].

The information provided herein consists of the following:

170.36(c)(1)(ii) [*Proposed*]: Common or Usual Name of the Substance

170.36(c)(1)(iii) [*Proposed*]: Applicable Conditions of Use [including foods in which the notified substance is to be used, levels of use in such foods, purpose for which the substance is used, and (if appropriate), description of the population expected to consume the substance.

170.36(c)(2) [*Proposed*]: Detailed information about the identity of the notified substance.

170.36(c)(3) [*Proposed*]: Information on any self-limiting levels of use.

170.36(c)(4) [*Proposed*]: Detailed summary of the basis for the Notifier's determination that the notified substance is GRAS.

170.36(c)(4)(i)(A) [*Proposed*]: Comprehensive discussion of, and citations to, generally available and accepted scientific data, information, methods, or principles the Notifier relies on to establish safety, including a discussion of the probable consumption of the substance and the effects thereof.

170.36(c)(4)(i)(B) [*Proposed*]: Comprehensive discussion of reports of investigations or other information that may appear to be inconsistent with the GRAS determination.

170.36(c)(4)(i)(C) [*Proposed*]: The basis for concluding, in light of the data and information under paragraphs (c)(1), (c)(2), (c)(3), (c)(4)(i)(A), and (c)(4)(i)(B) that there is consensus among experts qualified by scientific training and experience to evaluate the safety of substances added to food that there is reasonable certainty that the substance is not harmful under the intended conditions of use.

170.36(c)(1)(ii) [*Proposed*]: Common or Usual Name of the Substance

The common or usual name of the notified substance is -

Volatile Oil of Mustard

and it is also commonly known as -

Volatile mustard oil
Mustard oil
Oil of black mustard
Oleum sinapis
Senfoel
Allyl mustard oil
Allyl Isothiocyanate (natural)
Allyl thioisocyanate (natural)

This substance is referred to in the balance of this Information Attachment as "VOM".

170.36(c)(1)(iii) [*Proposed*]: Applicable Conditions of Use [including foods in which the notified substance is to be used, levels of use in such foods, purpose for which the substance is used, and (if appropriate), description of the population expected to consume the substance.

The specific use of VOM which is claimed as exempt from premarket approval requirements on the basis that the Notifier has determined that such use is GRAS is the use of VOM in or on certain foods (meats, poultry, fish and shellfish, baked goods and food products derived therefrom and either in bulk, in holding and transport containers, in display cases, or in consumer packaged forms) at levels below the level which would impart noticeable flavor to a specific subject food but at a level sufficient to provide for shelf life extension and/or an anti-spoilage effect in the specific subject food.

Foods in Which the Substance is to be Used:

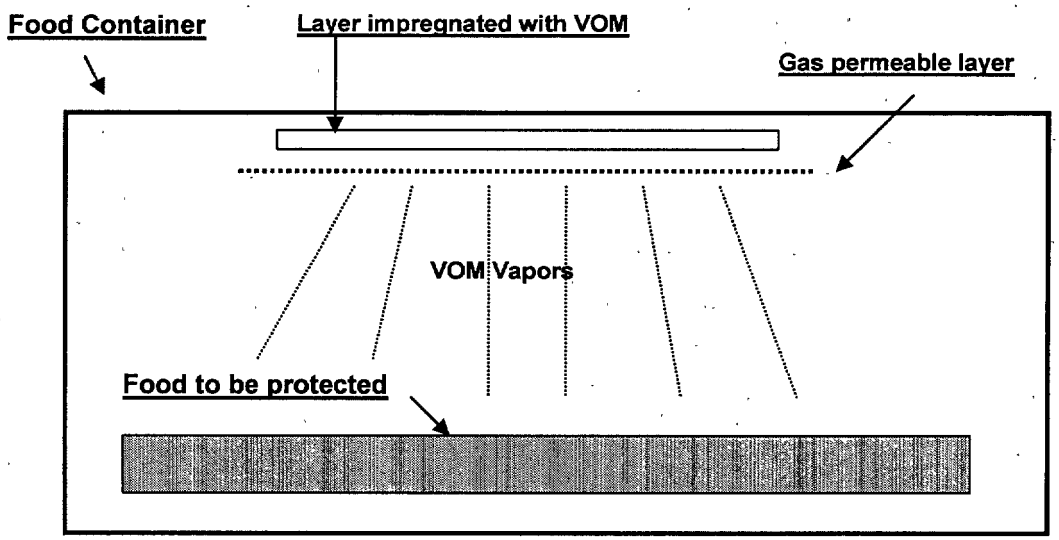
The foods in which the Notified Substance is to be used include meats, poultry, fish and shellfish, baked goods and food products derived therefrom and either in

bulk, in holding and transport containers, in display cases, or in consumer packaged forms.

VOM will be used in such foods by being incorporated into one of the layers (either the adhesive layer or a structural layer) of either a plastic film, paper tape, or labeling film which will serve as a delivery vehicle and reservoir for the VOM and which are sufficiently gas permeable as to allow the contained VOM to volatilize over time. For use, the VOM containing film, tape, or labeling is affixed to the inside of a food container or display case or used as film wrap for food. The volatilization of the VOM from the VOM impregnated material provides for exposure of the food to VOM vapors for the purpose of protection from spoilage. An schematic illustration of this concept is provided below.

As an alternative mode of use, VOM containing "packets" are prepared by impregnation of cellulose beads with VOM and enclosing these within a gas permeable, multilayer film container.

Figure: VOM gaseous delivery schematic representation



Levels of Use for the Notified Substance

The notified substance, VOM, will be used at levels below those which produce a noticeable flavor effect in the subject food but sufficient to provide the intended technical effect of shelf life extension and/or spoilage retardation ("anti-spoilage")

Usual VOM levels in some foods (selected from FASEB 1975, Table 1) which result from the flavoring uses of mustard oil are:

	Usual	Maximum
Meat products	35 ppm	61 ppm
Baked goods	14 ppm	29 ppm
Gravies, sauces	46 ppm	201 ppm

Therefore, the VOM levels in foods would be less than the above-cite "usual" flavoring levels.

The shelf life extension / anti-spoilage use has only the intended technical effect of retarding the spoilage of food with the primary intention not being to affect the organoleptic properties of the food. Published literature (see the Bibliography[1]) establishes that the levels needed to inhibit the growth of microorganisms in or on foods are from:

2 - 6 ppm for fungi/molds;
10 ppm for bacteria; and,
20 - 35 ppm for yeast

in the growth zone for these organisms.

Therefore, while VOM concentrations which inhibit the growth of yeast can approach the "usual" level for flavoring: (a) inhibition of fungi/molds and bacteria is attained at much less usual typical flavoring levels; and, (b) adequate inhibitory action against yeast can be obtained at less then the "usual" flavoring levels for VOM.

Also, since the inhibitory concentration needs only to be achieved in the potential growth zone for the microorganisms, VOM concentration is not distributed throughout the whole food being protected. This is readily conceptualized when one considers the example of a piece of meat in a VOM atmosphere. Transfer of VOM across the meat / air interface will be efficient as there is no diffusion barrier. Further diffusion of the VOM into the meat from the interface zone will be slower due to the need for the VOM to diffuse into a semi-solid matrix. Therefore, VOM concentrations near the surface of the meat (i.e., in the potential

[1] Foter and Golick, 1938; Tokuoka *et al.*, 1992; Isshiki *et al.*, 1992; Tokuoka and Isshiki, 1994; Holley, 1997; Delaquis and Sholberg, 1997; Ward *et al.*, 1998; Park *et al.*, 2000

microbial growing zone) will be higher than they will be deeper into the meat itself. Accordingly, flavoring of the entire food is not intended nor expected and this is borne out by the notification sponsor's experience with it's brand of VOM (Wasa Ouro) in use outside the U.S. Data developed on various foods support that levels in foods as consumed will be $\leq$ 8.6 ppm in whole meats, $\leq$ 4.5 ppm in sausages cooked before eating and in and ground meats, $\leq$ 51 ppm in bologna and related "cold cuts, $\leq$ 7.1 ppm in poultry and poultry products, and $\leq$ 5.1 ppm in pies.

Purpose for which the Notified Substance is to be Used

The purpose for which VOM will be used under the claimed GRAS exemption from premarket approval requirements is for shelf life extension and/or spoilage retardation in or on certain foods (meats, poultry, fish and shellfish, baked goods and food products derived therefrom and either in bulk, in holding and transport containers, in display cases, or in consumer packaged forms).

Description of the Population Expected to Consume the Substance

Due to the broad nature of the foods in which the Notified Substance is to be used the population expected to consume the substance is the entire U.S. population and all age groups within this population.

170.36(c)(2) [*Proposed*]: Detailed information about the identity of the notified substance.

(a) Common Names

Volatile Oil of Mustard

Also known as -
Mustard oil
Oil of black mustard
Oleum sinapis
Senfoel
Volatile mustard oil
Allyl mustard oil

(b) Chemical Name (of main consituent)

1-Propene, 3-isothiocyanato- (9CI) (CA INDEX NAME)

Also known as -
Isothiocyanic acid, allyl ester (6CI, 8CI)
2-Propenyl isothiocyanate
3-Isothiocyanato-1-propene

Allyl isothiocyanate
Allyl thioisocyanate

(c) Chemical Abstract Service (CAS) Registry Number

57-06-7

(d) Empirical Formula

$C_4 H_5 N S$

(e) Molecular Weight

99

(f) Structural Formula

$S = C = N - CH_2 - CH = CH_2$

(g) Specifications for Food Grade Material

Per the Food Chemicals Codex (FCC), Food Grade volatile oil of mustard shall meet the specifications below –

Assay	≥ 93% allyl isothiocyanate
Arsenic (as As)	≤ 3 ppm (0.0003%)
Heavy Metals (as Pb)	≤ 40 ppm (0.004%)
Phenols	Passes FCC Specified Test

(h) Quantitative Composition

Allyl isothiocyanate	≥ 95%
Allyl cyanide (AC)	< 2%
Carbon disulfide (CS)	< 2%
AC + CS Total	2% - 3%
Water	≤ 1%

(i) Manufacturing Process (excluding trade secrets)

The VOM which is the subject of this Notification is produced in Japan. This is done by the Notifier:

CAREX, Inc.
Office:
1-1-47 Chuo, Joto-ku
Osaka, 536-0005 Japan

Contact: Mr. Yasushi Sekiyama
Telephone: 81-6-6937-9255

The production process is conducted in accordance with current international (ISO) hygienic standards and good manufacturing practices for the manufacture of food chemicals and is as follows:

(1) The seeds of brown mustard (*Brassica juncea*) are pressed to remove non-volatile oils.

(2) The residue of pressed seeds is macerated with warm, deionized water and allowed to stand for one hour. This allows the endogenous myrosinase activity to liberate AIT from the endogenous sinigrin (a glucoside also known as potassium myronate).

(3) The macerate is distilled (i.e., steam distillation) to yield a volatile fraction which is typically $\geq$ 95% AIT

(4) Quality control inspections are conducted to assure that the product meets the following release specifications:

AIT Content	Minimum 94% (by GLC)
Specific Gravity, 15 ^{O}C	1.014 - 1.022
Refractive Index, 20 ^{O}C	1.523 - 1.529
Optical Rotation	$\pm 0^{O}$
Solubility in Water	Soluble in 160 to 300 parts of water
Solubility in 90% EtOH	1 : 0.5
Boiling Range	148 ^{O}C - 154 ^{O}C
Arsenic (as As)	$\leq$ 3 ppm (0.0003%)

Heavy Metals (as Pb)	≤ 40 ppm (0.004%)
Phenols	Passes FCC Specified Test

170.36(c)(3) [*Proposed*]: Information on any self-limiting levels of use.

The notified substance, VOM, will have self-limiting use levels. This limitation is created by that fact that use of VOM, for the herein claimed GRAS use, at levels which produce a noticeable flavor effect in the subject food will lead in most cases to food which is rejected by the consumer on the basis or taste and/or odor (of VOM).

Usual VOM levels in some foods (selected from FASEB 1975, Table 1) which result from the flavoring uses of mustard oil are:

	Usual	Maximum
Meat products	35 ppm	61 ppm
Baked goods	14 ppm	29 ppm
Gravies, sauces	46 ppm	201 ppm

Therefore, the VOM levels in foods would be less than the above-cite "usual" flavoring levels.

The shelf life extension / anti-spoilage use has only the intended technical effect of retarding the spoilage of food with the primary intention not being to affect the organoleptic properties of the food. Published literature (see the Bibliography[2]) establishes that the levels needed to inhibit the growth of microorganisms in or on foods are from:

2 - 6 ppm for fungi/molds;
10 ppm for bacteria; and,
20 - 35 ppm for yeast

in the growth zone for these organisms.

Therefore, while VOM concentrations which inhibit the growth of yeast can approach the "usual" level for flavoring: (a) inhibition of fungi/molds and bacteria is attained at much less usual typical flavoring levels; and, (b) adequate inhibitory action against yeast can be obtained at less then the "usual" flavoring levels for VOM.

[2] Foter and Golick, 1938; Tokuoka *et al.*, 1992; Isshiki *et al.*, 1992; Tokuoka and Isshiki, 1994; Holley, 1997; Delaquis and Sholberg, 1997; Ward *et al.*, 1998; Park *et al.*, 2000

170.36(c)(4) [*Proposed*]: Detailed summary of the basis for the Notifier's determination that the notified substance is GRAS.

The following is a detailed summary of the basis for the Notifier's determination that the notified substance is GRAS.

170.36(c)(4)(i)(A) [*Proposed*]: Comprehensive discussion of, and citations to, generally available and accepted scientific data, information, methods, or principles the Notifier relies on to establish safety, including a discussion of the probable consumption of the substance and the effects thereof.

and

170.36(c)(4)(i)(B) [*Proposed*]: Comprehensive discussion of reports of investigations or other information that may appear to be inconsistent with the GRAS determination.

Note: These two information items are provided in an integrated manner as they are inter-related for Volatile Oil of Mustard

Mustard, mustard oil, and other spices and spice essential oils have been used since the dawn of recorded history for their food preserving properties as well as their flavoring effects. Indeed, one is hard pressed to know whether the now-desired flavor effect of spices originated from human acculturation to consuming food preserved with mustard / mustard oil and other spices / spice oils or whether spices / spice oils where first sought for their flavoring properties and then – once in common use – their preservative properties became understood.

Various anti-spoilage uses of VOM have been reported in the technical / scientific literature since at least the 1920's. These include uses in preserving fruit juices, bread, and wine on a regular or regionally popular basis, as well as other foods on a more limited scale. (For references, see the Bibliography)

The use of VOM vapor for its anti-bacterial effects has been known since at least 1938 (Foter and Golick, 1938). Since the early 1990's a considerable amount of new research has been published on vapor-phase transfer delivery of VOM so as to achieve its known anti-spoilage effect (e.g.: Tokuoka *et al.*, 1992; Isshiki *et al.*, 1992; Tokuoka and Isshiki, 1994; Holley, 1997; Delaquis and Sholberg, 1997; Ward *et al.*, 1998; Park *et al.*, 2000).

This vapor phase method of using VOM for its anti-spoilage effect is identical to that used in the Wasa Ouro technology above-described. It consists of exposing the food to be protected to an atmosphere containing VOM vapor, during which exposure a small amount of VOM adsorbs onto the surface of the food and provides a surface protection

against the growth of bacteria and fungi (both pathogens and spoilage agents). Effective control of microbial growth has been reported at VOM air concentrations ranging from 1 μg VOM / L air to 720 μg VOM / L air (see the above references). Technical studies with Wasa Ouro brand VOM establish the following vapor MIC's for VOM's effects on various microorganisms:

		(ppm in Air)	μg / L air
MOLDS	*Alternaria alternata*	≤ 20 ppm	≤ 81
	Mucor racemosus	≤ 20 ppm	≤ 81
	Penicillium chrysogenum	20 - 40 ppm	81 - 162
	other *Penicillium* spp.	≤ 20 ppm	≤ 81
	Aspergillus spp.	20 - 60 ppm	81 - 242
	Cladosporidium spp.	≤ 20 ppm	≤ 81
	Rhizopus javanicus	40 - 90 ppm	162 - 364
	Fusarium spp.	≤ 20 ppm	≤ 81
YEASTS	*Candida albicans*	≤ 20 ppm	≤ 81
	Candida tropicalis	≤ 20 ppm	≤ 81
	Torulopsis versatilis	≤ 20 ppm	≤ 81
	Debarymyces kloeckeri	21 - 60 ppm	81 - 242
GRAM (+) BACTERIA	*Staphylococcus aureus*	40 - 90 ppm	162 - 364
	Staphylococcus epidermidis	40 - 90 ppm	162 - 364
	Bacillus spp.	21 - 90 ppm	81 - 364
	Streptococcus faecalis	61 - 120 ppm	242 - 485
	Pediococcus spp.	≥ 361 ppm	≥ 1460
	Lactobacillus spp.	≥ 361 ppm	≥ 1460
GRAM (-) BACTERIA	*Escherichia coli*	≤ 20 ppm	≤ 81
	Salmonella typhimurium	20 - 40 ppm	81 - 162
	Salmonella paratyphi B	21 - 60 ppm	81 - 242
	Other *Salmonella* spp.	20 - 40 ppm	81 - 162
	Shigella fexneri 1b	21 - 60 ppm	81 - 262
	Vibrio cholerae	≤ 20 ppm	≤ 81
	Vibrio parahaemolyticus	20 - 40 ppm	81 - 162
	Pseudomonas aeruginosa	≤ 20 ppm	≤ 81

Note: 1 ppm VOM in air = 4.04 μg AIT / L air

The above air-borne MIC's can be compared to solid and liquid culture media inhibitory concentrations of VOM reported in various scientific publications and summarized in Delaquis and Mazza's review (P.J. Delaquis and G. Mazza, "Antimicrobial properties of isothiocyanates in food preservation", Food Technology, Nov. 1995: 73 - 78):

Yeasts	20 - 35 μg/ml	(viable cells)
Fungi	2 - 6 μg/ml	(mycellar growth)
	150 - 600 μg/ml	(conidial germination)
Bacteria	*ca.* 10 μg/ml	(growth)

The actual concentration of VOM in a protected food will be a function of the food type, the duration of exposure to VOM, and the air concentration of VOM. With this in mind, the levels of VOM which are required within the effective microbial growing zone on a food need to be in the above-stated range of 2 - 10 μg/ml (or 2 - 10 ppm) to achieve effective inhibition of bacterial and fungal / mold growth and 20 - 35 μg/ml for yeasts.

The use which is here determined by the Notifier to be GRAS is for shelf life extension / anti-spoilage protection of foods. In the U.S. such foods will primarily be meats (i.e., beef, pork, lamb, veal, chicken, turkey, etc.), fish and shellfish, and baked goods (primarily pies). Meats to be protected can be protected both in typical pre-package forms or when displayed in meat counter cases. For pre-packaged meats, the packaging would include an VOM-containing matrix (not in direct contact with the meat) which would generate and sustain the desired VOM air concentration inside the packaging. For display case use, the VOM-containing matrix would be placed inside the display and would generate an VOM atmosphere inside the display case. Baked goods (pies) would have a VOM in-package delivery system essentially like that for pre-packaged meats.

For most meat, it is expected that the absorbed VOM would be driven off during cooking of the meat prior to consumption. Possible exceptions would be ham, bologna, salami, or other processed meats eaten without being first cooked or heated. For baked goods (pies), VOM loss due to cooking / heating is not expected to be significant since these baked goods are pre-baked. VOM loss, therefore, is expected only when the consumer pre-heated the item prior to consumption.

Beyond this, and of importance, is that only adherence to current good practice in the manufacture, processing, handling, and storage of food is necessary to assure low levels of VOM in foods as consumed. This is because foods which acquire a flavor from VOM or an odor of a more than very transient nature will be rejected by consumers. Experience with the use of Wasa Ouro brand VOM-containing packaging in Japan has shown that the packaging / holding container delivery system for AIT must be tailored to the type and amount of food in the package / holding container as well as to the package / holding container materials and volume so as to provide the desired anti-spoilage effect without imparting undesired flavor or odor. This integration of delivery system design with specific food packaging / holding systems assures that foods treated with airborne VOM as an anti-spoilage agent will not contain excessive levels of VOM (i.e., sufficient to impart noticeable flavor or odor to the food). In this way, the proposed uses is VOM is self-limiting.

The VOM levels in various foods exposed to VOM atmospheres have been determined by the Notifier and are as represented in the table below.

Table: Expected worst case VOM levels in foods after a 10-day exposure to 150 ppm VOM in air and in foods as "as consumed"

Food Type	Uptake Factor	10-Day VOM Level	Reduction on Cooking	"As Consumed " VOM Level
Beef, ground (representing also whole cuts of beef and veal and lamb, ground and cuts of)	≤ 0.34	≤ 51 ppm	5.9	≤ 8.6 ppm
Chicken, ground (representing also whole chicken and turkey, ground and whole)	≤ 0.24	≤ 36 ppm	5.1	≤ 7.1 ppm
Pork sausage (representing also ground pork and cuts of pork and ham)	≤ 0.10	< 15 ppm	3.3	≤ 4.5 ppm
Bologna, ground (representing sliced deli meats)	≤ 0.34	≤ 51 ppm	N/A	≤ 51 ppm
Pecan pie, ground (representing nut and fruit pies)	≤ 0.03	≤ 4.5 ppm	N/A	≤ 4.5 ppm

Note: 150 ppm in air will be a typical VOM atmosphere for this use

The estimated dietary intake associated with shelf-life extension / anti-spoilage uses of VOM on relevant foods is provided in Tables D-1A through D-10B (preceding the Bibliography). Tables D-1A, D-1B, D-2A, and D-2B provide the estimated daily dietary VOM intake associated with all VOM treated foods and for all age groups. The other tables provide the daily intake of VOM associated with specific food types.

The basic food consumption data are from USDA's Home Economics Research Repot No. 44, "Foods Commonly Eaten by Individuals". Age-specific average daily consumption of a given food type is obtained by multiplying the average amount consumed per eating occasion (for individuals consuming the food at least one in any given three day period) by the percent of individuals consuming the food at least once during any given three day period. The average daily intake of VOM for a given food type is then estimated by multiplying the average daily consumption (by age) for the food type by the concentration of VOM in the food. This type of assessment approach is one normally relied upon by experts in the evaluation of exposures to substances in foods.

For purposes of the estimation of daily dietary intake of VOM the following assumptions were made:

(a) 100% of any given food type was assumed to be treated with VOM. This is a conservative assumption because not all foods of any given type will be exposed to VOM for anti-spoilage purposes.

(b) Flavoring uses of VOM were assumed to produce an average 35 ppm VOM in food. This is a reasonable, fact-based assumption.

(c) Anti-Spoilage uses of VOM were assumed to produce 9 ppm in the entire food item for meats and fish, 7.1 ppm in the entire food item for poultry, and 4.5 ppm in the entire food item for pies (based on the data from the table above). This is a conservative assumption because the VOM will generally be in the surface VOM diffusion zone for a food (as opposed to being uniformly distributed throughout the food).

As can be seen from Tables D-1A through D-2B, the total VOM dietary load (as parts per billion [ppb] in the total daily diet) for the U.S. population which is associated with both flavoring and shelf life extension / anti-spoilage uses is:

Age Group	Flavoring		Anti-Spoilage	
	Male (D-1A)	*Female (D-2A)*	*Male (D-1B)*	*Female (D-2B)*
0 - 1 yr.	1.06 ppm	1.06 ppm	0.27 ppm	0.27 ppm
1 - 2 yr.	1.69 ppm	1.69 ppm	0.44 ppm	0.44 ppm
3 - 5 yr.	1.47 ppm	1.47 ppm	0.38 ppm	0.38 ppm
6 - 8 yr.	1.37 ppm	1.37 ppm	0.35 ppm	0.35 ppm
9 - 14 yr.	1.48 ppm	1.26 ppm	0.38 ppm	0.32 ppm
15 - 18 yr.	1.64 ppm	1.17 ppm	0.42 ppm	0.30 ppm
19 - 34 yr.	1.85 ppm	1.21 ppm	0.48 ppm	0.31 ppm
35 - 64 yr.	1.90 ppm	1.32 ppm	0.49 ppm	0.34 ppm
65 - 74 yr.	1.59 ppm	1.15 ppm	0.41 ppm	0.30 ppm

The flavor uses of VOM are, therefore, reasonably estimated to produce to dietary loads of > 1 ppm for all age and sex groups and between 1.4 - 1.9 ppm for all age groups other than infants (0 - 1 yr.).

In contrast, the shelf life extension / anti-spoilage uses is reasonably expected to lead to produce dietary loads of < 0.5 ppm for all age and sex groups.

The flavor uses of VOM are already listed as GRAS at 21 CFR 182.20. The shelf life extension / anti-spoilage uses, would of necessity be safe (due to an even lower dietary exposure) and this is generally recognized to be the case by experts qualified in the evaluation of the safety of substances added to food (see information for 170.36(c)(4)(i)(C), below).

Further to the safety of VOM, the dietary safety of VOM (including, also, that of synthetic ally isothiocyanate) was extensively reviewed as part of the Agency's review of GRAS substances in the 1970's (Food & Drug Research Labs, 1972; FASEB, 1975). As noted in the conclusion of this extensive review report:

There is no evidence in the available information on allyl isothiocyanate, p-hydroxybenzyl isothiocyanate, and brown and yellow mustard that demonstrates or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current of that might reasonably be expected in the future. **(FASEB, 1975 at p. 15)**

The above, quite clear cut, safety picture was complicated in 1982 by the results of carcinogencity testing carried out by the National Toxicology Program (NTP, 1982). In this study, conducted with synthetic allyl isothiocyanate ("AIT") as opposed to the natural product obtained from mustard (*Brassica* spp.), the result for AIT was negative in male and female mice and equivocal in female rats. In male rats, the result was positive on the basis of transitional cell papillomas of the urinary bladder.

The literature review provided in the NTP study report serves to update the mutagenicity / gene toxicity data base for AIT at the time. Since the FASEB review in 1975 (FASEB, 1975), bacterial mutagenicity tests in *B. subtillis* H17 and M45 were negative as were Ames tests in *S. typhimurium* TA98, TA100, TA 1535 and TA 1537 and the *rec* A test in *E. coli* WP2. These negative mutagenicity and gene toxicity tests, together with the above-reported negative uniformity of earlier mutagenicity and gene toxicity tests for AIT (FASEB, 1975) strongly suggest that AIT will not act, *in vivo*, through any direct, genotoxic carcinogenic mechanism.

In this context, it must also be taken into account that there was a corresponding and marked dose- related increase in hyperplasia of the urinary bladder epithelium in these male rats (0/49, 1/49, & 6/49 for control, low, and high dose groups respectively). In these male rats, the incidences of transitional cell papillomas were: 0/49, 2/49, and 4/49 for controls, low dose, and high dose groups, respectively. In female rats the incidence of transitional cell papillomas of the bladder was 0/49, 0/49, & 1/50 for controls, low dose, and high dose and the incidence of epithelial hyperplasia of the bladder was 0/49, 0/49, & 1/50 for control, low, and high dose groups, respectively. In both male and female mice, no epithelial hyperplasia of the urinary bladder was reported in any of the study animals and no transitional cell papillomas of the bladder are observed.

These data suggest a connection between the occurrence of epithelial hyperplasia and transitional papillomas in these test species, in fact the incidences of the two types of lesions appear to mirror each other. The fact that a transitional cell papilloma was observed in a female of the high dose group precludes any possible argument that perhaps female rats do not form transitional cell papillomas.

An earlier study by Major (1970), using the mouse two-stage skin carcinogenesis model, showed that synthetic AIT, when applied to mouse skin at an irritant dose level, induced a transient hyperplasia and an elevated mitotic index in the skin.

IARC (1985) reviewed a mouse skin carcinogenesis study which included natural VOM (as opposed to synthetic AIT). Volatile Oil of Mustard, which contained > 90%

allyl isothiocyanate, was tested in a two stage mouse skin assay. Three control groups of 12-16 'S' strain mice (actual strain, age and sex unspecified) received 0.3 ml of 0.1-0.15% 7,12-dimethylbenz(a)anthracene (DMBA) on the skin of the back followed by no treatment (two groups) or 21 days later by twice weekly applications for 12 weeks and weekly applications for 15 weeks of acetone. A fourth group of 16 mice received an initial skin application of 0.3 ml of 0.1% DMBA followed 39 days later by applications of a 3-4% solution of mustard oil in acetone weekly for 20 weeks. The incidence of skin papillomas among animals surviving to the end of the experiment was 4/21, 1/12 and 1/16 in the combined DMBA control groups with no secondary treatment, in controls receiving DMBA followed by acetone, and in treated mice receiving DMBA and mustard oil, respectively. In this study, **natural** VOM was neither carcinogenic nor co-carcinogenic.

The suggestion from both the data reported above is that: (a) the transitional cell papillomas which were observed were secondary to the effect of hyperplasia on promotion of pre-existing tumor precursor cells and/or increasing the susceptibility of normal bladder stem cells to transformation due to increased time spent cell cycle phases in which chromatin is un-condensed and more exposed; (b) that such effects would not be expected at lower, non-irritant doses of AIT; (c) that the result in the NTP biosassy was not mediated via a genotoxic mechanism of carcinogenesis and, therefore, will have a low dose threshold; and, (d) that there may be a difference in effect between natural Volatile Oil of Mustard and synthetic AIT.

Since the NTP Bioassay, no additional reliable *in vivo* evidence suggesting an AIT or VOM carcinogenic dietary risk to humans has been reported.

Kasamaki *et al.* (1987) reported than AIT (synthetic) was able to induce cell transformation of Chinese hamster B241 cells. This study, however, did not include adequate controls for the effects of the extensive passaging which was required to observe the low level of transformants reported.

Musk and Johnson (1993) reported that AIT (synthetic) did not produce chromosome aberrations *in vitro* under conditions in which benzyl-, phenethyl-, and phenyl-isothiocyanates produced chromosomal aberrations.

Bechtel, *et al.* (1998) reported that AIT (synthetic) did not induce unscheduled DNA synthesis in male rats when given at up to 125 mg/kg. This supports that AIT lacks the ability to directly induce cancer by a genotoxic mechanism *in vivo*.

Kassie and Knasmuller (2000) reported on an *in vitro* gene-toxicity battery study of AIT (synthetic) and phenethyl isothiocyanate (PEITC). The test battery included Ames tests in TA 98 and TA 100, the DNA repair test in E. coli, and a micronucleus test with human derive Hep G2 cells. AIT was reported to be more active in the bacterial test systems than in the mammalian cell test system. When tested *in vivo* at very high doses (90 and 270 mg/kg), using an E. coli marker, AIT's activity was significantly attenuated. This attenuation was suggested to be due to protein binding of AIT under *in vivo* conditions. Further investigations *in vitro* suggested that the formation of reactive

oxygen species could be mediated by AIT and PEITC. The suggestion that AIT might mediate the formation of reactive oxygen species has also been investigated by Murata at al. (2000) and Yonezawa et al., (1997) in *in vitro* studies.

Since the time of the NTP bioassay the metabolism of AIT has, also, been shown to differ between rat and the mouse and human (with the human metabolizing AIT like the mouse - which was negative in the NTP bioassay - and not like the rat).

Bollard et al. (1997) report that urinary metabolites account for the bulk of administered AIT (synthetic) doses in mice and rats (80% and 75% respectively). In mice, there are three major urinary species: inorganic thiocyanate ion, the glutathione conjugate[3], and the cysteine conjugate[4]. In contrast, rats do not form the cysteine conjugate and, also, show a greater retention of ^{14}C-AIT than do mice at 4 days (18-24% in rats *versus* 2-5% in mice). The results of this study suggest that in mice, AIT is primarily metabolized via hydrolysis whereas in rats glutathione conjugation is the predominant metabolic route. Of marked interest to the urinary papilloma picture for rats, a sex difference was reported for AIT in bladder tissue of rats, with higher levels of AIT / AIT-metabolites seen in the bladder tissue of male rats than in female rats. This finding would correlate with the observed differences in both bladder hyperplasia and papilloma incidence seen between male and female rats. In humans, as in mice, the major urinary metabolite is the cysteine conjugate and glutathione conjugation appears to be at most a minor pathway for the human metabolism (Jiao *et al.*, 1994).

The International Agency for Research on Cancer (IARC), has reviewed the overall weight of the evidence for carcinogencity of AIT and concluded that the evidence in animals is "limited" and in humans is "not classifiable" (IARC, 1999).

Beyond this immediate safety issue, AIT is probably an anti-carcinogen in the human diet. Therefore, its incorporation in the human diet may present potential public health benefits. Some selected citations follow.

Musk and Johnson (1993) reported on the selective toxicity of AIT to the human colorectal cell line HT29. A later report by Smith *et al.*, (1996) extended this in vitro finding to in vivo suppression of aberrant crypt foci in the colonic mucosa of rats.

Hasegawa *et al.*, (1993) reported on AIT inhibition of the malignant human HeLa cell line.

Xu and Thornalley (2000) have reported on the inhibition of human leukemia cells by AIT. As these authors note, the anti-cancer effect of consumption of cruciferous vegatables – which are rich in AIT – is well known.

---

[3] Allylthiocarbamoylmercapturic acid

[4] Allylthiocarbamoylcysteine

Another issue which may affect safety is the potential concern that the proposed use not, inadvertently, create an opportunity for growth of botulism organisms. This concern arises due to the presence of a modified atmosphere or in the case of vacuum packed foods (due to the anaerobic atmosphere created).

With regard to the first issue, the air concentrations of VOM in the proposed use will be *circa* 150 ppm but not greater. At this VOM level, there is no significant change in the oxygen concentration in air and a fully aerobic conditions, which prevents the growth of botulism, will be maintained.

With regard to the second issue, the proposed use of VOM is not compatible with vacuum packed foods as the VOM would be evacuated in the vacuum packing process. Therefore, this is not an applicable concern for this use.

Therefore, the current general recognition among experts as to the safety of VOM can be summarized as follows:

- AIT and/or VOM is consistently reported as non-genotoxic *in vivo* and usually is reported to be non-genotoxic *in vitro*.

- AIT has irritant properties which have been reported to produce transient hyperplasia of mouse skin when applied to mouse skin.

- In dermal mouse skin carcinogenicity bioassays, VOM was not carcinogenic.

- In a corn oil gavage carcinogencity bioassay in mice and rats, AIT was not carcinogenic in male or female mice, was considered equivocal in female rats, and was considered to increase the incidence of transitional cell papillomas in male rats. In male rats, the incidence of transitional cell papillomas mirrored that of bladder epithelial hyperplasia. In female rats, a single animal with transitional cell papilloma of the bladder was observed in the high dose group and, again, the incidence of bladder epithelial hyperplasia mirrored that of transitional cell papillomas in female rats.

- The metabolism of AIT differs significantly between rats and mice. In rats, glutathione conjugation is the major pathway and there is a sex difference in the accumulation of AIT / AIT-metabolites in the rat bladder with males showing higher levels than females. In mice, hydrolysis with formation of thiocyanate ion and of the cysteine conjugate is the major pathway for AIT. Humans appear to metabolize AIT similarly to mice.

- The weight of the evidence strongly supports that the increased incidence of transitional cell papillomas seen in male rats in the NTP bioassay is specific to the male rat under these test conditions and is probably due to accumulation of levels of the AIT-glutathione conjugate which induce epithelial hyperplasia in the male rat

under the NTP test conditions and that the papillomas are secondary to this hyperplasia. Female rats were observed, in the NTP bioassay, to be capable of forming transitional cell papillomas in the bladder but the incidence of epithelial hyperplasia was much lower than male rats and, accordingly, so was the incidence of papillomas (1/50 in high dose females *vs.* 0/49 in control females for both hyperplasia and papillomas). This is most likely a reflection of the fact that female rats do not accumulate AIT metabolites in the bladder to the same extent that male rats do. Given the known differences in AIT metabolism between rats and humans and the similarities between mouse and human AIT metabolism, the NTP bioassay actually suggests that AIT will be non-carcinogenic in humans, as was the case for the mice in the NTP bioassay.

- The NTP bioassay results in rats are not, therefore, an appropriate predictor, in this case, for AIT carcinogenic risk to humans at the levels presently consumed in the daily diet. The NTP bioassay results for mice, which metabolize AIT differently from rats but similarly to humans, indicate that AIT is not expected to be carcinogenic in the human diet and at the low levels associated with current food uses of AIT or of VOM both are generally considered as safe for human consumption.

- The proposed use will not increase the risk of botulism growth.

170.36(c)(4)(i)(C) [*Proposed*]: The basis for concluding, in light of the data and information under paragraphs (c)(1), (c)(2), (c)(3), (c)(4)(i)(A), and (c)(4)(i)(B) that there is consensus among experts qualified by scientific training and experience to evaluate the safety of substances added to food that there is reasonable certainty that the substance is not harmful under the intended conditions of use.

A number of scientific studies which have been published in the open literature clearly establish that there is a current consensus, i.e., general recognition, of the safety of AIT (synthetic) and VOM (natural) as currently consumed in the human diet. This is that at current consumption levels, AIT is toxicologically safe for humans and, to a reasonable degree of scientific certainty, does not have any expected carcinogenic potential. The anti-spoilage use of AIT will not significantly alter human dietary consumption of AIT as the resultant levels in foods, of necessity, are lower than current levels for use in imparting flavor to relevant foods.

IARC – an internationally recognized body of experts – in 1999 re-stated its conclusions as to the dietary safety of AIT (and VOM) and lack of weight of evidence for a carcinogenic risk to humans.

The U.S. Food and Drug Administration currently lists the flavor uses (and other potential but unspecified uses) as GRAS uses (21 CFR 182.20). The above referenced IARC expert review addresses the dietary safety of VOM at these low levels of use associated with flavoring and, of necessity, yet lower levels of VOM in the diet. As is discussed and supported above, the dietary VOM levels estimated, on a worst case basis, to be associated with the use for which the Notifier has made a GRAS determination and for which the Notifier is claiming a GRAS exemption from premarket approval requirements are much lower than those associated with flavoring uses and are, therefore, also generally recognized as safe for human consumption.

APPENDIX 1

Tables for Dietary Exposures to VOM Associated with both Flavoring and Anti-Spoilage Uses

000113 [redacted]

Table D-1A: Total Dietary Loading from Consumption of Foods
Containing 35 ppm Volatile Oil of Mustard (i.e.: flavoring use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	66.20	92.30	104.125	106.12	78.84
Poultry	5.64	13.93	16.19	19.39	24.81	29.78	30.324	30.60	27.56
Fish & Shellfish	0.35	3.81	5.51	7.20	8.80	9.71	15.56	16.68	17.05
Pies	0.11	1.74	3.12	3.65	5.92	8.43	8.84	9.42	13.07
TOTALS:	**15.15**	**48.41**	**62.91**	**78.46**	**105.72**	**140.22**	**158.85**	**162.82**	**136.52**
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Total Foods per Kg-day	1.87	4.28	3.77	3.38	2.83	2.34	2.18	2.09	1.75
Volatile Oil of Mustard Level in Food	**35 ppm**		**100% of Food Assumed to Contain Volatile Oil of Mustard**						
Dietary Load as mg/Kg/day	0.0653	0.1498	0.1318	0.1184	0.0992	0.0819	0.0762	0.0731	0.0613
Dietary Load as mg/day	0.530	1.694	2.202	2.746	3.700	4.908	5.560	5.699	4.778
Dietary Load as ppm in Total Diet	1.06	1.69	1.47	1.37	1.48	1.64	1.85	1.90	1.59
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT				NOEL = 8.6 mg/Kg/day					
	132	57	65	73	87	105	113	118	140

Table D-1B: Total Dietary Loading from Consumption of Foods
Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	66.20	92.30	104.125	106.12	78.84
Poultry	5.64	13.93	16.19	19.39	24.81	29.78	30.324	30.60	27.56
Fish & Shellfish	0.35	3.81	5.51	7.20	8.80	9.71	15.56	16.68	17.05
Pies	0.11	1.74	3.12	3.65	5.92	8.43	8.84	9.42	13 07
TOTALS:	**15.15**	**48.41**	**62.91**	**78.46**	**105.72**	**140.22**	**158.85**	**162.82**	**136.52**
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Total Foods per Kg-day	1.87	4.28	3.77	3.38	2.83	2.34	2.18	2.09	1.75
Volatile Oil of Mustard Level in Food	**9 ppm**	**100% of Food Assumed to Contain Volatile Oil of Mustard**							
	Assumed equal in all foods to worst case meat levels								
Dietary Load as mg/Kg/day	0.0168	0.0385	0.0339	0.0304	0.0255	0.0211	0.0196	0.0188	0.0158
Dietary Load as mg/day	0.136	0.436	0.566	0.706	0.951	1.262	1.430	1.465	1.229
Dietary Load As ppm in Total Diet	0.27	0.44	0.38	0.35	0.38	0.42	0.48	0.49	0.41
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	**NOEL = 8.6 mg/Kg/day**								
	512	223	254	283	337	408	439	458	546

Table D-2A: Dietary Loading from Consumption of Foods
Containing 35 ppm Volatile Oil of Mustard (i.e.: flavoring use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	56.41	62.78	64.038	68.88	52.46
Poultry	5.64	13.93	16.19	19.39	20.68	22.29	23.328	24.53	24.81
Fish & Shellfish	0.35	3.81	5.51	7.20	7.56	10.89	11.308	13.72	12.35
Pies	0.11	1.74	3.12	3.65	5.62	4.60	5.1	6.43	8.91
TOTALS:	**15.15**	**48.41**	**62.91**	**78.46**	**90.27**	**100.55**	**103.77**	**113.56**	**98.52**
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Total Foods per Kg-day	2.02	4.40	3.86	3.34	2.45	1.80	1.73	1.75	1.43
Volatile Oil of Mustard Level In Food	**35 ppm**		**100% of Food Assumed to Contain Volatile Oil of Mustard**						
Dietary Load as mg/Kg/day	0.0709	0.1540	0.1351	0.1169	0.0859	0.0630	0.0605	0.0611	0.0500
Dietary Load as mg/day	0.530	1.694	2.202	2.746	3.159	3.519	3.632	3.974	3.448
Dietray Load As ppm in Total Diet	1.06	1.69	1.47	1.37	1.26	1.17	1.21	1.32	1.15
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT				**NOEL = 8.6 mg/Kg/day**					
	121	56	64	74	100	137	142	141	172

Table D-2B: Dietary Loading from Consumption of Foods
Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	56.41	62.78	64.038	68.88	52.46
Poultry	5.64	13.93	16.19	19.39	20.68	22.29	23.328	24.53	24.81
Fish & Shellfish	0.35	3.81	5.51	7.20	7.56	10.89	11.308	13.72	12.35
Pies	0.11	1.74	3.12	3.65	5.62	4.60	5.1	6.43	8.91
TOTALS:	**15.15**	**48.41**	**62.91**	**78.46**	**90.27**	**100.55**	**103.77**	**113.56**	**98.52**
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Total Foods per Kg-day	2.02	4.40	3.86	3.34	2.45	1.80	1.73	1.75	1.43
Volatile Oil of Mustard Level in Food	9 ppm	100% of Food Assumed to Contain Volatile Oil of Mustard							
	Assumed equal in all foods to worst case meat levels								
Dietary Load as mg/Kg/day	0.0182	0.0396	0.0347	0.0300	0.0221	0.0162	0.0156	0.0157	0.0128
Dietary Load as mg/day	0.136	0.436	0.566	0.706	0.812	0.905	0.934	1.022	0.887
Dietray Load As ppm in Total Diet	0.27	0.44	0.38	0.35	0.32	0.30	0.31	0.34	0.30
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	472	217	248	286	390	531	552	547	669

Table D-3A: Dietary Loading from Consumption of Beef, Pork, Lamb, and Veal (all forms) Containing 35 ppm Volatile Oil of Mustard (i.e.: flavoring use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	66.20	92.30	104.125	106.12	78.84
TOTALS:	**9.05**	**28.93**	**38.09**	**48.22**	**66.20**	**92.30**	**104.13**	**106.12**	**78.84**
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Meat Foods per Kg-day	1.11	2.56	2.28	2.08	1.77	1.54	1.43	1.36	1.01
Volatile Oil of Mustard Level in Food	35 ppm			100% of Food Assumed to Contain Volatile Oil of Mustard					
Dietary Load as mg/Kg/day	0.0390	0.0895	0.0798	0.0727	0.0621	0.0539	0.0499	0.0476	0.0354
Dietary Load as mg/day	0.317	1.013	1.333	1.688	2.317	3.230	3.644	3.714	2.760
Dietary Load as ppm in Total Diet	0.63	1.01	0.89	0.84	0.93	1.08	1.21	1.24	0.92
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	221	96	108	118	138	159	172	181	243

Table D-3B: Dietary Loading from Consumption of Beef, Pork, Lamb, and Veal (all forms) Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	66.20	92.30	104.125	106.12	78.84
TOTALS:	**9.05**	**28.93**	**38.09**	**48.22**	**66.20**	**92.30**	**104.13**	**106.12**	**78.84**
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Meat Foods per Kg-day	1.11	2.56	2.28	2.08	1.77	1.54	1.43	1.36	1.01
Volatile Oil of Mustard Level in Food	9 ppm 100% of Food Assumed to be Contain Volatile Oil of Mustard *Based on worst case meat values*								
Dietary Load as mg/Kg/day	0.0100	0.0230	0.0205	0.0187	0.0160	0.0139	0.0128	0.0122	0.0091
Dietary Load as mg/day	0.081	0.260	0.343	0.434	0.596	0.831	0.937	0.955	0.710
Dietary Load As ppm in Total Diet	0.16	0.26	0.23	0.22	0.24	0.28	0.31	0.32	0.24
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	858	374	419	460	538	620	670	702	945

Table D-4A: Dietary Loading from Consumption of Beef, Pork, Lamb, and Veal (all forms) Containing 35 ppm Volatile Oil of Mustard (i.e.: flavoring use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	56.41	62.78	64.038	68.88	52.46
TOTALS:	**9.05**	**28.93**	**38.09**	**48.22**	**56.41**	**62.78**	**64.04**	**68.88**	**52.46**
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Meat Foods per Kg-day	1.21	2.63	2.34	2.05	1.53	1.12	1.07	1.06	0.76
Volatile Oil of Mustard Level in Food	35 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard							
Dietary Load as mg/Kg/day	0.0423	0.0921	0.0818	0.0718	0.0537	0.0393	0.0374	0.0371	0.0266
Dietary Load as mg/day	0.317	1.013	1.333	1.688	1.974	2.197	2.241	2.411	1.836
Dietary Load As ppm in Total Diet	0.63	1.01	0.89	0.84	0.79	0.73	0.75	0.80	0.61
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	203	93	105	120	160	219	230	232	323

Table D-4B: Dietary Loading from Consumption of Beef, Pork, Lamb, and Veal (all forms) Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	56.41	62.78	64.038	68.88	52.46
TOTALS:	**9.05**	**28.93**	**38.09**	**48.22**	**56.41**	**62.78**	**64.04**	**68.88**	**52.46**
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Meat Foods per Kg-day	1.21	2.63	2.34	2.05	1.53	1.12	1.07	1.06	0.76
Volatile Oil of Mustard Level in Food	9 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard							
	Based on worst case meat values								
Dietary Load as mg/Kg/day	0.0109	0.0237	0.0210	0.0185	0.0138	0.0101	0.0096	0.0095	0.0068
Dietary Load as mg/day	0.081	0.260	0.343	0.434	0.508	0.565	0.576	0.620	0.472
Dietary Load As ppm in Total Diet	0.16	0.26	0.23	0.22	0.20	0.19	0.19	0.21	0.16
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	790	363	409	466	623	851	895	902	1,257

Table D-5A: Dietary Loading from Consumption of Poultry (all forms)
Containing 35 ppm Volatile Oil of Mustard (i.e.: flavoring use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Poultry	5.64	13.93	16.19	19.39	24.81	29.78	30.324	30.60	27.56
TOTALS:	**5.64**	**13.93**	**16.19**	**19.39**	**24.81**	**29.78**	**30.32**	**30.60**	**27.56**
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Poultry Foods per Kg-day	0.69	1.23	0.97	0.84	0.67	0.50	0.42	0.39	0.35
Volatile Oil of Mustard Level in Food	35 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard							
Dietary Load as mg/Kg/day	0.0243	0.0431	0.0339	0.0293	0.0233	0.0174	0.0145	0.0137	0.0124
Dietary Load as mg/day	0.197	0.487	0.567	0.679	0.868	1.042	1.061	1.071	0.965
Dietary Load As ppm in Total Diet	0.39	0.49	0.38	0.34	0.35	0.35	0.35	0.36	0.32
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	354	200	253	294	369	494	592	626	695

Table D-5B: Dietary Loading from Consumption of Poultry (all forms)
Containing 7.1 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Poultry	5.64	13.93	16.19	19.39	24.81	29.78	30.324	30.60	27.56
TOTALS:	**5.64**	**13.93**	**16.19**	**19.39**	**24.81**	**29.78**	**30.32**	**30.60**	**27.56**
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Poultry Foods per Kg-day	0.69	1.23	0.97	0.84	0.67	0.50	0.42	0.39	0.35
Volatile Oil of Mustard Level in Food	7.1 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard *Based on worst case poultry values*							
Dietary Load as mg/Kg/day	0.0049	0.0087	0.0069	0.0059	0.0047	0.0035	0.0029	0.0028	0.0025
Dietary Load as mg/day	0.040	0.099	0.115	0.138	0.176	0.211	0.215	0.217	0.196
Dietary Load As ppm in Total Diet	0.08	0.10	0.08	0.07	0.07	0.07	0.07	0.07	0.07
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	1,743	984	1,250	1,449	1,821	2,436	2,916	3,087	3,428

Table D-6A: Dietary Loading from Consumption of Poultry (all forms)
Containing 35 ppm Volatile Oil of Mustard (i.e.: flavor use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Poultry	5.64	13.93	16.19	19.39	20.68	22.29	23.328	24.53	24.81
TOTALS:	**5.64**	**13.93**	**16.19**	**19.39**	**20.68**	**22.29**	**23.33**	**24.53**	**24.81**
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Poultry Foods per Kg-day	0.75	1.27	0.99	0.83	0.56	0.40	0.39	0.38	0.36
Volatile Oil of Mustard Level In Food	35 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard							
Dietary Load as mg/Kg/day	0.0264	0.0443	0.0348	0.0289	0.0197	0.0140	0.0136	0.0132	0.0126
Dietary Load as mg/day	0.197	0.487	0.567	0.679	0.724	0.780	0.816	0.859	0.868
Dietary Load As ppm in Total Diet	0.39	0.49	0.38	0.34	0.29	0.26	0.27	0.29	0.29
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	326	194	247	298	437	616	632	651	684

Table D-6B: Dietary Loading from Consumption of Poultry (all forms)
Containing 7.1 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Poultry	5.64	13.93	16.19	19.39	20.68	22.29	23.328	24.53	24.81
TOTALS:	**5.64**	**13.93**	**16.19**	**19.39**	**20.68**	**22.29**	**23.33**	**24.53**	**24.81**
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Poultry Foods per Kg-day	0.75	1.27	0.99	0.83	0.56	0.40	0.39	0.38	0.36
Volatile Oil of Mustard Level in Food	7.1 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard							
	Based on worst case poultry values								
Dietary Load as mg/Kg/day	0.0054	0.0090	0.0071	0.0059	0.0040	0.0028	0.0028	0.0027	0.0026
Dietary Load as mg/day	0.040	0.099	0.115	0.138	0.147	0.158	0.166	0.174	0.176
Dietary Load As ppm in Total Diet	0.08	0.10	0.08	0.07	0.06	0.05	0.06	0.06	0.06
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	1,606	957	1,220	1,468	2,155	3,038	3,115	3,210	3,369

Table D-7A: Dietary Loading from Consumption of Fish and Shellfish (all forms) Containing 35 ppm Volatile Oil of Mustard (i.e.: flavor use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Fish & Shellfish	0.35	3.81	5.51	7.20	8.80	9.71	15.56	16.68	17.05
TOTALS:	**0.35**	**3.81**	**5.51**	**7.20**	**8.80**	**9.71**	**15.56**	**16.68**	**17.05**
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Sea Foods per Kg-day	0.04	0.34	0.33	0.31	0.24	0.16	0.21	0.21	0.22
Volatile Oil of Mustard Level in Food	35 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard							
Dietary Load as mg/Kg/day	0.0015	0.0118	0.0115	0.0109	0.0083	0.0057	0.0075	0.0075	0.0077
Dietary Load as mg/day	0.012	0.133	0.193	0.252	0.308	0.340	0.545	0.584	0.597
Dietary Load As ppm in Total Diet	0.02	0.13	0.13	0.13	0.12	0.11	0.18	0.19	0.20
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	5,783	730	745	792	1,041	1,516	1,153	1,149	1,124

Table D-7B: Dietary Loading from Consumption of Fish and Shellfish (all forms)
Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Fish & Shellfish	0.35	3.81	5.51	7.20	8.80	9.71	15.56	16.68	17.05
TOTALS:	**0.35**	**3.81**	**5.51**	**7.20**	**8.80**	**9.71**	**15.56**	**16.68**	**17.05**
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Sea Foods per Kg-day	0.04	0.34	0.33	0.31	0.24	0.16	0.21	0.21	0.22
Volatile Oil of Mustard Level in Food	9 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard *Based on worst case meats values*							
Dietary Load as mg/Kg/day	0.0004	0.0030	0.0030	0.0028	0.0021	0.0015	0.0019	0.0019	0.0020
Dietary Load as mg/day	0.003	0.034	0.050	0.065	0.079	0.087	0.140	0.150	0.153
Dietary Load As ppm in Total Diet	0.01	0.03	0.03	0.03	0.03	0.03	0.05	0.05	0.05
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	22,490	2,840	2,897	3,079	4,050	5,897	4,482	4,469	4,371

Table D-8A: Dietary Loading from Consumption of Fish and Shellfish (all forms) Containing 35 ppm Volatile Oil of Mustard (i.e.: flavoring use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Fish & Shellfish	0.35	3.81	5.51	7.20	7.56	10.89	11.308	13.72	12.35
TOTALS:	0.35	3.81	5.51	7.20	7.56	10.89	11.31	13.72	12.35
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Sea Foods per Kg-day	0.05	0.35	0.34	0.31	0.21	0.19	0.19	0.21	0.18
Volatile Oil of Mustard Level in Food	35 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard							
Dietary Load as mg/Kg/day	0.0016	0.0121	0.0118	0.0107	0.0072	0.0068	0.0066	0.0074	0.0063
Dietary Load as mg/day	0.012	0.133	0.193	0.252	0.264	0.381	0.396	0.480	0.432
Dietary Load As ppm in Total Diet	0.02	0.13	0.13	0.13	0.11	0.13	0.13	0.16	0.14
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	5,327	710	727	802	1,197	1,261	1,304	1,164	1,373

Table D-8B: Dietary Loading from Consumption of Fish and Shellfish (all forms) Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extensiom use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Fish & Shellfish	0.35	3.81	5.51	7.20	7.56	10.89	11.308	13.72	12.35
TOTALS:	**0.35**	**3.81**	**5.51**	**7.20**	**7.56**	**10.89**	**11.31**	**13.72**	**12.35**
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Sea Foods per Kg-day	0.05	0.35	0.34	0.31	0.21	0.19	0.19	0.21	0.18
Volatile Oil of Mustard Level in Food	9 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard *Based on worst case meats values*							
Dietary Load as mg/Kg/day	0.0004	0.0031	0.0030	0.0028	0.0018	0.0018	0.0017	0.0019	0.0016
Dietary Load as mg/day	0.003	0.034	0.050	0.065	0.068	0.098	0.102	0.123	0.111
Dietary Load As ppm in Total Diet	0.01	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.04
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	20,718	2,762	2,828	3,119	4,653	4,905	5,070	4,527	5,339

Table D-9A: Dietary Loading from Consumption of Pies (all forms)
Containing 35 ppm Volatile Oil of Mustard (i.e.: flavor use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Pies	0.11	1.74	3.12	3.65	5.92	8.43	8.84	9.42	13.07
TOTALS:	**0.11**	**1.74**	**3.12**	**3.65**	**5.92**	**8.43**	**8.84**	**9.42**	**13.07**
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Pies per Kg-day	0.01	0.15	0.19	0.16	0.16	0.14	0.12	0.12	0.17
Volatile Oil of Mustard Level In Food	35 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard							
Dietary Load as mg/Kg/day	0.0005	0.0054	0.0065	0.0055	0.0056	0.0049	0.0042	0.0042	0.0059
Dietary Load as mg/day	0.004	0.061	0.109	0.128	0.207	0.295	0.309	0.330	0.457
Dietary Load As ppm in Total Diet	0.01	0.06	0.07	0.06	0.08	0.10	0.10	0.11	0.15
Dietary Load Safety Factor to NOEL In Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	18,138	1,598	1,314	1,564	1,549	1,746	2,029	2,035	1,467

Table D-9B: Dietary Loading from Consumption of Pies (all forms)
Containing 4.5 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Pies	0.11	1.74	3.12	3.65	5.92	8.43	8.84	9.42	13.07
TOTALS:	**0.11**	**1.74**	**3.12**	**3.65**	**5.92**	**8.43**	**8.84**	**9.42**	**13.07**
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Pies per Kg-day	0.01	0.15	0.19	0.16	0.16	0.14	0.12	0.12	0.17
Volatile Oil of Mustard Level in Food	4.5 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard							
	Based on worst case pie values								
Dietary Load as mg/Kg/day	0.0001	0.0007	0.0008	0.0007	0.0007	0.0006	0.0005	0.0005	0.0008
Dietary Load as mg/day	0.0005	0.0078	0.0141	0.0164	0.0266	0.0379	0.0398	0.0424	0.0588
Dietary Load As ppm in Total Diet	0.001	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	141,075	12,429	10,216	12,164	12,049	13,576	15,782	15,824	11,410

Table D-10A: Dietary Loading from Consumption of Pies (all forms)
Containing 35 ppm Volatile Oil of Mustard (i.e.: flavor use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Pies	0.11	1.74	3.12	3.65	5.62	4.60	5.1	6.43	8.91
TOTALS:	**0.11**	**1.74**	**3.12**	**3.65**	**5.62**	**4.60**	**5.10**	**6.43**	**8.91**
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Pies per Kg-day	0.01	0.16	0.19	0.16	0.15	0.08	0.09	0.10	0.13
Volatile Oil of Mustard Level in Food	35 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard							
Dietary Load as mg/Kg/day	0.0005	0.0055	0.0067	0.0054	0.0053	0.0029	0.0030	0.0035	0.0045
Dietary Load as mg/day	0.004	0.061	0.109	0.128	0.197	0.161	0.179	0.225	0.312
Dietary Load As ppm In Total Diet	0.01	0.06	0.07	0.06	0.08	0.05	0.06	0.07	0.10
Dietary Load Safety Factor to NOEL In Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	16,709	1,554	1,282	1,584	1,610	2,986	2,891	2,485	1,904

Table D-10B: Dietary Loading from Consumption of Pies (all forms)
Containing 4.5 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Pies	0.11	1.74	3.12	3.65	5.62	4.60	5.1	6.43	8.91
TOTALS:	**0.11**	**1.74**	**3.12**	**3.65**	**5.62**	**4.60**	**5.10**	**6.43**	**8.91**
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Pies per Kg-day	0.01	0.16	0.19	0.16	0.15	0.08	0.09	0.10	0.13
Volatile Oil of Mustard Level in Food	4.5 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard							
	Based on worst pie values								
Dietary Load as mg/Kg/day	0.0001	0.0007	0.0009	0.0007	0.0007	0.0004	0.0004	0.0004	0.0006
Dietary Load as mg/day	0.0005	0.0078	0.0141	0.0164	0.0253	0.0207	0.0230	0.0289	0.0401
Dietary Load As ppm In Total Diet	0.001	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Dietary Load Safety Factor to NOEL In Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	129,956	12,089	9,972	12,321	12,518	23,224	22,484	19,331	14,806

APPENDIX 2

Bibliography

Prior to 1958

1. Anderson, E.E., et al. 1951. Pasteurized fresh whole pickles. II. Thermal resistance of microorganisms and peroxidase. Food Technol. 5(9): 364-368.

2. Anderson, E.E., et al. 1953. Effect of essential oils on the inhibition and thermal resistance of microorganisms in acid food products. Food Research. 18: 40-47.

3. Blum, H.B. and F.W. Fabian. 1943. Spice oils and their components for controlling microbiol surface growth. Fruit Products J. 22: 326-329, 347.

4. Delage, J.C. 1923. Use of mustard meal and of mustard oil in enology, Ann. Fals. 16: 483-491.

5. Ferre, L. 1925. Action of allyl mustard oil as a wine and must preservative. Ann. fals. 18: 34-37.

6. Kosker, O.; et al. 1949. Mustard as a preservative for fruit juices. The Glass Packer. 28: 818-820, 836, 838, 841.

7. Kosker, O.; et al. 1951. Effect of allyl isothiocyanate and related substances on the thermal resistance of Aspergillus niger, Saccharomyces ellipsoideus, and Bacillus thermoacidurans. Fd. Res. 16(6): 510-514.

8. Malvrzin and G. Bidart. 1923. The action of standard allyl mustard oil solutions in preventing secondary fermentations. Ann. fuls. 16: 534-542.

9. Perazzo, A. and L. de prado. 1937. The action of mustard essence on wines. Its use as antifermentative agent. Rev. assoc. bioquim. argentina. 5: 54-61.

10. Roos, L. and E. Hugues. 1924. Allyl mustard oil in enology. Ann. Fals. 17: 413-421.

11. Vaughan, J.G. and J.S. Hemingway. 1959. The utilization of mustards. Econ. Bot. 13(3): 196-204.

12. Vincens. 1924. Experiments on the use of mustard oil (allyl) in enology. Ann. fals. 17: 541-551.

13. Walker, J.C.; et al. 1937. Toxicity of mustard oils and related sulfur compounds to certain fungi. Am. J. Bot. 24(8); 536-541 (2 fig.).

Post 1958 References

Bechtel D; Henderson L; and Proudlock R (1998) "Lack of UDS activity in the livers of rats exposed to allylisothiocyanate." Teratogenesis, Carcinogenesis, And Mutagenesis, 18 (5): 209-217.

Bollard M; Stribbling S; Mitchell S; Caldwell J (1997) "The disposition of allyl isothiocyanate in the rat and mouse." Food And Chemical Toxicology, 35 (10-11): 933-943.

Delaquis P J and Sholberg P L (1997) "Antimicrobial activity of gaseous allyl isothiocyanate." Journal Of Food Protection, 60 (8): 943-947.

FASEB [Federation of American Societies for Experimental Biology] (1975) "Evaluation of the Health Aspects of Mustard and Oil of Mustard as Food Ingredients" National Technical Information Service, No. PB-254528

Food and Drug Research Labs., Inc. (1972) "GRAS (Generally Recognized as Safe) Food Ingredients – Oil of Mustard and Allyl Isothiocyanate", National Technical Information Service, No. PB-221215

Holley R A (1997) "Impact of slicing hygiene upon shelf life and distribution of spoilage bacteria in vacuum packaged cured meats" Food Microbiology (London), 14 (3): 201-211

IARC (1999) "Allyl isothiocyanate" IARC Monographs On The Evaluation Of Carcinogenic Risks To Humans, 73: 37-48.

IARC (1985) "Allyl isothiocyanate" IARC Monographs On The Evaluation Of Carcinogenic Risks To Humans, 36: pp. 60 ff.

Isshiki K; Tokuoka K; Mori R; and Chiba S (1992) "Preliminary examination of allyl isothiocyanate vapor for food preservation." Biosci Biotechnol Biochem, 56 (9): 1476-1477.

Jiao D; Ho C T; Foiles P; Chung F L (1994) "Identification and quantification of the N-acetylcysteine conjugate of allyl isothiocyanate in human urine after ingestion of mustard." Cancer Epidemiology, Biomarkers And Prevention, 3(6): 487-92.

Kasamaki A; Yasuhara T; Urasawa S, (1987) "Neoplastic transformation of Chinese hamster cells in vitro after treatment with flavoring agents." Journal Of Toxicological Sciences, 12(4): 383-96.

Kassie F; Knasmuller S (2000) "Genotoxic effects of allyl isothiocyanate (AITC) and phenethyl isothiocyanate (PEITC)." Chemico-Biological Interactions, 127(2): 163-80.

Murata M; Yamashita N; Inoue S; Kawanishi S (2000)
"Mechanism of oxidative DNA damage induced by carcinogenic allyl isothiocyanate." Free Radical Biol. Med., 28(5): pp. 797-805.

Musk S R; Johnson I T (1993) "The clastogenic effects of isothiocyanates." Mutation Research, 300(2): 111-117.

Musk SR R; Johnson IT (1993) "Allyl isothiocyanate is selectively toxic to transformed cells of the human colorectal tumor line HT29." Carcinogenesis, 14(10): pp. 2079-83.

Hasegawa T; Nishino H; Iwashima A (1993) "Isothiocyanates inhibit cell cycle progression of HeLa cells at G2/M phase." Anti-Cancer Drugs, 4(2):, pp. 273-9.

National Toxicology Program (1982) "Carcinogensis Bioassay of Allyl Isothiocyanate (CAS No. 57-06-7) in F344/N Rats and B6C3F1 Mice (Gavage Study)", NTP Technical Report No. 234 / National Technical Information Service, No. PB-83-144238.

Park C M; Taormina P J; and Beuchat L R (2000) "Efficacy of allyl isothiocyanate in killing enterohemorrhagic Escherichia coli O157:H7 on alfalfa seeds." International Journal Of Food Microbiology, 56 (1): 13-20.

Smith T; Musk S R; Johnson I T (1996) "Allyl isothiocyanate selectively kills undifferentiated HT29 cells in vitro and suppresses aberrant crypt foci in the colonic mucosa of rats." Biochemical Society Transactions, 24(3): 381S.

Tokuoka K; Mori R; and Isshiki K (1992) "Inhibitory effects of volatile mustard extract on the growth of yeasts." Journal Of The Japanese Society for Food Science and Technology, 39 (1): 68-71.

Tokuoka K and Isshiki K (1994) "Possibility of application of allylisothiocyanate vapor for food preservation." Journal Of The Japanese Society for Food Science and Technology, 41 (9): 595-599.

Ward S M; Delaquis P J; Holley R A; and Mazza G (1998) "Inhibition of spoilage and pathogenic bacteria on agar and pre-cooked roast beef by volatile horseradish distillates." Food Research International, 31(1): 19-26.

Yonezawa Y; Nishikawa K; Nishioka H (1997) "Implication of intracellular reactive oxygen species in DNA damage by allyl isothiocyanate." Sci. Eng. Rev. Doshisha Univ, 38(1): pp. 1-8.

Xu K; Thornalley PJ (2000) "Studies on the mechanism of the inhibition of human leukemia cell growth by dietary isothiocyanates and their cysteine adducts in vitro." Biochem. Pharmacol., 60(2): pp. 221-231.

000137

SRS INTERNATIONAL® CORPORATION

1901 L Street, NW • Suite 250 • Washington, DC 20036-3503
Telephone (202) 223-0157/0298 • Fax (202) 835-8970
E-Mail: mainsrs@srsinternational.com http://www.srsinternational.com

FAX TRANSMISSION

DESTINATION NO.	202-418-3131
ATTENTION OF:	Dr Negash Belay
COMPANY NAME:	FDA
NUMBER OF PAGES:	52 *(Including Cover Page)*
AUTHORIZED BY:	Michael Farrow
DATE:	11-6-03

COMMENTS

This fax is being sent in 2 sections due to its length

PART I

Letter – 5 pgs.
Report – 21 pgs

SRS INTERNATIONAL® CORPORATION

1901 L Street, NW • Suite 250 • Washington, DC 20036-3503
Telephone (202) 223-0157/0298 • Fax (202) 835-8970
E-Mail: mainsrs@srsinternational.com http://www.srsinternational.com

Hand Delivered by Courier October 14, 2003

Office of Premarket Approval
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, NW
Washington, DC 20204

Attention: Dr. Negash Belay

- Office Address -
  Office of Premarket Approval (HFS-255)
  1110 Vermont Avenue, Room 1295
  Washington, DC 20006

- Courier Delivery Address -
  Office of Food Additive Safety
  U.S. FDA
  5100 Paint Branch Parkway
  College Park, MD 20740

Subject: Notice of Claim of GRAS Exemption from
Premarket Approval Requirements
Revisions to June 27, 2003 Submission per Agency comments and discussion by telephone September 15, 2003

Dear Dr. Belay:

Pursuant to the procedures and guidance provided in 21 CFR 170.36 [*Proposed Rule: 62 FR 18937 - 18964, April 17, 1997*] and additional guidance provided by the Agency in a meeting on May 16, 2003, we are submitting certain revisions to a Claim of GRAS Exemption from Premarket Approval Requirements previously submitted dated June 27, 2003. The revisions are as follows:

The baked goods claim should be narrowed to pies and dry baked goods should not appear on the claim.

This has been addressed in the revised GRAS Claim which follows

[redacted]

000139

"Real numbers' should be used for anticipated VOM levels in foods as a result of the proposed use.

This has now been done both at pages 13 – 14 of the revised Information Attachment as well as in Appendix 1 to same (Dietary Exposure Tables).

A brief discussion of why the use would not inadvertently elevate the risk of botulism growth should be included. This concern arose due to the use of a modified food packaging atmosphere or in the event of use with vacuum packed foods.

This has now been done at page 18 of the revised Information Attachment.

The dietary exposure tables should be reviewed in detail so as to assure consistent use of units of measure and to incorporate data-based VOM levels for the assessment of dietary exposure associated with the proposed use.

This has now been done both in Appendix 1 to the revised Information Attachment (Dietary Exposure Tables).

The REVISED GRAS claim follows below -

> CAREX, Inc. (as Notifier) through its agent SRS International Corporation (as Notifier's Agent) make a claim that Volatile Oil of Mustard (*Brassica spp.*), when used in or on certain foods (meats, poultry, fish and shellfish, pies and moisture containing baked goods) and food products derived therefrom and either in bulk, in holding and transport containers, in display cases, or in consumer packaged forms at levels below the level which would impart noticeable flavor to a specific subject food but at a level sufficient to provide for shelf life extension and/or an anti-spoilage effect in the specific subject food, is exempt from the premarket approval requirements of the federal Food, Drug, and Cosmetic Act because the Notifier has determined such use is GRAS.

The Notifier provides below the information required under 21 CFR 170.36 [*Proposed Rule: 62 FR 18937 - 18964, April 17, 1997*] to be included in a claim of GRAS exemption from premarket approval requirements:

170.36(c)(1) [*Proposed*]: Claim of GRAS exemption, dated and signed by Notifier or Notifier's agent

> This is provided by the present letter making such claim as above stated and is signed and dated by the Notifier's Agent.

170.36(c)(1)(i) [*Proposed*]: Name and address of the Notifier

Notifier - CAREX, Inc.
1-1-47, Chuo, Joto-ku
Osaka, 536-0005, JAPAN

Contact: Mr. Yashushi Sekiyama
Director, Marketing and Business Development

Agent - SRS International Corporation
1901 L Street NW
Suite 250
Washington, DC 20036

Contact: Dr. John A. Todhunter
Tel. (202) 223 - 0157
Fax (202) 835 - 8970
e-mail: todhunter@srsinternational.com

170.36(c)(1)(ii) [*Proposed*]: Common or Usual Name of the Substance

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(1)(iii) [*Proposed*]: Applicable Conditions of Use [including foods in which the notified substance is to be used, levels of use in such foods, purpose for which the substance is used, and (if appropriate), description of the population expected to consume the substance.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(1)(iv) [*Proposed*]: Basis for the GRAS Determination

The Notifier has relied on scientific procedures for its determination of the GRAS status of the use which is claimed as exempt.

170.36(c)(1)(v) [*Proposed*]: Statement as to availability for FDA review and copying of data and information that are the basis for the Notifier's GRAS determination

The data and information that are the basis of the Notifier's GRAS determination are available for the Food and Drug Administration's (FDA) review and copying at reasonable times at the address specified below

SRS International Corporation
1901 L Street, NW / Suite 250 Tel. (202) 223 - 0157
Washington, DC 20036 Fax (202) 835-8970

or will be sent to FDA upon request.

170.36(c)(2) [*Proposed*]: Detailed information about the identity of the notified substance.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(3) [*Proposed*]: Information on any self-limiting levels of use.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(4) [*Proposed*]: Detailed summary of the basis for the Notifier's determination that the notified substance is GRAS.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(4)(i)(A) [*Proposed*]: Comprehensive discussion of, and citations to, generally available and accepted scientific data, information, methods, or principles the Notifier relies on to establish safety, including a discussion of the probable consumption of the substance and the effects thereof.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(4)(i)(B) [*Proposed*]: Comprehensive discussion of reports of investigations or other information that may appear to be inconsistent with the GRAS determination.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

170.36(c)(4)(i)(C) [*Proposed*]: The basis for concluding, in light of the data and information under paragraphs (c)(1), (c)(2), (c)(3), (c)(4)(i)(A), and (c)(4)(i)(B) that there is consensus among experts qualified by scientific training and experience to evaluate the safety of substances added to food that there is reasonable certainty that the substance is not harmful under the intended conditions of use.

This is provided in the Information Attachment to this Notice of GRAS Exemption Claim (which attachment is incorporated herein by reference)

Respectfully submitted on behalf of CAREX, Inc.

John A. Todhunter, Ph.D.
President,
SRS International Corporation
Agents for CAREX, Inc.

10/14/03
Date

INFORMATION ATTACHMENT TO NOTICE OF GRAS EXEMPTION CLAIM FOR:

VOLATILE OIL OF MUSTARD (VOM) AS A SHELF LIFE EXTENSION AND ANTI-SPOILAGE AGENT FOR CERTAIN FOODS

NOTIFIER:

CAREX, INC.
1-1-47, Chuo, Joto-ku
Osaka, 536-0005, Japan

NOTIFIER'S AGENT:

SRS INTERNATIONAL CORPORATION
1901 L Street, NW / Suite 250
Washington, DC 20036

Original: June 27, 2003
Revised: October 14, 2003

TABLE OF CONTENTS

REFERENCED GRAS EXEMPTION CLAIM

This Information Attachment is incorporated by reference into a GRAS Exemption Claim dated June 27, 2003 and submitted on behalf of the Notifier (CAREX, Inc.) by the Notifier's Agent (SRS International Corporation) to the Office of Premarket Approval (HFS-200), Center for Food Safety and Applied Nutrition. Food and Drug Administration, 200C Street, SW Washington, DC 20204.

The referenced GRAS Exemption Claim makes a claim that Volatile Oil of Mustard (*Brassica spp.*), when used in or on certain foods (meats, poultry, fish and shellfish, baked goods and food products derived therefrom and either in bulk, in holding and transport containers, in display cases, or in consumer packaged forms) at levels below the level which would impart noticeable flavor to a specific subject food but at a level sufficient to provide for shelf life extension and/or an anti-spoilage effect in the specific subject food, is exempt from the premarket approval requirements of the federal Food, Drug, and Cosmetic Act because the Notifier has determined such use is GRAS.

This Information Attachment provides certain information, specified in the referenced GRAS Exemption Claim, which is required to be provided as part of such a GRAS Exemption Claim by 21 CFR 170.36 [*Proposed Rule: 62 FR 18937 - 18964, April 17, 1997*].

The information provided herein consists of the following:

170.36(c)(1)(ii) [*Proposed*]: Common or Usual Name of the Substance

170.36(c)(1)(iii) [*Proposed*]: Applicable Conditions of Use [including foods in which the notified substance is to be used, levels of use in such foods, purpose for which the substance is used, and (if appropriate), description of the population expected to consume the substance.

170.36(c)(2) [*Proposed*]: Detailed information about the identity of the notified substance.

170.36(c)(3) [*Proposed*]: Information on any self-limiting levels of use.

170.36(c)(4) [*Proposed*]: Detailed summary of the basis for the Notifier's determination that the notified substance is GRAS.

170.36(c)(4)(i)(A) [*Proposed*]: Comprehensive discussion of, and citations to, generally available and accepted scientific data, information, methods, or principles the Notifier relies on to establish safety, including a discussion of the probable consumption of the substance and the effects thereof.

170.36(c)(4)(i)(B) [*Proposed*]: Comprehensive discussion of reports of investigations or other information that may appear to be inconsistent with the GRAS determination.

170.36(c)(4)(i)(C) [*Proposed*]: The basis for concluding, in light of the data and information under paragraphs (c)(1), (c)(2), (c)(3), (c)(4)(i)(A), and (c)(4)(i)(B) that there is consensus among experts qualified by scientific training and experience to evaluate the safety of substances added to food that there is reasonable certainty that the substance is not harmful under the intended conditions of use.

170.36(c)(1)(ii) [*Proposed*]: Common or Usual Name of the Substance

The common or usual name of the notified substance is -

Volatile Oil of Mustard

and it is also commonly known as -

Volatile mustard oil
Mustard oil
Oil of black mustard
Oleum sinapis
Senfoel
Allyl mustard oil
Allyl Isothiocyanate (natural)
Allyl thioisocyanate (natural)

This substance is referred to in the balance of this Information Attachment as "VOM".

170.36(c)(1)(iii) [*Proposed*]: Applicable Conditions of Use [including foods in which the notified substance is to be used, levels of use in such foods, purpose for which the substance is used, and (if appropriate), description of the population expected to consume the substance.

The specific use of VOM which is claimed as exempt from premarket approval requirements on the basis that the Notifier has determined that such use is GRAS is the use of VOM in or on certain foods (meats, poultry, fish and shellfish, baked goods and food products derived therefrom and either in bulk, in holding and transport containers, in display cases, or in consumer packaged forms) at levels below the level which would impart noticeable flavor to a specific subject food but at a level sufficient to provide for shelf life extension and/or an anti-spoilage effect in the specific subject food.

Foods in Which the Substance is to be Used:

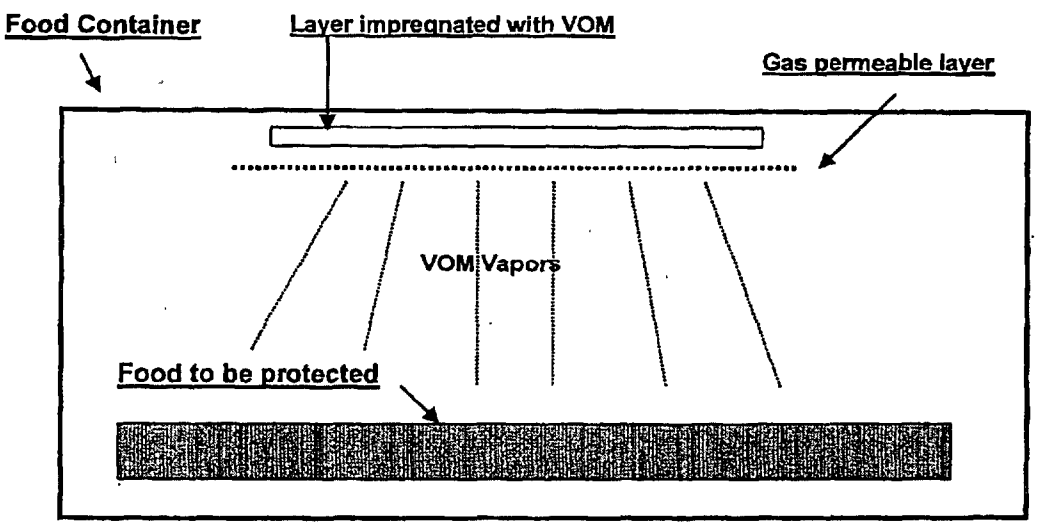
The foods in which the Notified Substance is to be used include meats, poultry, fish and shellfish, baked goods and food products derived therefrom and either in

bulk, in holding and transport containers, in display cases, or in consumer packaged forms.

VOM will be used in such foods by being incorporated into one of the layers (either the adhesive layer or a structural layer) of either a plastic film, paper tape, or labeling film which will serve as a delivery vehicle and reservoir for the VOM and which are sufficiently gas permeable as to allow the contained VOM to volatilize over time. For use, the VOM containing film, tape, or labeling is affixed to the inside of a food container or display case or used as film wrap for food. The volatilization of the VOM from the VOM impregnated material provides for exposure of the food to VOM vapors for the purpose of protection from spoilage. An schematic illustration of this concept is provided below.

As an alternative mode of use, VOM containing "packets" are prepared by impregnation of cellulose beads with VOM and enclosing these within a gas permeable, multilayer film container.

Figure: VOM gaseous delivery schematic representation



Levels of Use for the Notified Substance

The notified substance, VOM, will be used at levels below those which produce a noticeable flavor effect in the subject food but sufficient to provide the intended technical effect of shelf life extension and/or spoilage retardation ("anti-spoilage")

Usual VOM levels in some foods (selected from FASEB 1975, Table 1) which result from the flavoring uses of mustard oil are:

	Usual	Maximum
Meat products	35 ppm	61 ppm
Baked goods	14 ppm	29 ppm
Gravies, sauces	46 ppm	201 ppm

Therefore, the VOM levels in foods would be less than the above-cite "usual" flavoring levels.

The shelf life extension / anti-spoilage use has only the intended technical effect of retarding the spoilage of food with the primary intention not being to affect the organoleptic properties of the food. Published literature (see the Bibliography[1]) establishes that the levels needed to inhibit the growth of microorganisms in or on foods are from:

2 - 6 ppm for fungi/molds;
10 ppm for bacteria; and,
20 - 35 ppm for yeast

in the growth zone for these organisms.

Therefore, while VOM concentrations which inhibit the growth of yeast can approach the "usual" level for flavoring: (a) inhibition of fungi/molds and bacteria is attained at much less usual typical flavoring levels; and, (b) adequate inhibitory action against yeast can be obtained at less then the "usual" flavoring levels for VOM.

Also, since the inhibitory concentration needs only to be achieved in the potential growth zone for the microorganisms, VOM concentration is not distributed throughout the whole food being protected. This is readily conceptualized when one considers the example of a piece of meat in a VOM atmosphere. Transfer of VOM across the meat / air interface will be efficient as there is no diffusion barrier. Further diffusion of the VOM into the meat from the interface zone will be slower due to the need for the VOM to diffuse into a semi-solid matrix. Therefore, VOM concentrations near the surface of the meat (i.e., in the potential

[1] Foter and Golick, 1938; Tokuoka *et al.*, 1992; Isshiki *et al.*, 1992; Tokuoka and Isshiki, 1994; Holley, 1997; Delaquis and Sholberg, 1997; Ward *et al.*, 1998; Park *et al.*, 2000

microbial growing zone) will be higher than they will be deeper into the meat itself. Accordingly, flavoring of the entire food is not intended nor expected and this is borne out by the notification sponsor's experience with it's brand of VOM (Wasa Ouro) in use outside the U.S. Data developed on various foods support that levels in foods as consumed will be ≤ 8.6 ppm in whole meats, ≤ 4.5 ppm in sausages cooked before eating and in and ground meats, ≤ 51 ppm in bologna and related "cold cuts, ≤ 7.1 ppm in poultry and poultry products, and ≤ 5.1 ppm in pies.

Purpose for which the Notified Substance is to be Used

The purpose for which VOM will be used under the claimed GRAS exemption from premarket approval requirements is for shelf life extension and/or spoilage retardation in or on certain foods (meats, poultry, fish and shellfish, baked goods and food products derived therefrom and either in bulk, in holding and transport containers, in display cases, or in consumer packaged forms).

Description of the Population Expected to Consume the Substance

Due to the broad nature of the foods in which the Notified Substance is to be used the population expected to consume the substance is the entire U.S. population and all age groups within this population.

170.36(c)(2) [*Proposed*]: Detailed information about the identity of the notified substance.

(a) Common Names

Volatile Oil of Mustard

Also known as -
Mustard oil
Oil of black mustard
Oleum sinapis
Senfoel
Volatile mustard oil
Allyl mustard oil

(b) Chemical Name (of main consituent)

1-Propene, 3-isothiocyanato- (9CI) (CA INDEX NAME)

Also known as -
Isothiocyanic acid, allyl ester (6CI, 8CI)
2-Propenyl isothiocyanate
3-Isothiocyanato-1-propene

Allyl isothiocyanate
Allyl thioisocyanate

(c) Chemical Abstract Service (CAS) Registry Number

57-06-7

(d) Empirical Formula

$C_4 H_5 N S$

(e) Molecular Weight

99

(f) Structural Formula

$S = C = N - CH_2 - CH = CH_2$

(g) Specifications for Food Grade Material

Per the Food Chemicals Codex (FCC), Food Grade volatile oil of mustard shall meet the specifications below –

Assay	≥ 93% allyl isothiocyanate
Arsenic (as As)	≤ 3 ppm (0.0003%)
Heavy Metals (as Pb)	≤ 40 ppm (0.004%)
Phenols	Passes FCC Specified Test

(h) Quantitative Composition

Allyl isothiocyanate	≥ 95%
Allyl cyanide (AC)	< 2%
Carbon disulfide (CS)	< 2%
AC + CS Total	2% - 3%
Water	≤ 1%

(i) Manufacturing Process (excluding trade secrets)

The VOM which is the subject of this Notification is produced in Japan. This is done by the Notifier:

CAREX, Inc.
Office:
1-1-47 Chuo, Joto-ku
Osaka, 536-0005 Japan

Contact: Mr. Yasushi Sekiyama
Telephone: 81-6-6937-9255

The production process is conducted in accordance with current international (ISO) hygienic standards and good manufacturing practices for the manufacture of food chemicals and is as follows:

(1) The seeds of brown mustard (*Brassica juncea*) are pressed to remove non-volatile oils.

(2) The residue of pressed seeds is macerated with warm, deionized water and allowed to stand for one hour. This allows the endogenous myrosinase activity to liberate AIT from the endogenous sinigrin (a glucoside also known as potassium myronate).

(3) The macerate is distilled (i.e., steam distillation) to yield a volatile fraction which is typically $\geq$ 95% AIT

(4) Quality control inspections are conducted to assure that the product meets the following release specifications:

AIT Content	Minimum 94% (by GLC)
Specific Gravity, 15 ^{o}C	1.014 - 1.022
Refractive Index, 20 ^{o}C	1.523 - 1.529
Optical Rotation	$\pm 0^{o}$
Solubility in Water	Soluble in 160 to 300 parts of water
Solubility in 90% EtOH	1 : 0.5
Boiling Range	148 ^{o}C - 154 ^{o}C
Arsenic (as As)	$\leq$ 3 ppm (0.0003%)

Heavy Metals (as Pb)	≤ 40 ppm (0.004%)
Phenols	Passes FCC Specified Test

170.36(c)(3) [*Proposed*]: Information on any self-limiting levels of use.

The notified substance, VOM, will have self-limiting use levels. This limitation is created by that fact that use of VOM, for the herein claimed GRAS use, at levels which produce a noticeable flavor effect in the subject food will lead in most cases to food which is rejected by the consumer on the basis or taste and/or odor (of VOM).

Usual VOM levels in some foods (selected from FASEB 1975, Table 1) which result from the flavoring uses of mustard oil are:

	Usual	Maximum
Meat products	35 ppm	61 ppm
Baked goods	14 ppm	29 ppm
Gravies, sauces	46 ppm	201 ppm

Therefore, the VOM levels in foods would be less than the above-cite "usual" flavoring levels.

The shelf life extension / anti-spoilage use has only the intended technical effect of retarding the spoilage of food with the primary intention not being to affect the organoleptic properties of the food. Published literature (see the Bibliography[2]) establishes that the levels needed to inhibit the growth of microorganisms in or on foods are from:

2 - 6 ppm for fungi/molds;
10 ppm for bacteria; and,
20 - 35 ppm for yeast

in the growth zone for these organisms.

Therefore, while VOM concentrations which inhibit the growth of yeast can approach the "usual" level for flavoring: (a) inhibition of fungi/molds and bacteria is attained at much less usual typical flavoring levels; and, (b) adequate inhibitory action against yeast can be obtained at less then the "usual" flavoring levels for VOM.

[2] Foter and Golick, 1938; Tokuoka *et al.*, 1992; Isshiki *et al.*, 1992; Tokuoka and Isshiki, 1994; Holley, 1997; Delaquis and Sholberg, 1997; Ward *et al.*, 1998; Park *et al.*, 2000

170.36(c)(4) [*Proposed*]: Detailed summary of the basis for the Notifier's determination that the notified substance is GRAS.

The following is a detailed summary of the basis for the Notifier's determination that the notified substance is GRAS.

170.36(c)(4)(i)(A) [*Proposed*]: Comprehensive discussion of, and citations to, generally available and accepted scientific data, information, methods, or principles the Notifier relies on to establish safety, including a discussion of the probable consumption of the substance and the effects thereof.

and

170.36(c)(4)(i)(B) [*Proposed*]: Comprehensive discussion of reports of investigations or other information that may appear to be inconsistent with the GRAS determination.

***Note:* These two information items are provided in an integrated manner as they are inter-related for Volatile Oil of Mustard**

Mustard, mustard oil, and other spices and spice essential oils have been used since the dawn of recorded history for their food preserving properties as well as their flavoring effects. Indeed, one is hard pressed to know whether the now-desired flavor effect of spices originated from human acculturation to consuming food preserved with mustard / mustard oil and other spices / spice oils or whether spices / spice oils where first sought for their flavoring properties and then – once in common use – their preservative properties became understood.

Various anti-spoilage uses of VOM have been reported in the technical / scientific literature since at least the 1920's. These include uses in preserving fruit juices, bread, and wine on a regular or regionally popular basis, as well as other foods on a more limited scale. (For references, see the Bibliography)

The use of VOM vapor for its anti-bacterial effects has been known since at least 1938 (Foter and Golick, 1938). Since the early 1990's a considerable amount of new research has been published on vapor-phase transfer delivery of VOM so as to achieve its known anti-spoilage effect (e.g.: Tokuoka *et al.*, 1992; Isshiki *et al.*, 1992; Tokuoka and Isshiki, 1994; Holley, 1997; Delaquis and Sholberg, 1997; Ward *et al.*, 1998; Park *et al.*, 2000).

This vapor phase method of using VOM for its anti-spoilage effect is identical to that used in the Wasa Ouro technology above-described. It consists of exposing the food to be protected to an atmosphere containing VOM vapor, during which exposure a small amount of VOM adsorbs onto the surface of the food and provides a surface protection

against the growth of bacteria and fungi (both pathogens and spoilage agents). Effective control of microbial growth has been reported at VOM air concentrations ranging from 1 µg VOM / L air to 720 µg VOM / L air (see the above references). Technical studies with Wasa Ouro brand VOM establish the following vapor MIC's for VOM's effects on various microorganisms:

		(ppm in Air)	µg / L air
MOLDS	*Alternaria alternata*	≤ 20 ppm	≤ 81
	Mucor racemosus	≤ 20 ppm	≤ 81
	Penicillium chrysogenum	20 - 40 ppm	81 - 162
	other *Penicillium* spp.	≤ 20 ppm	≤ 81
	Aspergillus spp.	20 - 60 ppm	81 - 242
	Cladosporidium spp.	≤ 20 ppm	≤ 81
	Rhizopus javanicus	40 - 90 ppm	162 - 364
	Fusarium spp.	≤ 20 ppm	≤ 81
YEASTS	*Candida albicans*	≤ 20 ppm	≤ 81
	Candida tropicalis	≤ 20 ppm	≤ 81
	Torulopsis versatilis	≤ 20 ppm	≤ 81
	Debarymyces kloeckeri	21 - 60 ppm	81 - 242
GRAM (+) BACTERIA	*Staphylococcus aureus*	40 - 90 ppm	162 - 364
	Staphylococcus epidermidis	40 - 90 ppm	162 - 364
	Bacillus spp.	21 - 90 ppm	81 - 364
	Streptococcus faecalis	61 - 120 ppm	242 - 485
	Pediococcus spp.	≥ 361 ppm	≥ 1460
	Lactobacillus spp.	≥ 361 ppm	≥ 1460
GRAM (-) BACTERIA	*Escherichia coli*	≤ 20 ppm	≤ 81
	Salmonella typhimurium	20 - 40 ppm	81 - 162
	Salmonella paratyphi B	21 - 60 ppm	81 - 242
	Other *Salmonella* spp.	20 - 40 ppm	81 - 162
	Shigella fexneri 1b	21 - 60 ppm	81 - 262
	Vibrio cholerae	≤ 20 ppm	≤ 81
	Vibrio parahaemolyticus	20 - 40 ppm	81 - 162
	Pseudomonas aeruginosa	≤ 20 ppm	≤ 81

Note: 1 ppm VOM in air = 4.04 µg AIT / L air

The above air-borne MIC's can be compared to solid and liquid culture media inhibitory concentrations of VOM reported in various scientific publications and summarized in Delaquis and Mazza's review (P.J. Delaquis and G. Mazza, "Antimicrobial properties of isothiocyanates in food preservation", Food Technology, Nov. 1995: 73 - 78):

Yeasts	20 - 35 µg/ml	(viable cells)
Fungi	2 - 6 µg/ml	(mycellar growth)
	150 - 600 µg/ml	(conidial germination)
Bacteria	*ca.* 10 µg/ml	(growth)

The actual concentration of VOM in a protected food will be a function of the food type, the duration of exposure to VOM, and the air concentration of VOM. With this in mind, the levels of VOM which are required within the effective microbial growing zone on a food need to be in the above-stated range of 2 - 10 μg/ml (or 2 - 10 ppm) to achieve effective inhibition of bacterial and fungal / mold growth and 20 - 35 μg/ml for yeasts.

The use which is here determined by the Notifier to be GRAS is for shelf life extension / anti-spoilage protection of foods. In the U.S. such foods will primarily be meats (i.e., beef, pork, lamb, veal, chicken, turkey, etc.), fish and shellfish, and baked goods (primarily pies). Meats to be protected can be protected both in typical pre-package forms or when displayed in meat counter cases. For pre-packaged meats, the packaging would include an VOM-containing matrix (not in direct contact with the meat) which would generate and sustain the desired VOM air concentration inside the packaging. For display case use, the VOM-containing matrix would be placed inside the display and would generate an VOM atmosphere inside the display case. Baked goods (pies) would have a VOM in-package delivery system essentially like that for pre-packaged meats.

For most meat, it is expected that the absorbed VOM would be driven off during cooking of the meat prior to consumption. Possible exceptions would be ham, bologna, salami, or other processed meats eaten without being first cooked or heated. For baked goods (pies), VOM loss due to cooking / heating is not expected to be significant since these baked goods are pre-baked. VOM loss, therefore, is expected only when the consumer pre-heated the item prior to consumption.

Beyond this, and of importance, is that only adherence to current good practice in the manufacture, processing, handling, and storage of food is necessary to assure low levels of VOM in foods as consumed. This is because foods which acquire a flavor from VOM or an odor of a more than very transient nature will be rejected by consumers. Experience with the use of Wasa Ouro brand VOM-containing packaging in Japan has shown that the packaging / holding container delivery system for AIT must be tailored to the type and amount of food in the package / holding container as well as to the package / holding container materials and volume so as to provide the desired anti-spoilage effect without imparting undesired flavor or odor. This integration of delivery system design with specific food packaging / holding systems assures that foods treated with airborne VOM as an anti-spoilage agent will not contain excessive levels of VOM (i.e., sufficient to impart noticeable flavor or odor to the food). In this way, the proposed uses is VOM is self-limiting.

The VOM levels in various foods exposed to VOM atmospheres have been determined by the Notifier and are as represented in the table below.

Table: Expected worst case VOM levels in foods after a 10-day exposure to 150 ppm VOM in air and in foods as "as consumed"

Food Type	Uptake Factor	10-Day VOM Level	Reduction on Cooking	"As Consumed" VOM Level
Beef, ground (representing also whole cuts of beef and veal and lamb, ground and cuts of)	≤ 0.34	≤ 51 ppm	5.9	≤ 8.6 ppm
Chicken, ground (representing also whole chicken and turkey, ground and whole)	≤ 0.24	≤ 36 ppm	5.1	≤ 7.1 ppm
Pork sausage (representing also ground pork and cuts of pork and ham)	≤ 0.10	< 15 ppm	3.3	≤ 4.5 ppm
Bologna, ground (representing sliced deli meats)	≤ 0.34	≤ 51 ppm	N/A	≤ 51 ppm
Pecan pie, ground (representing nut and fruit pies)	≤ 0.03	≤ 4.5 ppm	N/A	≤ 4.5 ppm

Note: 150 ppm in air will be a typical VOM atmosphere for this use

The estimated dietary intake associated with shelf-life extension / anti-spoilage uses of VOM on relevant foods is provided in Tables D-1A through D-10B (preceding the Bibliography). Tables D-1A, D-1B, D-2A, and D-2B provide the estimated daily dietary VOM intake associated with all VOM treated foods and for all age groups. The other tables provide the daily intake of VOM associated with specific food types.

The basic food consumption data are from USDA's Home Economics Research Repot No. 44, "Foods Commonly Eaten by Individuals". Age-specific average daily consumption of a given food type is obtained by multiplying the average amount consumed per eating occasion (for individuals consuming the food at least one in any given three day period) by the percent of individuals consuming the food at least once during any given three day period. The average daily intake of VOM for a given food type is then estimated by multiplying the average daily consumption (by age) for the food type by the concentration of VOM in the food. This type of assessment approach is one normally relied upon by experts in the evaluation of exposures to substances in foods.

For purposes of the estimation of daily dietary intake of VOM the following assumptions were made:

(a) 100% of any given food type was assumed to be treated with VOM. This is a conservative assumption because not all foods of any given type will be exposed to VOM for anti-spoilage purposes.

000157

(b) Flavoring uses of VOM were assumed to produce an average 35 ppm VOM in food. This is a reasonable, fact-based assumption.

(c) Anti-Spoilage uses of VOM were assumed to produce 9 ppm in the entire food item for meats and fish, 7.1 ppm in the entire food item for poultry, and 4.5 ppm in the entire food item for pies (based on the data from the table above). This is a conservative assumption because the VOM will generally be in the surface VOM diffusion zone for a food (as opposed to being uniformly distributed throughout the food).

As can be seen from Tables D-1A through D-2B, the total VOM dietary load (as parts per billion [ppb] in the total daily diet) for the U.S. population which is associated with both flavoring and shelf life extension / anti-spoilage uses is:

Age Group	Flavoring		Anti-Spoilage	
	Male (D-1A)	*Female (D-2A)*	*Male (D-1B)*	*Female (D-2B)*
0 - 1 yr.	1.06 ppm	1.06 ppm	0.27 ppm	0.27 ppm
1 - 2 yr.	1.69 ppm	1.69 ppm	0.44 ppm	0.44 ppm
3 - 5 yr.	1.47 ppm	1.47 ppm	0.38 ppm	0.38 ppm
6 - 8 yr.	1.37 ppm	1.37 ppm	0.35 ppm	0.35 ppm
9 - 14 yr.	1.48 ppm	1.26 ppm	0.38 ppm	0.32 ppm
15 - 18 yr.	1.64 ppm	1.17 ppm	0.42 ppm	0.30 ppm
19 - 34 yr.	1.85 ppm	1.21 ppm	0.48 ppm	0.31 ppm
35 - 64 yr.	1.90 ppm	1.32 ppm	0.49 ppm	0.34 ppm
65 - 74 yr.	1.59 ppm	1.15 ppm	0.41 ppm	0.30 ppm

The flavor uses of VOM are, therefore, reasonably estimated to produce to dietary loads of > 1 ppm for all age and sex groups and between 1.4 - 1.9 ppm for all age groups other than infants (0 - 1 yr.).

In contrast, the shelf life extension / anti-spoilage uses is reasonably expected to lead to produce dietary loads of < 0.5 ppm for all age and sex groups.

The flavor uses of VOM are already listed as GRAS at 21 CFR 182.20. The shelf life extension / anti-spoilage uses, would of necessity be safe (due to an even lower dietary exposure) and this is generally recognized to be the case by experts qualified in the evaluation of the safety of substances added to food (see information for 170.36(c)(4)(i)(C), below).

Further to the safety of VOM, the dietary safety of VOM (including, also, that of synthetic ally isothiocyanate) was extensively reviewed as part of the Agency's review of GRAS substances in the 1970's (Food & Drug Research Labs, 1972; FASEB, 1975). As noted in the conclusion of this extensive review report:

There is no evidence in the available information on allyl isothiocyanate, p-hydroxybenzyl isothiocyanate, and brown and yellow mustard that demonstrates or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current of that might reasonably be expected in the future. **(FASEB, 1975 at p. 15)**

The above, quite clear cut, safety picture was complicated in 1982 by the results of carcinogencity testing carried out by the National Toxicology Program (NTP, 1982). In this study, conducted with synthetic allyl isothiocyanate ("AIT") as opposed to the natural product obtained from mustard (*Brassica* spp.), the result for AIT was negative in male and female mice and equivocal in female rats. In male rats, the result was positive on the basis of transitional cell papillomas of the urinary bladder.

The literature review provided in the NTP study report serves to update the mutagenicity / gene toxicity data base for AIT at the time. Since the FASEB review in 1975 (FASEB, 1975), bacterial mutagenicity tests in *B. subtillis* H17 and M45 were negative as were Ames tests in *S. typhimurium* TA98, TA100, TA 1535 and TA 1537 and the *rec* A test in *E. coli* WP2. These negative mutagenicity and gene toxicity tests, together with the above-reported negative uniformity of earlier mutagenicity and gene toxicity tests for AIT (FASEB, 1975) strongly suggest that AIT will not act, *in vivo*, through any direct, genotoxic carcinogenic mechanism.

In this context, it must also be taken into account that there was a corresponding and marked dose- related increase in hyperplasia of the urinary bladder epithelium in these male rats (0/49, 1/49, & 6/49 for control, low, and high dose groups respectively). In these male rats, the incidences of transitional cell papillomas were: 0/49, 2/49, and 4/49 for controls, low dose, and high dose groups, respectively. In female rats the incidence of transitional cell papillomas of the bladder was 0/49, 0/49, & 1/50 for controls, low dose, and high dose and the incidence of epithelial hyperplasia of the bladder was 0/49, 0/49, & 1/50 for control, low, and high dose groups, respectively. In both male and female mice, no epithelial hyperplasia of the urinary bladder was reported in any of the study animals and no transitional cell papillomas of the bladder are observed.

These data suggest a connection between the occurrence of epithelial hyperplasia and transitional papillomas in these test species, in fact the incidences of the two types of lesions appear to mirror each other. The fact that a transitional cell papilloma was observed in a female of the high dose group precludes any possible argument that perhaps female rats do not form transitional cell papillomas.

An earlier study by Major (1970), using the mouse two-stage skin carcinogenesis model, showed that synthetic AIT, when applied to mouse skin at an irritant dose level, induced a transient hyperplasia and an elevated mitotic index in the skin.

IARC (1985) reviewed a mouse skin carcinogenesis study which included natural VOM (as opposed to synthetic AIT). Volatile Oil of Mustard, which contained > 90%

allyl isothiocyanate, was tested in a two stage mouse skin assay. Three control groups of 12-16 'S' strain mice (actual strain, age and sex unspecified) received 0.3 ml of 0.1-0.15% 7,12-dimethylbenz(a)anthracene (DMBA) on the skin of the back followed by no treatment (two groups) or 21 days later by twice weekly applications for 12 weeks and weekly applications for 15 weeks of acetone. A fourth group of 16 mice received an initial skin application of 0.3 ml of 0.1% DMBA followed 39 days later by applications of a 3-4% solution of mustard oil in acetone weekly for 20 weeks. The incidence of skin papillomas among animals surviving to the end of the experiment was 4/21, 1/12 and 1/16 in the combined DMBA control groups with no secondary treatment, in controls receiving DMBA followed by acetone, and in treated mice receiving DMBA and mustard oil, respectively. In this study, **natural** VOM was neither carcinogenic nor co-carcinogenic.

The suggestion from both the data reported above is that: (a) the transitional cell papillomas which were observed were secondary to the effect of hyperplasia on promotion of pre-existing tumor precursor cells and/or increasing the susceptibility of normal bladder stem cells to transformation due to increased time spent cell cycle phases in which chromatin is un-condensed and more exposed; (b) that such effects would not be expected at lower, non-irritant doses of AIT; (c) that the result in the NTP biosassy was not mediated via a genotoxic mechanism of carcinogenesis and, therefore, will have a low dose threshold; and, (d) that there may be a difference in effect between natural Volatile Oil of Mustard and synthetic AIT.

Since the NTP Bioassay, no additional reliable *in vivo* evidence suggesting an AIT or VOM carcinogenic dietary risk to humans has been reported.

Kasamaki *et al.* (1987) reported than AIT (synthetic) was able to induce cell transformation of Chinese hamster B241 cells. This study, however, did not include adequate controls for the effects of the extensive passaging which was required to observe the low level of transformants reported.

Musk and Johnson (1993) reported that AIT (synthetic) did not produce chromosome aberrations *in vitro* under conditions in which benzyl-, phenethyl-, and phenyl-isothiocyanates produced chromosomal aberrations.

Bechtel, *et al.* (1998) reported that AIT (synthetic) did not induce unscheduled DNA synthesis in male rats when given at up to 125 mg/kg. This supports that AIT lacks the ability to directly induce cancer by a genotoxic mechanism *in vivo*.

Kassie and Knasmuller (2000) reported on an *in vitro* gene-toxicity battery study of AIT (synthetic) and phenethyl isothiocyanate (PEITC). The test battery included Ames tests in TA 98 and TA 100, the DNA repair test in E. coli, and a micronucleus test with human derive Hep G2 cells. AIT was reported to be more active in the bacterial test systems than in the mammalian cell test system. When tested *in vivo* at very high doses (90 and 270 mg/kg), using an E. coli marker, AIT's activity was significantly attenuated. This attenuation was suggested to be due to protein binding of AIT under *in vivo* conditions. Further investigations *in vitro* suggested that the formation of reactive

oxygen species could be mediated by AIT and PEITC. The suggestion that AIT might mediate the formation of reactive oxygen species has also been investigated by Murata at al. (2000) and Yonezawa et al., (1997) in *in vitro* studies.

Since the time of the NTP bioassay the metabolism of AIT has, also, been shown to differ between rat and the mouse and human (with the human metabolizing AIT like the mouse - which was negative in the NTP bioassay - and not like the rat).

Bollard et al. (1997) report that urinary metabolites account for the bulk of administered AIT (synthetic) doses in mice and rats (80% and 75% respectively). In mice, there are three major urinary species: inorganic thiocyanate ion, the glutathione conjugate[3], and the cysteine conjugate[4]. In contrast, rats do not form the cysteine conjugate and, also, show a greater retention of ^{14}C-AIT than do mice at 4 days (18-24% in rats *versus* 2-5% in mice). The results of this study suggest that in mice, AIT is primarily metabolized via hydrolysis whereas in rats glutathione conjugation is the predominant metabolic route. Of marked interest to the urinary papilloma picture for rats, a sex difference was reported for AIT in bladder tissue of rats, with higher levels of AIT / AIT-metabolites seen in the bladder tissue of male rats than in female rats. This finding would correlate with the observed differences in both bladder hyperplasia and papilloma incidence seen between male and female rats. In humans, as in mice, the major urinary metabolite is the cysteine conjugate and glutathione conjugation appears to be at most a minor pathway for the human metabolism (Jiao *et al.*, 1994).

The International Agency for Research on Cancer (IARC), has reviewed the overall weight of the evidence for carcinogencity of AIT and concluded that the evidence in animals is "limited" and in humans is "not classifiable" (IARC, 1999).

Beyond this immediate safety issue, AIT is probably an anti-carcinogen in the human diet. Therefore, its incorporation in the human diet may present potential public health benefits. Some selected citations follow.

Musk and Johnson (1993) reported on the selective toxicity of AIT to the human colorectal cell line HT29. A later report by Smith *et al.*, (1996) extended this in vitro finding to in vivo suppression of aberrant crypt foci in the colonic mucosa of rats.

Hasegawa *et al.*, (1993) reported on AIT inhibition of the malignant human HeLa cell line.

Xu and Thornalley (2000) have reported on the inhibition of human leukemia cells by AIT. As these authors note, the anti-cancer effect of consumption of cruciferous vegatables – which are rich in AIT – is well known.

---

[3] Allylthiocarbamoylmercapturic acid
[4] Allylthiocarbamoylcysteine

Another issue which may affect safety is the potential concern that the proposed use not, inadvertently, create an opportunity for growth of botulism organisms. This concern arises due to the presence of a modified atmosphere or in the case of vacuum packed foods (due to the anaerobic atmosphere created).

With regard to the first issue, the air concentrations of VOM in the proposed use will be *circa* 150 ppm but not greater. At this VOM level, there is no significant change in the oxygen concentration in air and a fully aerobic conditions, which prevents the growth of botulism, will be maintained.

With regard to the second issue, the proposed use of VOM is not compatible with vacuum packed foods as the VOM would be evacuated in the vacuum packing process. Therefore, this is not an applicable concern for this use.

Therefore, the current general recognition among experts as to the safety of VOM can be summarized as follows:

- AIT and/or VOM is consistently reported as non-genotoxic *in vivo* and usually is reported to be non-genotoxic *in vitro*.
- AIT has irritant properties which have been reported to produce transient hyperplasia of mouse skin when applied to mouse skin.
- In dermal mouse skin carcinogenicity bioassays, VOM was not carcinogenic.
- In a corn oil gavage carcinogencity bioassay in mice and rats, AIT was not carcinogenic in male or female mice, was considered equivocal in female rats, and was considered to increase the incidence of transitional cell papillomas in male rats. In male rats, the incidence of transitional cell papillomas mirrored that of bladder epithelial hyperplasia. In female rats, a single animal with transitional cell papilloma of the bladder was observed in the high dose group and, again, the incidence of bladder epithelial hyperplasia mirrored that of transitional cell papillomas in female rats.
- The metabolism of AIT differs significantly between rats and mice. In rats, glutathione conjugation is the major pathway and there is a sex difference in the accumulation of AIT / AIT-metabolites in the rat bladder with males showing higher levels than females. In mice, hydrolysis with formation of thiocyanate ion and of the cysteine conjugate is the major pathway for AIT. Humans appear to metabolize AIT similarly to mice.
- The weight of the evidence strongly supports that the increased incidence of transitional cell papillomas seen in male rats in the NTP bioassay is specific to the male rat under these test conditions and is probably due to accumulation of levels of the AIT-glutathione conjugate which induce epithelial hyperplasia in the male rat

under the NTP test conditions and that the papillomas are secondary to this hyperplasia. Female rats were observed, in the NTP bioassay, to be capable of forming transitional cell papillomas in the bladder but the incidence of epithelial hyperplasia was much lower than male rats and, accordingly, so was the incidence of papillomas (1/50 in high dose females *vs.* 0/49 in control females for both hyperplasia and papillomas). This is most likely a reflection of the fact that female rats do not accumulate AIT metabolites in the bladder to the same extent that male rats do. Given the known differences in AIT metabolism between rats and humans and the similarities between mouse and human AIT metabolism, the NTP bioassay actually suggests that AIT will be non-carcinogenic in humans, as was the case for the mice in the NTP bioassay.

- The NTP bioassay results in rats are not, therefore, an appropriate predictor, in this case, for AIT carcinogenic risk to humans at the levels presently consumed in the daily diet. The NTP bioassay results for mice, which metabolize AIT differently from rats but similarly to humans, indicate that AIT is not expected to be carcinogenic in the human diet and at the low levels associated with current food uses of AIT or of VOM both are generally considered as safe for human consumption.

- The proposed use will not increase the risk of botulism growth.

170.36(c)(4)(i)(C) [*Proposed*]: The basis for concluding, in light of the data and information under paragraphs (c)(1), (c)(2), (c)(3), (c)(4)(i)(A), and (c)(4)(i)(B) that there is consensus among experts qualified by scientific training and experience to evaluate the safety of substances added to food that there is reasonable certainty that the substance is not harmful under the intended conditions of use.

A number of scientific studies which have been published in the open literature clearly establish that there is a current consensus, i.e., general recognition, of the safety of AIT (synthetic) and VOM (natural) as currently consumed in the human diet. This is that at current consumption levels, AIT is toxicologically safe for humans and, to a reasonable degree of scientific certainty, does not have any expected carcinogenic potential. The anti-spoilage use of AIT will not significantly alter human dietary consumption of AIT as the resultant levels in foods, of necessity, are lower than current levels for use in imparting flavor to relevant foods.

IARC – an internationally recognized body of experts – in 1999 re-stated its conclusions as to the dietary safety of AIT (and VOM) and lack of weight of evidence for a carcinogenic risk to humans.

The U.S. Food and Drug Administration currently lists the flavor uses (and other potential but unspecified uses) as GRAS uses (21 CFR 182.20). The above referenced IARC expert review addresses the dietary safety of VOM at these low levels of use associated with flavoring and, of necessity, yet lower levels of VOM in the diet. As is discussed and supported above, the dietary VOM levels estimated, on a worst case basis, to be associated with the use for which the Notifier has made a GRAS determination and for which the Notifier is claiming a GRAS exemption from premarket approval requirements are much lower than those associated with flavoring uses and are, therefore, also generally recognized as safe for human consumption.

SRS INTERNATIONAL® CORPORATION

1901 L Street, NW • Suite 250 • Washington, DC 20036-3503
Telephone (202) 223-0157/0298 • Fax (202) 835-8970
E-Mail: mainsrs@srsinternational.com http://www.srsinternational.com

FAX TRANSMISSION

DESTINATION NO.	202-418-3131
ATTENTION OF:	Negash Belay
COMPANY NAME:	FDA
NUMBER OF PAGES:	***(Including Cover Page)***
AUTHORIZED BY:	Michael G Farrow
DATE:	11-6-03

COMMENTS

PART II

Appendix 1 - 21 pgs
Appendix 2 - 4 pgs

APPENDIX 1

Tables for Dietary Exposures to VOM Associated with both Flavoring and Anti-Spoilage Uses

Table D-1A: Total Dietary Loading from Consumption of Foods Containing 35 ppm Volatile Oil of Mustard (i.e.: flavoring use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	66.20	92.30	104.125	106.12	78.84
Poultry	5.64	13.93	16.19	19.39	24.81	29.78	30.324	30.60	27.56
Fish & Shellfish	0.35	3.81	5.51	7.20	8.80	9.71	15.56	16.68	17.05
Pies	0.11	1.74	3.12	3.65	5.92	8.43	8.84	9.42	13.07
TOTALS:	15.15	48.41	62.91	78.46	105.72	140.22	158.85	162.82	136.52
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Total Foods per Kg-day	1.87	4.28	3.77	3.38	2.83	2.34	2.18	2.09	1.75
Volatile Oil of Mustard Level in Food	35 ppm	100% of Food Assumed to Contain Volatile Oil of Mustard							
Dietary Load as mg/Kg/day	0.0653	0.1498	0.1318	0.1184	0.0992	0.0819	0.0762	0.0731	0.0613
Dietary Load as mg/day	0.530	1.694	2.202	2.746	3.700	4.908	5.560	5.698	4.778
Dietary Load as ppm in Total Diet	1.06	1.69	1.47	1.37	1.48	1.64	1.85	1.90	1.59
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	132	57	65	73	87	105	113	118	140

Table D-1B: Total Dietary Loading from Consumption of Foods
Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	66.20	92.30	104.125	106.12	78.84
Poultry	5.64	13.93	16.19	19.39	24.81	29.78	30.324	30.60	27.56
Fish & Shellfish	0.35	3.81	5.51	7.20	8.80	9.71	15.56	16.68	17.05
Pies	0.11	1.74	3.12	3.65	5.92	8.43	8.84	9.42	13.07
TOTALS:	15.15	48.41	62.91	78.46	105.72	140.22	158.85	162.82	136.52
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Total Foods per Kg-day	1.87	4.28	3.77	3.38	2.83	2.34	2.18	2.09	1.75
Volatile Oil of Mustard Level in Food	9 ppm	100% of Food Assumed to Contain Volatile Oil of Mustard *Assumed equal in all foods to worst case meat levels*							
Dietary Load as mg/Kg/day	0.0168	0.0385	0.0339	0.0304	0.0255	0.0211	0.0196	0.0188	0.0158
Dietary Load as mg/day	0.136	0.436	0.566	0.706	0.951	1.262	1.430	1.465	1.229
Dietary Load As ppm in Total Diet	0.27	0.44	0.38	0.35	0.38	0.42	0.48	0.49	0.41
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	512	223	254	283	337	408	439	458	546

Table D-2A: Dietary Loading from Consumption of Foods
Containing 35 ppm Volatile Oil of Mustard (i.e.: flavoring use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	56.41	62.78	64.038	68.88	52.46
Poultry	5.64	13.93	16.19	19.39	20.68	22.29	23.328	24.53	24.81
Fish & Shellfish	0.35	3.81	5.51	7.20	7.56	10.89	11.308	13.72	12.35
Pies	0.11	1.74	3.12	3.65	5.62	4.60	5.1	6.43	8.91
TOTALS:	15.15	48.41	62.91	78.46	90.27	100.55	103.77	113.56	98.52
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Total Foods per Kg-day	2.02	4.40	3.86	3.34	2.45	1.80	1.73	1.75	1.43
Volatile Oil of Mustard Level in Food	35 ppm		100% of Food Assumed to Contain Volatile Oil of Mustard						
Dietary Load as mg/Kg/day	0.0709	0.1540	0.1351	0.1169	0.0859	0.0630	0.0605	0.0611	0.0500
Dietary Load as mg/day	0.530	1.694	2.202	2.746	3.159	3.519	3.632	3.974	3.448
Dietray Load As ppm in Total Diet	1.06	1.69	1.47	1.37	1.26	1.17	1.21	1.32	1.15
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	121	56	64	74	100	137	142	141	172

Table D-2B: Dietary Loading from Consumption of Foods
Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	56.41	62.78	64.038	68.88	52.46
Poultry	5.64	13.93	16.19	19.39	20.68	22.29	23.328	24.53	24.81
Fish & Shellfish	0.35	3.81	5.51	7.20	7.56	10.89	11.308	13.72	12.35
Pies	0.11	1.74	3.12	3.65	5.62	4.60	5.1	6.43	8.91
TOTALS:	15.15	48.41	62.91	78.46	90.27	100.55	103.77	113.56	98.52
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Total Foods per Kg-day	2.02	4.40	3.86	3.34	2.45	1.80	1.73	1.75	1.43
Volatile Oil of Mustard Level in Food	9 ppm	100% of Food Assumed to Contain Volatile Oil of Mustard *Assumed equal in all foods to worst case meat levels*							
Dietary Load as mg/Kg/day	0.0182	0.0396	0.0347	0.0300	0.0221	0.0162	0.0156	0.0157	0.0128
Dietary Load as mg/day	0.136	0.436	0.566	0.706	0.812	0.905	0.934	1.022	0.887
Dietray Load As ppm in Total Diet	0.27	0.44	0.38	0.35	0.32	0.30	0.31	0.34	0.30
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	472	217	248	286	390	NOEL = 8.6 mg/Kg/day 531	552	547	669

Table D-3A: Dietary Loading from Consumption of Beef, Pork, Lamb, and Veal (all forms) Containing 35 ppm Volatile Oil of Mustard (i.e.: flavoring use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	66.20	92.30	104.125	106.12	78.84
TOTALS:	9.05	28.93	38.09	48.22	66.20	92.30	104.13	106.12	78.84
	MID-POINT BODY WEIGHT, Kg								
	6.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Meat Foods per Kg-day	1.11	2.56	2.28	2.08	1.77	1.54	1.43	1.36	1.01
Volatile Oil of Mustard Level in Food	35 ppm	100% of Food Assumed to Contain Volatile Oil of Mustard							
Dietary Load as mg/Kg/day	0.0390	0.0895	0.0798	0.0727	0.0621	0.0539	0.0499	0.0478	0.0354
Dietary Load as mg/day	0.317	1.013	1.333	1.688	2.317	3.230	3.644	3.714	2.760
Dietary Load as ppm in Total Diet	0.63	1.01	0.89	0.84	0.93	1.08	1.21	1.24	0.92
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	221	96	108	118	NOEL = 8.6 mg/Kg/day 138	159	172	181	243

Table D-3B: Dietary Loading from Consumption of Beef, Pork, Lamb, and Veal (all forms) Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	66.20	92.30	104.125	106.12	78.84
TOTALS:	9.05	28.93	38.09	48.22	66.20	92.30	104.13	106.12	78.84
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Meat Foods per Kg-day	1.11	2.56	2.28	2.08	1.77	1.54	1.43	1.36	1.01
Volatile Oil of Mustard Level in Food	9 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard *Based on worst case meat values*							
Dietary Load as mg/Kg/day	0.0100	0.0230	0.0205	0.0187	0.0160	0.0139	0.0128	0.0122	0.0091
Dietary Load as mg/day	0.081	0.260	0.343	0.434	0.596	0.831	0.937	0.955	0.710
Dietary Load As ppm in Total Diet	0.16	0.26	0.23	0.22	0.24	0.28	0.31	0.32	0.24
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	858	374	419	460	NOEL = 6.6 mg/Kg/day 538	620	670	702	945

Table D-4A: Dietary Loading from Consumption of Beef, Pork, Lamb, and Veal (all forms) Containing 35 ppm Volatile Oil of Mustard (i.e.: flavoring use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	56.41	62.78	64.038	68.88	52.46
TOTALS:	9.05	28.93	38.09	48.22	56.41	62.78	64.04	68.88	52.46
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Meat Foods per Kg-day	1.21	2.63	2.34	2.05	1.53	1.12	1.07	1.08	0.76
Volatile Oil of Mustard Level in Food	35 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard							
Dietary Load as mg/Kg/day	0.0423	0.0921	0.0618	0.0718	0.0537	0.0393	0.0374	0.0371	0.0266
Dietary Load as mg/day	0.317	1.013	1.333	1.688	1.974	2.197	2.241	2.411	1.836
Dietary Load As ppm in Total Diet	0.63	1.01	0.89	0.84	0.79	0.73	0.75	0.80	0.61
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	203	93	105	120	160	219	230	232	323

Table D-4B: Dietary Loading from Consumption of Beef, Pork, Lamb, and Veal (all forms) Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Beef, Pork, Lamb & Veal	9.05	28.93	38.09	48.22	56.41	62.78	64.038	68.88	52.46
TOTALS:	9.05	28.93	38.09	48.22	56.41	62.78	64.04	68.88	52.46
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Meat Foods per Kg-day	1.21	2.63	2.34	2.05	1.53	1.12	1.07	1.06	0.76
Volatile Oil of Mustard Level in Food	9 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard *Based on worst case meat values*							
Dietary Load as mg/Kg/day	0.0109	0.0237	0.0210	0.0185	0.0138	0.0101	0.0096	0.0095	0.0068
Dietary Load as mg/day	0.081	0.260	0.343	0.434	0.508	0.565	0.576	0.620	0.472
Dietary Load As ppm in Total Diet	0.16	0.26	0.23	0.22	0.20	0.19	0.19	0.21	0.16
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.8 mg/Kg/day								
	790	363	409	466	623	851	895	902	1,257

Table D-5A: Dietary Loading from Consumption of Poultry (all forms)
Containing 35 ppm Volatile Oil of Mustard (i.e.: flavoring use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Poultry	5.64	13.93	16.19	19.39	24.81	29.78	30.324	30.60	27.56
TOTALS:	5.64	13.93	16.19	19.39	24.81	29.78	30.32	30.60	27.56
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Poultry Foods per Kg-day	0.69	1.23	0.97	0.84	0.67	0.50	0.42	0.39	0.35
Volatile Oil of Mustard Level in Food	35 ppm		100% of Food Assumed to be Contain Volatile Oil of Mustard						
Dietary Load as mg/Kg/day	0.0243	0.0431	0.0339	0.0293	0.0233	0.0174	0.0145	0.0137	0.0124
Dietary Load as mg/day	0.197	0.487	0.567	0.679	0.868	1.042	1.061	1.071	0.965
Dietary Load As ppm in Total Diet	0.39	0.49	0.38	0.34	0.35	0.35	0.35	0.36	0.32
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT				NOEL = 8.6 mg/Kg/day					
	354	200	253	294	369	494	592	626	695

Table D-5B: Dietary Loading from Consumption of Poultry (all forms)
Containing 7.1 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Poultry	5.64	13.93	16.19	19.39	24.81	29.78	30.324	30.60	27.56
TOTALS:	5.64	13.93	16.19	19.39	24.81	29.78	30.32	30.60	27.56
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Poultry Foods per Kg-day	0.69	1.23	0.97	0.84	0.67	0.50	0.42	0.39	0.35
Volatile Oil of Mustard Level in Food	7.1 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard *Based on worst case poultry values*							
Dietary Load as mg/Kg/day	0.0049	0.0087	0.0069	0.0059	0.0047	0.0035	0.0029	0.0028	0.0025
Dietary Load as mg/day	0.040	0.099	0.115	0.138	0.176	0.211	0.215	0.217	0.196
Dietary Load As ppm in Total Diet	0.08	0.10	0.08	0.07	0.07	0.07	0.07	0.07	0.07
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	1,743	984	1,250	1,449	1,821	2,436	2,916	3,087	3,428

Table D-6A: Dietary Loading from Consumption of Poultry (all forms)
Containing 35 ppm Volatile Oil of Mustard (i.e.: flavor use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Poultry	5.64	13.93	16.19	19.39	20.68	22.29	23.328	24.53	24.81
TOTALS:	5.64	13.93	16.19	19.39	20.68	22.29	23.33	24.53	24.81
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Poultry Foods per Kg-day	0.75	1.27	0.99	0.83	0.56	0.40	0.39	0.38	0.36
Volatile Oil of Mustard Level in Food	35 ppm		100% of Food Assumed to be Contain Volatile Oil of Mustard						
Dietary Load as mg/Kg/day	0.0264	0.0443	0.0348	0.0289	0.0197	0.0140	0.0136	0.0132	0.0126
Dietary Load as mg/day	0.197	0.487	0.567	0.679	0.724	0.780	0.816	0.859	0.868
Dietary Load As ppm in Total Diet	0.39	0.49	0.38	0.34	0.29	0.26	0.27	0.29	0.29
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	326	194	247	298	NOEL = 8.6 mg/Kg/day 437	616	632	651	684

Table D-6B: Dietary Loading from Consumption of Poultry (all forms)
Containing 7.1 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Poultry	5.64	13.93	16.19	19.39	20.68	22.29	23.328	24.53	24.81
TOTALS:	5.64	13.93	16.19	19.39	20.68	22.29	23.33	24.53	24.81
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Poultry Foods per Kg-day	0.75	1.27	0.99	0.83	0.56	0.40	0.39	0.38	0.36
Volatile Oil of Mustard Level in Food	7.1 ppm 100% of Food Assumed to be Contain Volatile Oil of Mustard *Based on worst case poultry values*								
Dietary Load as mg/Kg/day	0.0054	0.0090	0.0071	0.0059	0.0040	0.0028	0.0028	0.0027	0.0026
Dietary Load as mg/day	0.040	0.099	0.115	0.138	0.147	0.158	0.166	0.174	0.176
Dietary Load As ppm in Total Diet	0.08	0.10	0.08	0.07	0.06	0.05	0.06	0.06	0.06
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	1,606	957	1,220	1,468	2,155	3,038	3,115	3,210	3,369

Table D-7A: Dietary Loading from Consumption of Fish and Shellfish (all forms) Containing 35 ppm Volatile Oil of Mustard (i.e.: flavor use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Fish & Shellfish	0.35	3.81	5.51	7.20	8.80	9.71	15.56	16.68	17.05
TOTALS:	0.35	3.81	5.51	7.20	8.80	9.71	15.56	16.68	17.05
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Sea Foods per Kg-day	0.04	0.34	0.33	0.31	0.24	0.16	0.21	0.21	0.22
Volatile Oil of Mustard Level in Food	35 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard							
Dietary Load as mg/Kg/day	0.0015	0.0118	0.0115	0.0109	0.0083	0.0057	0.0075	0.0075	0.0077
Dietary Load as mg/day	0.012	0.133	0.193	0.252	0.308	0.340	0.545	0.584	0.597
Dietary Load As ppm in Total Diet	0.02	0.13	0.13	0.13	0.12	0.11	0.18	0.19	0.20
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	5,783	730	745	792	NOEL = 8.6 mg/Kg/day 1,041	1,516	1,153	1,149	1,124

Table D-7B: Dietary Loading from Consumption of Fish and Shellfish (all forms) Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Fish & Shellfish	0.35	3.81	5.51	7.20	8.80	9.71	15.56	16.68	17.05
TOTALS:	0.35	3.81	5.51	7.20	8.80	9.71	15.56	16.68	17.05
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Sea Foods per Kg-day	0.04	0.34	0.33	0.31	0.24	0.16	0.21	0.21	0.22
Volatile Oil of Mustard Level in Food	9 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard							
	Based on worst case meats values								
Dietary Load as mg/Kg/day	0.0004	0.0030	0.0030	0.0028	0.0021	0.0015	0.0019	0.0019	0.0020
Dietary Load as mg/day	0.003	0.034	0.050	0.065	0.079	0.087	0.140	0.150	0.153
Dietary Load As ppm in Total Diet	0.01	0.03	0.03	0.03	0.03	0.03	0.05	0.05	0.05
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 6.6 mg/Kg/day								
	22,490	2,840	2,897	3,079	4,050	5,897	4,482	4,469	4,371

Table D-8A: Dietary Loading from Consumption of Fish and Shellfish (all forms) Containing 35 ppm Volatile Oil of Mustard (i.e.: flavoring use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Fish & Shellfish	0.35	3.81	5.51	7.20	7.56	10.89	11.308	13.72	12.35
TOTALS:	0.35	3.81	5.51	7.20	7.56	10.89	11.31	13.72	12.35
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Sea Foods per Kg-day	0.05	0.35	0.34	0.31	0.21	0.19	0.19	0.21	0.18
Volatile Oil of Mustard Level in Food	35 ppm			100% of Food Assumed to be Contain Volatile Oil of Mustard					
Dietary Load as mg/Kg/day	0.0016	0.0121	0.0118	0.0107	0.0072	0.0068	0.0066	0.0074	0.0063
Dietary Load as mg/day	0.012	0.133	0.193	0.252	0.264	0.381	0.396	0.480	0.432
Dietary Load As ppm in Total Diet	0.02	0.13	0.13	0.13	0.11	0.13	0.13	0.16	0.14
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	5,327	710	727	802	1,197	1,261	1,304	1,164	1,373

Table D-8B: Dietary Loading from Consumption of Fish and Shellfish (all forms)
Containing 9 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Fish & Shellfish	0.35	3.81	5.51	7.20	7.56	10.89	11.308	13.72	12.35
TOTALS:	0.35	3.81	5.51	7.20	7.56	10.89	11.31	13.72	12.35
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Sea Foods per Kg-day	0.05	0.35	0.34	0.31	0.21	0.19	0.19	0.21	0.18
Volatile Oil of Mustard Level in Food	9 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard *Based on worst case meats values*							
Dietary Load as mg/Kg/day	0.0004	0.0031	0.0030	0.0026	0.0018	0.0018	0.0017	0.0019	0.0016
Dietary Load as mg/day	0.003	0.034	0.050	0.065	0.068	0.098	0.102	0.123	0.111
Dietary Load As ppm in Total Diet	0.01	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.04
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day.								
	20,718	2,762	2,828	3,119	4,653	4,905	5,070	4,527	5,339

Table D-9A: Dietary Loading from Consumption of Pies (all forms)
Containing 35 ppm Volatile Oil of Mustard (i.e.: flavor use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Pies	0.11	1.74	3.12	3.65	5.92	6.43	6.84	9.42	13.07
TOTALS:	0.11	1.74	3.12	3.65	5.92	6.43	6.84	9.42	13.07
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Pies per Kg-day	0.01	0.15	0.19	0.16	0.16	0.14	0.12	0.12	0.17
Volatile Oil of Mustard Level in Food	35 ppm			100% of Food Assumed to be Contain Volatile Oil of Mustard					
Dietary Load as mg/Kg/day	0.0005	0.0054	0.0065	0.0055	0.0056	0.0049	0.0042	0.0042	0.0059
Dietary Load as mg/day	0.004	0.061	0.109	0.126	0.207	0.295	0.309	0.330	0.457
Dietary Load As ppm in Total Diet	0.01	0.06	0.07	0.06	0.08	0.10	0.10	0.11	0.15
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	18,138	1,598	1,314	1,564	NOEL = 8.6 mg/Kg/day 1,549	1,746	2,029	2,035	1,467

Table D-9B: Dietary Loading from Consumption of Pies (all forms)
Containing 4.5 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Males, by Age Group

MALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Pies	0.11	1.74	3.12	3.65	5.92	8.43	8.84	9.42	13.07
TOTALS:	0.11	1.74	3.12	3.65	5.92	8.43	8.84	9.42	13.07
	MID-POINT BODY WEIGHT, Kg								
	8.12	11.3	16.7	23.2	37.3	59.9	73	78	78
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Pies per Kg-day	0.01	0.15	0.19	0.16	0.16	0.14	0.12	0.12	0.17
Volatile Oil of Mustard Level in Food	4.5 ppm			100% of Food Assumed to be Contain Volatile Oil of Mustard					
	Based on worst case pie values								
Dietary Load as mg/Kg/day	0.0001	0.0007	0.0008	0.0007	0.0007	0.0006	0.0005	0.0005	0.0008
Dietary Load as mg/day	0.0005	0.0078	0.0141	0.0164	0.0266	0.0379	0.0398	0.0424	0.0588
Dietary Load As ppm in Total Diet	0.001	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.8 mg/Kg/day								
	141,075	12,429	10,216	12,164	12,049	13,576	15,782	15,824	11,410

Table D-10A: Dietary Loading from Consumption of Pies (all forms)
Containing 35 ppm Volatile Oil of Mustard (i.e.: flavor use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Pies	0.11	1.74	3.12	3.65	5.62	4.60	5.1	6.43	8.91
TOTALS:	0.11	1.74	3.12	3.65	5.62	4.60	5.10	6.43	8.91
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Pies per Kg-day	0.01	0.16	0.19	0.16	0.15	0.08	0.09	0.10	0.13
Volatile Oil of Mustard Level in Food	35 ppm			100% of Food Assumed to be Contain Volatile Oil of Mustard					
Dietary Load as mg/Kg/day	0.0005	0.0055	0.0067	0.0054	0.0053	0.0029	0.0030	0.0035	0.0045
Dietary Load as mg/day	0.004	0.061	0.109	0.128	0.197	0.161	0.179	0.225	0.312
Dietary Load As ppm in Total Diet	0.01	0.06	0.07	0.06	0.08	0.05	0.06	0.07	0.10
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 8.6 mg/Kg/day								
	16,709	1,554	1,282	1,584	1,610	2,986	2,891	2,485	1,904

Table D-10B: Dietary Loading from Consumption of Pies (all forms)
Containing 4.5 ppm Volatile Oil of Mustard (i.e.: shelf-life extension use levels) for Females, by Age Group

FEMALES (Average)	AGE GROUP								
	0 - 1 yrs	1 - 2 yrs	3 - 5 yrs	6 - 8 yrs	9 - 14 yrs	15 - 18 yrs	19 - 34 yrs	35 - 64 yrs	65 - 74 yrs
Food Source	CONSUMPTION: g/day								
Pies	0.11	1.74	3.12	3.65	5.62	4.60	5.1	6.43	8.91
TOTALS:	0.11	1.74	3.12	3.65	5.62	4.60	5.10	6.43	8.91
	MID-POINT BODY WEIGHT, Kg								
	7.48	11.0	16.3	23.5	36.8	55.9	60	65	69
	TOTAL DAILY DIET (Kg)								
	0.5	1.0	1.5	2	2.5	3	3	3	3
g Pies per Kg-day	0.01	0.16	0.19	0.16	0.15	0.08	0.09	0.10	0.13
Volatile Oil of Mustard Level in Food	4.5 ppm	100% of Food Assumed to be Contain Volatile Oil of Mustard *Based on worst pie values*							
Dietary Load as mg/Kg/day	0.0001	0.0007	0.0009	0.0007	0.0007	0.0004	0.0004	0.0004	0.0006
Dietary Load as mg/day	0.0005	0.0078	0.0141	0.0164	0.0253	0.0207	0.0230	0.0289	0.0401
Dietary Load As ppm in Total Diet	0.001	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Dietary Load Safety Factor to NOEL in Rat NTP Bioassay of Synthetic AIT	NOEL = 6.6 mg/Kg/day								
	129,956	12,089	9,972	12,321	12,518	23,224	22,484	19,331	14,806

APPENDIX 2

Bibliography

Prior to 1958

1. Anderson, E.E., et al. 1951. Pasteurized fresh whole pickles. II. Thermal resistance of microorganisms and peroxidase. Food Technol. 5(9): 364-368.

2. Anderson, E.E., et al. 1953. Effect of essential oils on the inhibition and thermal resistance of microorganisms in acid food products. Food Research. 18: 40-47.

3. Blum, H.B. and F.W. Fabian. 1943. Spice oils and their components for controlling microbiol surface growth. Fruit Products J. 22: 326-329, 347.

4. Delage, J.C. 1923. Use of mustard meal and of mustard oil in enology, Ann. Fals. 16: 483-491.

5. Ferre, L. 1925. Action of allyl mustard oil as a wine and must preservative. Ann. fals. 18: 34-37.

6. Kosker, O.; et al. 1949. Mustard as a preservative for fruit juices. The Glass Packer. 28: 818-820, 836, 838, 841.

7. Kosker, O.; et al. 1951. Effect of allyl isothiocyanate and related substances on the thermal resistance of Aspergillus niger, Saccharomyces ellipsoideus, and Bacillus thermoacidurans. Fd. Res. 16(6): 510-514.

8. Malvrzin and G. Bidart. 1923. The action of standard allyl mustard oil solutions in preventing secondary fermentations. Ann. fuls. 16: 534-542.

9. Perazzo, A. and L. de prado. 1937. The action of mustard essence on wines. Its use as antifermentative agent. Rev. assoc. bioquim. argentina. 5: 54-61.

10. Roos, L. and E. Hugues. 1924. Allyl mustard oil in enology. Ann. Fals. 17: 413-421.

11. Vaughan, J.G. and J.S. Hemingway. 1959. The utilization of mustards. Econ. Bot. 13(3): 196-204.

12. Vincens. 1924. Experiments on the use of mustard oil (allyl) in enology. Ann. fals. 17: 541-551.

13. Walker, J.C.; et al. 1937. Toxicity of mustard oils and related sulfur compounds to certain fungi. Am. J. Bot. 24(8); 536-541 (2 fig.).

Post 1958 References

Bechtel D; Henderson L; and Proudlock R (1998) "Lack of UDS activity in the livers of rats exposed to allylisothiocyanate." Teratogenesis, Carcinogenesis, And Mutagenesis, 18 (5): 209-217.

Bollard M; Stribbling S; Mitchell S; Caldwell J (1997) "The disposition of allyl isothiocyanate in the rat and mouse." Food And Chemical Toxicology, 35 (10-11): 933-943.

Delaquis P J and Sholberg P L (1997) "Antimicrobial activity of gaseous allyl isothiocyanate." Journal Of Food Protection, 60 (8): 943-947.

FASEB [Federation of American Societies for Experimental Biology] (1975) "Evaluation of the Health Aspects of Mustard and Oil of Mustard as Food Ingredients" National Technical Information Service, No. PB-254528

Food and Drug Research Labs., Inc. (1972) "GRAS (Generally Recognized as Safe) Food Ingredients – Oil of Mustard and Allyl Isothiocyanate", National Technical Information Service, No. PB-221215

Holley R A (1997) "Impact of slicing hygiene upon shelf life and distribution of spoilage bacteria in vacuum packaged cured meats" Food Microbiology (London), 14 (3): 201-211

IARC (1999) "Allyl isothiocyanate" IARC Monographs On The Evaluation Of Carcinogenic Risks To Humans, 73: 37-48.

IARC (1985) "Allyl isothiocyanate" IARC Monographs On The Evaluation Of Carcinogenic Risks To Humans, 36: pp. 60 ff.

Isshiki K; Tokuoka K; Mori R; and Chiba S (1992) "Preliminary examination of allyl isothiocyanate vapor for food preservation." Biosci Biotechnol Biochem, 56 (9): 1476-1477.

Jiao D; Ho C T; Foiles P; Chung F L (1994) "Identification and quantification of the N-acetylcysteine conjugate of allyl isothiocyanate in human urine after ingestion of mustard." Cancer Epidemiology, Biomarkers And Prevention, 3(6): 487-92.

Kasamaki A; Yasuhara T; Urasawa S, (1987) "Neoplastic transformation of Chinese hamster cells in vitro after treatment with flavoring agents." Journal Of Toxicological Sciences, 12(4): 383-96.

Kassie F; Knasmuller S (2000) "Genotoxic effects of allyl isothiocyanate (AITC) and phenethyl isothiocyanate (PEITC)." Chemico-Biological Interactions, 127(2): 163-80.

Murata M; Yamashita N; Inoue S; Kawanishi S (2000)
"Mechanism of oxidative DNA damage induced by carcinogenic allyl isothiocyanate." Free Radical Biol. Med., 28(5): pp. 797-805.

Musk S R; Johnson I T (1993) "The clastogenic effects of isothiocyanates." Mutation Research, 300(2): 111-117.

Musk SR R; Johnson IT (1993) "Allyl isothiocyanate is selectively toxic to transformed cells of the human colorectal tumor line HT29." Carcinogenesis, 14(10): pp. 2079-83.

Hasegawa T; Nishino H; Iwashima A (1993) "Isothiocyanates inhibit cell cycle progression of HeLa cells at G2/M phase." Anti-Cancer Drugs, 4(2):, pp. 273-9.

National Toxicology Program (1982) "Carcinogensis Bioassay of Allyl Isothiocyanate (CAS No. 57-06-7) in F344/N Rats and B6C3F1 Mice (Gavage Study)", NTP Technical Report No. 234 / National Technical Information Service, No. PB-83-144238.

Park C M; Taormina P J; and Beuchat L R (2000) "Efficacy of allyl isothiocyanate in killing enterohemorrhagic Escherichia coli O157:H7 on alfalfa seeds." International Journal Of Food Microbiology, 56 (1): 13-20.

Smith T; Musk S R; Johnson I T (1996) "Allyl isothiocyanate selectively kills undifferentiated HT29 cells in vitro and suppresses aberrant crypt foci in the colonic mucosa of rats." Biochemical Society Transactions, 24(3): 381S.

Tokuoka K; Mori R; and Isshiki K (1992) "Inhibitory effects of volatile mustard extract on the growth of yeasts." Journal Of The Japanese Society for Food Science and Technology, 39 (1): 68-71.

Tokuoka K and Isshiki K (1994) "Possibility of application of allylisothiocyanate vapor for food preservation." Journal Of The Japanese Society for Food Science and Technology, 41 (9): 595-599.

Ward S M; Delaquis P J; Holley R A; and Mazza G (1998) "Inhibition of spoilage and pathogenic bacteria on agar and pre-cooked roast beef by volatile horseradish distillates." Food Research International, 31(1): 19-26.

Yonezawa Y; Nishikawa K; Nishioka H (1997) "Implication of intracellular reactive oxygen species in DNA damage by allyl isothiocyanate." Sci. Eng. Rev. Doshisha Univ, 38(1): pp. 1-8.

Xu K; Thornalley PJ (2000) "Studies on the mechanism of the inhibition of human leukemia cell growth by dietary isothiocyanates and their cysteine adducts in vitro." Biochem. Pharmacol., 60(2): pp. 221-231.

SRS INTERNATIONAL® CORPORATION

1901 L Street, NW • Suite 250 • Washington, DC 20036-3503
Telephone (202) 223-0157/0298 • Fax (202) 835-8970
E-Mail: mainsrs@srsinternational.com http://www.srsinternational.com

FAX TRANSMISSION

DESTINATION NO.	202-418-3131
ATTENTION OF:	Dr. Negash Belay
COMPANY NAME:	FDA
NUMBER OF PAGES:	4 *(Including Cover Page)*
AUTHORIZED BY:	Michael G Farrow
DATE:	11-24-03

COMMENTS

This is the 2nd fax.
Please disregard the first fax sent at 4:34 pm. today

SRS INTERNATIONAL® CORPORATION

1901 L Street, NW • Suite 250 • Washington, DC 20036-3503
Telephone (202) 223-0157/0298 • Fax (202) 835-8970
E-Mail: mainsrs@srsinternational.com http://www.srsinternational.com

MEMO

To: Dr. Negash Belay
From: Michael G. Farrow, Ph.D.
Date: November 24, 2003

RE: Correction to October 14, 2003 Claim of GRAS Exemption from Premarket Approval

We are faxing, as per your request in your telephone conversation today with Dr. John A. Todhunter, the two corrected pages (pages 13 and 18) in which the units used for VOM in air are now correctly stated as 150 µL. As was mentioned, this correct unit is, indeed, mentioned on page 11.

Thank you,

Michael G. Farrow, Ph.D.

The VOM levels in various foods exposed to VOM atmospheres have been determined by the Notifier and are as represented in the table below.

Table: Expected worst case VOM levels in foods after a 10-day exposure to 150 µL VOM in air and in foods as "as consumed"

Food Type	Uptake Factor	10-Day VOM Level	Reduction on Cooking	"As Consumed " VOM Level
Beef, ground (representing also whole cuts of beef and veal and lamb, ground and cuts of)	≤ 0.34	≤ 51 ppm	5.9	≤ 8.6 ppm
Chicken, ground (representing also whole chicken and turkey, ground and whole)	≤ 0.24	≤ 36 ppm	5.1	≤ 7.1 ppm
Pork sausage (representing also ground pork and cuts of pork and ham)	≤ 0.10	< 15 ppm	3.3	≤ 4.5 ppm
Bologna, ground (representing sliced deli meats)	≤ 0.34	≤ 51 ppm	N/A	≤ 51 ppm
Pecan pie, ground (representing nut and fruit pies)	≤ 0.03	≤ 4.5 ppm	N/A	≤ 4.5 ppm

Note: 150 µL in air will be a typical VOM atmosphere for this use

The estimated dietary intake associated with shelf-life extension / anti-spoilage uses of VOM on relevant foods is provided in Tables D-1A through D-10B (preceding the Bibliography). Tables D-1A, D-1B, D-2A, and D-2B provide the estimated daily dietary VOM intake associated with all VOM treated foods and for all age groups. The other tables provide the daily intake of VOM associated with specific food types.

The basic food consumption data are from USDA's Home Economics Research Repot No. 44, "Foods Commonly Eaten by Individuals". Age-specific average daily consumption of a given food type is obtained by multiplying the average amount consumed per eating occasion (for individuals consuming the food at least one in any given three day period) by the percent of individuals consuming the food at least once during any given three day period. The average daily intake of VOM for a given food type is then estimated by multiplying the average daily consumption (by age) for the food type by the concentration of VOM in the food. This type of assessment approach is one normally relied upon by experts in the evaluation of exposures to substances in foods.

For purposes of the estimation of daily dietary intake of VOM the following assumptions were made:

(a) 100% of any given food type was assumed to be treated with VOM. This is a conservative assumption because not all foods of any given type will be exposed to VOM for anti-spoilage purposes.

Another issue which may affect safety is the potential concern that the proposed use not, inadvertently, create an opportunity for growth of botulism organisms. This concern arises due to the presence of a modified atmosphere or in the case of vacuum packed foods (due to the anaerobic atmosphere created).

With regard to the first issue, the air concentrations of VOM in the proposed use will be *circa* 150 µL but not greater. At this VOM level, there is no significant change in the oxygen concentration in air and a fully aerobic conditions, which prevents the growth of botulism, will be maintained.

With regard to the second issue, the proposed use of VOM is not compatible with vacuum packed foods as the VOM would be evacuated in the vacuum packing process. Therefore, this is not an applicable concern for this use.

Therefore, the current general recognition among experts as to the safety of VOM can be summarized as follows:

- AIT and/or VOM is consistently reported as non-genotoxic *in vivo* and usually is reported to be non-genotoxic *in vitro*.
- AIT has irritant properties which have been reported to produce transient hyperplasia of mouse skin when applied to mouse skin.
- In dermal mouse skin carcinogenicity bioassays, VOM was not carcinogenic.
- In a corn oil gavage carcinogencity bioassay in mice and rats, AIT was not carcinogenic in male or female mice, was considered equivocal in female rats, and was considered to increase the incidence of transitional cell papillomas in male rats. In male rats, the incidence of transitional cell papillomas mirrored that of bladder epithelial hyperplasia. In female rats, a single animal with transitional cell papilloma of the bladder was observed in the high dose group and, again, the incidence of bladder epithelial hyperplasia mirrored that of transitional cell papillomas in female rats.
- The metabolism of AIT differs significantly between rats and mice. In rats, glutathione conjugation is the major pathway and there is a sex difference in the accumulation of AIT / AIT-metabolites in the rat bladder with males showing higher levels than females. In mice, hydrolysis with formation of thiocyanate ion and of the cysteine conjugate is the major pathway for AIT. Humans appear to metabolize AIT similarly to mice.
- The weight of the evidence strongly supports that the increased incidence of transitional cell papillomas seen in male rats in the NTP bioassay is specific to the male rat under these test conditions and is probably due to accumulation of levels of the AIT-glutathione conjugate which induce epithelial hyperplasia in the male rat