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March 17, 2017

Dr. Paulette Gaynor
Office of Food Additive Safety (FHS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Drive
College Park, MD 20740

Re: GRAS Notice for Dimethyl Ether

Dear Dr. Gaynor

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Callaghan Innovation hereby informs the United States Food and Drug Administration of the conclusion that the intended use of dimethyl ether as an extraction solvent in the processing of various food products is Generally Recognized as Safe (GRAS) as described in the enclosed notice, and thereby the intended use of dimethyl ether is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act. I hereby certify that the enclosed electronic files were scanned for viruses prior to submission and are thus certified as being virus-free using McAfee VirusScan 8.8.

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Yours sincerely

(b) (6)

Stephen Tallon, Ph.D.
Senior Research Engineer
Callaghan Innovation

GRAS Notice for the Use of Dimethyl Ether as an Extraction Solvent

Prepared for:

Office of Food Additive Safety (FHS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Drive
College Park, MD
20740

Submitted by:

Callaghan Innovation
69 Gracefield Road
Lower Hutt 5040
New Zealand

March 7, 2017

GRAS Notice for the Use of Dimethyl Ether as an Extraction Solvent

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GRAS Notice for the Use of Dimethyl Ether as an Extraction Solvent

Part 1. §170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Callaghan Innovation hereby informs the U.S. (United States) Food and Drug Administration (FDA) of the view that the use of dimethyl ether is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Callaghan Innovation's conclusion that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Part 1.3 below. In addition, as a responsible official of Callaghan Innovation, Stephen Tallon, hereby certifies that all data and information presented in this notice constitutes a complete, representative, and balanced submission, and which considered all unfavorable as well as favorable information known to Callaghan Innovation and pertinent to the evaluation of the safety and GRAS status of dimethyl ether as an extraction solvent in the processing of food ingredients, as described herein.

Signed,

Stephen Tallon, Ph.D.
Senior Research Engineer
Callaghan Innovation

8/3/2017
Date

1.1 Name and Address of Notifier

Stephen Tallon, Ph.D.
Senior Research Engineer
Callaghan Innovation
69 Gracefield Road
Lower Hutt 5040
New Zealand
Tel: +64 4 9313005
E-Mail: Stephen.Tallon@CallaghanInnovation.Govt.NZ

1.2 Common Name of Notified Substance

Dimethyl ether

1.3 Conditions of Use

Dimethyl ether is intended for use as an extraction solvent in the processing of various food products, such as marine lipids, egg phospholipids, bacteria-derived lipids, algae-derived lipids, plant lipids, egg proteins, plant proteins, meat proteins, and fruit sugars, which can then be added to food and dietary supplements. The intended use level of dimethyl ether in the extraction process is 5 to 6 volumes of dimethyl ether per unit volume of starting material (% v/v). Dimethyl ether is recycled internally and recovered, such that the net loss of dimethyl ether is approximately 2 to 5% of the amount of the extracted food products. Residual dimethyl ether that is not recovered in the process is diluted with air so that it is below its flammability limit and vented to the atmosphere.

The dimethyl ether-extracted food product may be used in the production of final food products at levels of approximately 0.5 to 10%, with some dehydrated or defatted foods potentially containing higher levels of the dimethyl ether-extracted product.

1.4 Basis for GRAS

Pursuant to 21 CFR § 170.30 (a) and (b) of the *Code of Federal Regulations* (CFR), the dimethyl ether, as used by Callaghan Innovation, has been concluded to have GRAS status for use as an extraction solvent in the processing of various food products, as described in Part 1.3, on the basis of scientific procedures.

1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notification will be made available to the FDA for review and copying upon request during business hours at the offices of:

Callaghan Innovation
69 Gracefield Road
Lower Hutt 5040
New Zealand

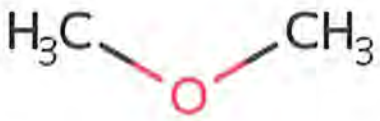
In addition, should the FDA have any questions or additional information requests regarding this notification during or after the Agency's review of the notice, Callaghan Innovation will supply these data and information as requested.

1.6 Freedom of Information Act, 5 U.S.C. Section 552

It is Callaghan Innovation's view that all data and information presented in parts 2 through 7 of this notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore all data and information presented herein are not exempt from the Freedom of Information Act, 5 U.S.C. Section 552.

Part 2. §170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identity

Chemical Name:	Methoxymethane
Other Names:	Dimethyl ether; Dimethyl oxide; oxybismethane; wood ether
Trade Names	Dymel A®, Demeon D®, Propel
Chemical Abstracts Service (CAS) Number:	115-10-6
Molecular Weight:	46.07 g/mol
Chemical Structure:	

2.2 Method of Manufacturing

2.2.1 Raw Materials and Processing Aids

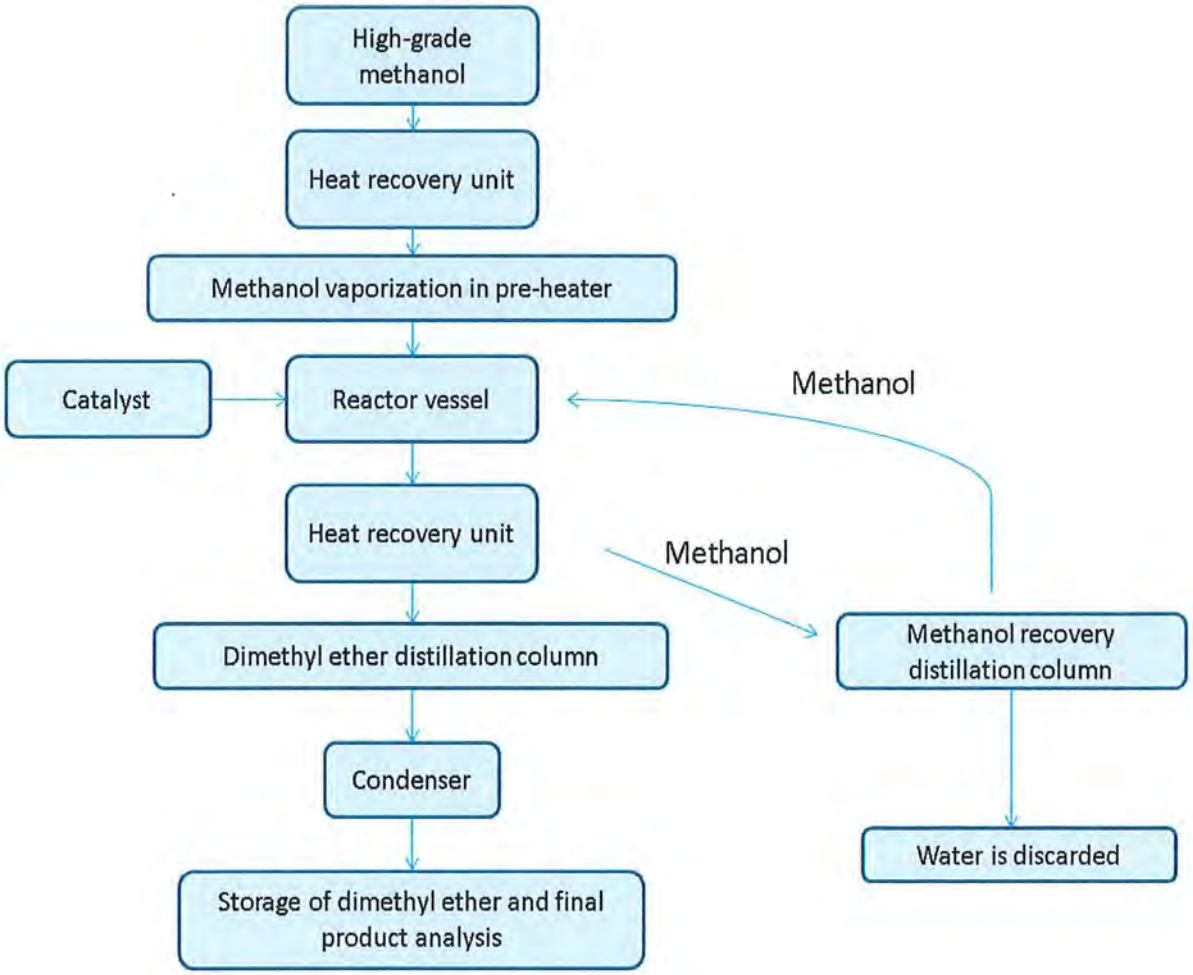
Methanol, the main reactant in the manufacture of dimethyl ether, currently has regulatory status in the manufacture of human foods in the U.S. under 21 CFR §173.250 and §175.105. The dimethyl ether used by Callaghan is produced by catalytic dehydration of methanol using a standard catalyst, such as γ-Al_2O_3, metal alloys, transition metals, or a strong acid (sulfuric acid) (Bai *et al.,* 2013; Jamshidi *et al.,* 2013). The catalyst is removed from the dimethyl ether following production of the solvent. No other reactants or processing aids are used in the manufacture of dimethyl ether.

2.2.2 Manufacturing Process

The dimethyl ether used by Callaghan Innovation is produced by catalytic dehydration of methanol under high temperature and pressure conditions. In the first step, pure, high-grade methanol is passed through a heat recovery unit and vaporized in a pre-heater. Next, 2 parts of methanol vapor are reacted in a fixed-bed reactor vessel under pressure and at elevated

temperatures in the presence of a catalyst to yield dimethyl ether and water. The reaction products (*i.e.,* dimethyl ether and water, and residual methanol) are then passed to a dimethyl ether distillation column *via* a heat recovery unit where dimethyl ether is separated from methanol and water. Dimethyl ether is passed through a condenser prior to storage, while methanol and water are separated in a methanol recovery distillation column. Methanol is recycled back into the reactor vessel and water is discarded. The dimethyl ether is analyzed to ensure compliance with the product specifications. The manufacturing process consistently produces a dimethyl ether with high purity (≥99.99%) (see Section 2.3.2). The final product is stored in food-grade dedicated gas cylinders or stainless steel tanks in a well-ventilated area away from heat, possible ignition sources, oxygen cylinders and other oxidizing materials. A schematic overview of the manufacturing process of the dimethyl ether is shown in Figure 2.2.2-1.

Figure 2.2.2-1 Schematic Overview of the Manufacturing Process for the Dimethyl Ether



2.3 Product Specifications and Batch Analyses

2.3.1 Product Specifications

Appropriate food-grade product specifications have been established for the dimethyl ether and are presented in Table 2.3.1-1 below. The product specifications are similar to the specifications of the dimethyl ether evaluated by the European Food Safety Authority (EFSA) in their review of dimethyl ether for use as a processing aid (EFSA, 2009, 2015). Dimethyl ether is supplied and stored in a liquid state in a pressurized cylinder, and therefore a specification parameter for color is included in the product specifications.

Table 2.3.1-1 Product Specifications for Dimethyl Ether

Parameter	Specification
Purity	≥99.99%
Color (APHA)	NMT 5
Methanol	<10 mg/kg
Water	<100 mg/kg
C1-C4 hydrocarbons (alkanes and CO_2)	<100 mg/kg

APHA = American Public Health Association; CO_2 = carbon dioxide; NMT = not more than

2.3.2 Batch Analyses

Five (5) non-consecutive batches of dimethyl ether were analyzed to verify that the manufacturing process produces a consistent product that meets the product specifications. The results of the batch analyses are provided in Table 2.3.2-1.

Table 2.3.2-1 Summary of Product Analysis for 5 Non-Consecutive Batches of the Dimethyl Ether

Parameter	Specification	Manufacturing Lot No. (Date of Analysis)				
		Lot 310790 (03/04/2016)	Lot 310996 (01/05/2016)	Lot 310482 (05/06/2016)	Lot TSC0224497 (11/09/2016)	Lot TSC0224488 (14/08/2016)
Purity (%)	≥99.99	≥99.99	≥99.99	≥99.99	≥99.99	≥99.99
Color (APHA)	NMT 5	NMT 5	NMT 5	NMT 5	NMT 5	NMT 5
Methanol (mg/kg)	<10	<10	<10	<10	<10	<10
Water (mg/kg)	<100	<100	<100	<100	<100	<100
C1-C4 hydrocarbons (alkanes and CO_2) (mg/kg)	<100	<100	<100	<100	<100	<100

APHA = American Public Health Association; CO_2 = carbon dioxide; NMT = not more than

2.3.3 Additional Analytical Information

2.3.3.1 Non-volatile Impurities

Non-volatile residues in dimethyl ether (less than 0.01%) obtained from two different manufacturers were analyzed by quadrupole-time-of-flight mass spectrometry (QToF-MS). Briefly, a sample of dimethyl ether is slowly evaporated into the atmosphere. The valve assembly is removed, the cylinder rinsed with ACS-grade absolute ethanol, and the sample is analyzed by QToF-MS *via* infusion in both negative and positive modes. The major peaks observed in the ethanol phase were faintly present in the evaporated dimethyl ether extracts, indicating no contamination.

The results of the analysis showed the presence of phthalates (sodium adduct of diisooctyl phthalate, didodecyl phthalate, and dibutyl phthalate), bis(butyldiglycol)adipate, various polyethylene glycols and polypropylene glycols, and a polyfluorinated compound that was not fully identified at low concentrations. The presence of these compounds were attributed to the seals of the storage container. It should be noted that dimethyl ether is recycled during the extraction process, which would limit the introduction of additional non-volatile residues into the food ingredient.

2.4 Stability

Unsubstituted ethers, such as dimethyl ether, are stable compounds due to the presence of alpha-hydrogens that protect the compound against oxidation that cause the formation of peroxides (Naito *et al.,* 2005; Sakuth *et al.,* 2012). Dimethyl ether has been shown to be chemically stable in that no peroxide formation occurred at temperatures up to 353K (~80°C) (Naito *et al.,* 2005). Spontaneous degradation of dimethyl ether has been observed in the presence of oxygen at temperatures higher than 500K (Rosado-Reyes *et al.,* 2005). The extraction process is performed in a closed environment with the temperature maintained at 40 to 50°C, limiting the potential for peroxide formation (see Section 3.1 for further details). The dimethyl ether used by Callaghan Innovation is manufactured in a closed environment, thereby limiting the presence of oxygen and ultimately the potential for peroxide formation. Dimethyl ether is not expected to undergo auto-oxidation. Following the manufacturing process, the dimethyl ether is stored in dedicated gas cylinders or tanks in a well-ventilated area away from sources of heat, ignition, and oxidizing compounds. Dimethyl ether does not degrade or contain any contaminants as demonstrated by analytical data showing the purity and absence of C1-C4 hydrocarbons, methanol, and water in the dimethyl ether (see Table 2.3.2-1). Furthermore, dimethyl ether is not expected to form reaction by-products with food substances due to its stable nature and is absent from various extracted food products (Table 3.2-1).

Part 3. §170.235 Dietary Exposure

3.1 Intended Conditions of Use of the Dimethyl Ether and Proposed Use Levels

Dimethyl ether is a powerful polar solvent as its density is similar to liquids and its viscosity is similar to gases. Callaghan Innovation intends to use dimethyl ether as an extraction solvent in the processing of various food products, such as preparing lipid extracts and defatted (non-lipid) products, derived from marine and animal products, plants, fruits, seeds, and micro-organisms. Examples of lipid extract products include marine lipids, egg phospholipids, bacteria-derived lipids, algae-derived lipids, and plant lipids. Examples of defatted (non-lipid) products include egg proteins, plant proteins, meat proteins, and fruit sugars. After processing, these food extracts may then be added to food and dietary supplements.

A general description of a typical extraction process is described as follows. The food or food product is loaded into stainless steel extraction vessels and liquid dimethyl ether is added (pressurized to 40 bar and heated to 40 to 50°C). The dimethyl ether phase containing the dissolved polar and non-polar extracts is passed through separation vessels where the pressure is decreased until the extracts are no longer soluble in dimethyl ether. The extract and dimethyl ether is recovered from the separation vessel; the extract is removed, whereas dimethyl ether is recycled back through the extraction vessel. The intended use level of dimethyl ether in the production process is 5 to 6 volumes of dimethyl ether per unit volume of starting material. Dimethyl ether is recycled internally and recovered such that the net loss of dimethyl ether is approximately 2 to 5% of the amount of the extracted food product. The dimethyl ether-extracted food product may be used in the production of final food products at levels of approximately 0.5 to 10%, with some dehydrated or defatted foods containing higher levels of the dimethyl ether-extracted products.

3.2 Residue Levels of the Dimethyl Ether in Extracted Food Products

Residual levels of dimethyl ether in various extracted food products were measured by gas chromatography-mass spectroscopy (GC-MS) or flame ionization detection (GC-FID). The residue levels of dimethyl ether were below the detection limit (2 ppm). As shown in Table 3.2-1 below, dimethyl ether was not detected in a number of extracted food products. Due to the chemical nature of dimethyl ether, the solvent dissipates from both lipid extracts and residual lipid-depleted biomass at room temperature and atmospheric conditions. It should be noted that no chemical by-products are expected to form in the extracted food products.

Table 3.2-1 Residual Levels of Dimethyl Ether in Various Extracted Food Products		
DME Extracted Product	**Time of Measurement**	**Residue Level**
Lipase enzyme powder	24 hours after processing	ND
Fermented bacterial biomass	24 hours after processing	ND
DME extract from fermented biomass	Immediately after processing	ND
Defatted aqueous protein/lipid solution	After spray drying	ND
Extract from aqueous protein/lipid solution fraction	After rotary evaporation of water	ND

DME = dimethyl ether; ND = not detected

Part 4. §170.240 Self-Limiting Levels of Use

No known self-limiting levels of use are associated with the dimethyl ether.

Part 5. §170.245 Experience Based on Common Use in Food Before 1958

Not applicable.

Part 6. §170.250 Narrative

The conclusion that dimethyl ether, as described herein, is GRAS under the conditions of its intended use as an extraction solvent in the processing of various food products is based on scientific procedures using generally available data and information on dimethyl ether. A comprehensive and detailed search of the published scientific literature was conducted through February 2017 to identify toxicology studies conducted with dimethyl ether to support the safety of the solvent. Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and Toxfile® served as the primary sources of published literature. Toxicology studies on dimethyl ether conducted *via* the oral route were not identified in the literature. Two (2) inhalation toxicology studies were identified in the literature search (Collins *et al.,* 1978; Reuzel *et al.,* 1981). These studies are summarized in Section 6.3 below. In addition to the published toxicology studies, a number of unpublished toxicology studies conducted *via* the inhalation route were identified which were extensively reviewed by EFSA and Food Standards Australia New Zealand (FSANZ) to support the safety of dimethyl ether when used as an extraction solvent in the processing of various food products (EFSA, 2009, 2015; FSANZ, 2011a,b). The results of the EFSA and FSANZ reviews corroborate the safety of dimethyl ether for use as an extraction solvent and are presented in Section 6.4 below.

6.1 Metabolic Fate

Given that dimethyl ether is a gas at body temperature, the majority of studies conducted from a safety standpoint have been *via* the inhalation route. Following inhalation as the route of administration, dimethyl ether has been shown to be rapidly absorbed, and distributed throughout the body prior to elimination (Kemper and Eckard, 1978; Eckard and Kemper, 1979; Daly and Kennedy, 1987). Furthermore, organ distribution of dimethyl ether has been reported to be directly proportional to the concentrations of exposure. Following exposure to concentrations up to 1,000 ppm, equivalent to 1,884 mg/m^3, for 60 minutes, levels of dimethyl ether were detected in muscle (14 mg/kg), lungs (15 mg/kg), liver (15 mg/kg), spleen (16 mg/kg), kidney (17 mg/kg), heart (17 mg/kg), brain (18 mg/kg), blood (19 mg/kg), and fat tissue (22 mg/kg) (Kemper and Eckard, 1978). In another inhalation study by Eckard and Kemper (1979), rats were exposed to dimethyl ether at concentrations up to 2,000 ppm, equivalent to 3,769 mg/m^3, for 180 minutes. In all tissues and organs (blood, heart, lungs, liver, spleen, kidneys, fat, muscle, and brain), levels of dimethyl ether were 16.4±1.4 mg/kg, except for muscles, where concentrations of dimethyl ether did not exceed 8 mg/kg. Daly and Kennedy (1987) reported that tissue concentrations of dimethyl ether rapidly increased following exposure to concentrations of 750 to 2,000 ppm, and rapidly decreased when exposure was ceased with no observed tissue storage. Steady-state levels of dimethyl ether were reached within 30 minutes of continuous inhalation exposure (Kemper and Eckard, 1978; Eckard and Kemper, 1979; Daly and Kennedy, 1987). Unchanged dimethyl ether is rapidly excreted *via* exhaled air in a biphasic manner, with levels returning to background levels within 90 minutes. The $t_{1/2\alpha}$ and $t_{1/2\beta}$ were reported to be 10 and 90 minutes, respectively (Kemper and Eckard, 1978; Daly and Kennedy, 1987). Dimethyl ether is a stable compound and is not known to be reactive due to the absence of reactive functional groups (see Section 2.4 for further details). As a result, dimethyl ether is not metabolized and is excreted as an unchanged molecule.

6.2 Applicability of Route-to-Route Extrapolation

As the majority of the data identified is *via* a route of administration not applicable to food ingredients, route-to-route extrapolation can be applied in the absence of route-specific toxicity data (Pepelko and Withey, 1985; Rennen *et al.,* 2004). In general, the most common use of this technique is through the extrapolation from the oral to the inhalation route and vice versa (Pepelko, 1987). The general principle of route-to-route extrapolation is to convert the external no-observed-adverse-effect level (NOAEL) (from inhalation studies) into an internal systemic dose, and to correct for the amount of the compound that does not enter the body due to incomplete absorption. This route-to-route extrapolation methodology is directly applicable for the solvent dimethyl ether in that:

1) The absorption and expression of toxicity are not influenced by local effects;
2) The absorption efficiency is the same between routes;

3) No metabolism occurs including chemical transformation by intestinal microflora or pulmonary macrophages takes place; and
4) The chemical is relatively soluble in body fluids.

As described in Section 6.1 above, dimethyl ether is rapidly absorbed and distributed when exposure is through the inhalation route, the kinetics of which is similar following oral exposure (Kemper and Eckard, 1978; Eckard and Kemper, 1979; Daly and Kennedy, 1987). Moreover, dimethyl ether is not metabolized and is eliminated unchanged in expired air. In addition, dimethyl ether does not cause toxicity at the site of contact (EFSA, 2009, 2015). Therefore, based on the pharmacokinetics and the available toxicity data of dimethyl ether, and the criteria described by Rennen *et al.* (2004), the available inhalation data on dimethyl ether can be bridged to support exposure through the oral route. Both EFSA (2009, 2015) and FSANZ (2011a,b) used the extrapolation methodology in support of the safe use of dimethyl ether as an extraction solvent (see Section 6.4 for further details).

6.3 Published Toxicology Studies

Two published toxicology studies supporting the safety of dimethyl ether were identified in the literature search (Collins *et al.,* 1978; Reuzel *et al.,* 1981). These studies were reviewed by EFSA and the U.S. Environmental Protection Agency (U.S. EPA) as part of their safety evaluations.

6.3.1 13-Week Toxicity Study

Reuzel *et al.* (1981) conducted an inhalation toxicity study in CPB Wistar rats (10/sex/group) exposed to dimethyl ether (99.8% purity) in chambers containing 0, 2,000, 10,000, or 20,000 ppm for 6 hours/day, 5 days/week, for 13 weeks. Analyses showed actual concentrations of dimethyl ether to be 0, 1,943, 9,734, or 19,466 ppm, respectively, equivalent to 0, 3,661, 18,341, or 36,679 mg/m^3 (ACGIH, 2016)[1]. In terms of internal dose, these concentrations are equivalent to 0, 728, 3,645, or 7,289 mg/kg body weight/day for males, respectively, and 0, 748, 3,748, or 7,495 mg/kg body weight/day for females, respectively. Food and water were provided *ad libitum* during the non-exposure periods. Animals were observed for clinical signs and behavior on a daily basis. Body weight and food consumption were measured weekly. The following hematological parameters were measured on week 13: hemoglobin level, packed cell volume (PCV), red blood cell (RBC) count, and white blood cell (WBC) count and differential. In addition, the following serum biochemistry parameters were measured: aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, total protein, and albumin. Urine samples were collected on weeks 6 and 13 and the following parameters were measured: volume, specific gravity, appearance, pH, sugar, protein, occult blood, ketones, and microscopy of the sediment. At the end of the study period, all animals were killed and the following organs were removed, weighed, and examined for gross pathological changes: spleen, adrenals, kidneys, liver, heart, lungs with trachea and larynx,

testes, ovaries, thymus, thyroid, brain, and pituitary. Microscopic examination of the following organs and tissues was conducted in all animals of the control and high-dose group: skin, axillary lymph nodes, preputial glands, skeletal muscle, femoral nerve, mammary glands, abdominal wall, coagulating glands, prostate, epididymides, caecum, colon, pancreas, mesenteric lymph nodes, urinary bladder, seminal vesicles, spinal cord, uterus, nasal cavity, sternum, esophagus, aorta, stomach, duodenum, jejunum, ileum, salivary glands (submaxillary, parotid, sublingual), exorbital lachrymal glands, and eyes. The lungs of animals of the low-dose and mid-dose groups were also microscopically examined.

No significant changes were reported on clinical signs, body weight, or food consumption. One female animal of the control group became ill on week 11 and was terminated. At autopsy, a tumor in the pituitary gland was observed in this animal.

A significant increase in percentage of neutrophils and a significant decrease in percentage of lymphocytes were observed in male and female animals of the high-dose group. A slight decrease in percentage of lymphocytes was observed in males of the low-dose ppm group. However, this effect was not considered treatment-related by the study authors as it was not observed in the higher concentration groups and occurred only in males. In females of the mid-dose group, an increase in erythrocyte count was observed. This change was within the normal range and was not considered treatment-related.

Males of the high-dose group showed a slight but significant increase in ALT levels. A slight but significant decrease in total protein was noted in females of the high-dose group.

A significant decrease in mean specific gravity of the urine was noted in females of the low-dose and mid-dose groups at week 6, while an increase was observed in males of the mid-dose group. Additionally, mean urine volume was significantly increased in females of the high-dose group at week 6 and decreased in males of the mid-dose group at week 13. The authors noted that all changes in urinalysis were within the normal range, only occurred in one sex, and were therefore of no toxicological significance.

The adrenal weight relative-to-body weight was slightly increased in males of the low-dose group. This change was not considered toxicologically significant as a similar effect was not observed in animals exposed to the higher concentrations. In females of the high-dose group, a low mean ovary weight was observed. The authors noted that the low organ weight may be due to two animals with “extremely low” ovary weight, and therefore, this change was not considered to be relevant. The authors noted that ovary weight is “known to be highly variable under normal conditions”. A number of changes were noted upon microscopic examination, however, they were minimal to slight, did not occur in a dose-related or sex-related manner, or occurred in a similar manner between the control and high-dose groups. Slight vacuolation of cells in the medulla and zona fasciculate of the adrenals was observed in 4 control and in 4 high-dose animals (both sexes). Based on the results of this study, the authors determined the no-

observed-adverse-effects concentration (NOAEC) to be 20,000 ppm, equivalent to 7,289 and 7,495 mg/kg body weight/day in male and female animals, respectively.

6.3.2 30-Week Toxicity Study

In the study by Collins *et al.* (1978), male and female Wistar rats (25/sex/group; 150 to 220 g body weight) were exposed to dimethyl ether (purity not reported) in chambers containing concentrations of 0, 0.02, 0.2, or 2% for 6 hours/day, 5 days/week, for 30 weeks. Analysis showed the mean concentration of dimethyl ether in each group to be 0, 197, 1,964, or 18,830 ppm, respectively, equivalent to 0, 371, 3,701, or 35,481 mg/m^3 (ACGIH, 2016)[1]. In terms of internal dose[2], the concentrations are equivalent to 0, 63, 631, 6,048 mg/kg body weight/day for males, respectively, and 0, 70, 701, 6,720 mg/kg body weight/day for females, respectively. Animals were provided food and water *ad libitum* when not in exposure chambers. Animals were observed for clinical signs twice daily and body weight and food consumption were measured on a weekly basis. Ophthalmological examination was conducted before exposure and on week 26 of the study period in all control and high-dose animals. Hematology, serum biochemistry, and urinalysis were conducted in control animals prior to initiation of the study and at week 24. Examinations were performed in high-dose animals only on week 24. The following hematological parameters were measured: total RBC and WBC, hemoglobin, packed cell volume, mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), differential WBC and platelet count, and prothrombin time. Serum biochemistry parameters evaluated include: blood urea nitrogen (BUN), alkaline phosphatase, glucose, serum proteins, and sodium and potassium concentrations. The urinalysis parameters measured include: volume, specific gravity, pH, protein, total reducing substances, glucose, ketones, bile pigments, urobilinogen, and blood pigments. Serum levels of ALT were measured in all groups on week 27 and 30 and AST levels were measured on week 30. All animals were killed at the end of the study period and the following organs were removed and weighed: brain, pituitary glands, heart, lungs, liver, spleen, kidneys, thyroid, adrenals, uterus, and gonads. Histopathological examination was conducted on the following organs and tissues from 10 animals/sex of the control and high-dose groups: nasal passages, larynx, trachea, lungs, heart, thymus, lymph nodes (cervical and mesenteric), liver, spleen, pancreas, kidneys, urinary bladder, gonads, uterus, thyroid, adrenals, salivary gland, stomach (glandular and non-glandular), duodenum, ileum, mid-colon, bone marrow, brain (cerebral cortex, cerebellum, medulla), pituitary and eyes.

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[1] Concentrations in ppm were converted to mg/m^3 according to ACGIH (2016) where the molecular weight of dimethyl ether is 46.07 g/mol and the molar volume of air at NTP (25°C, 760 Torr) is 24.45 L.
[2] Internal dose (mg/kg body weight/day) calculated as: (absorption efficiency x inhalation rate x duration of exposure x concentration) / body weight. Where the absorption efficiency is 75%, the exposure duration is 6 h/day, and inhalation rate and mean body weights for Wistar rats are reported by U.S EPA (1988).

No significant changes in clinical observations, body weight, food consumption, ophthalmologic examination, hematology, urinalysis, or histopathology were observed in any group. A significant increase in ALT was noted in males and females of the high-dose group at week 30, whereas a significant increase in AST was noted in males of the mid-dose group. When adjusted for body weight, a significant decrease in liver weight was observed in males of the high-dose group. The authors noted that the increase in ALT level and decrease in liver weight in males of the high-dose group may be attributed to hepatic fibrosis, which was "insufficiently severe" to be detected upon histopathological examination. Based on the elevations in AST observed at mid-dose in this study, the authors determined the NOAEC to be 0.02%, equivalent to 63 and 70 mg/kg body weight/day in male and female animals, respectively.

6.4 Safety Evaluations by Authoritative Bodies

In addition to the published toxicology studies summarized in Section 6.3 above, the unpublished inhalation toxicity studies reviewed by EFSA (2009, 2015) and FSANZ (2011a,b) are presented in the sections below. These studies corroborate the safety of dimethyl ether when used as an extraction solvent.

6.4.1 European Food Safety Authority (EFSA)

In 2009, EFSA published a scientific opinion on the safety of dimethyl ether and its use as an extraction solvent in the processing of animal proteins, specifically collagen, with a proposed residual limit of 9 µg/kg (EFSA, 2009). The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) noted that toxicology studies conducted *via* the oral route were not available, and, as such, their review was based on inhalation exposure studies. The CEF Panel considered the findings from the inhalation studies to be applicable to the safety evaluation of orally consumed dimethyl ether as the compound is rapidly absorbed and distributed to the systemic system following inhalation exposure, similar to absorption that may occur in the gastrointestinal tract following ingestion.

A number of sub-chronic and chronic studies conducted *via* the inhalation route were reviewed by the CEF Panel. Two (2) 13-week studies were identified whereby male and female Wistar rats were exposed to dimethyl ether at concentrations up to 20,000 ppm, equivalent to 37,685 mg/m^3, in the atmosphere for 6 hours/day for 5 days/week (CIVO, 1978, 1983). No significant changes were observed in behavior, body weight, relative organ weights, urinalysis, serum biochemistry, gross pathology, and histopathological examinations throughout the study period. Sporadic hematological findings were observed throughout treated animals, however, as they were not dose-related and no corresponding effects were observed upon histopathological examination, these effects were not considered to be treatment-related. Based on the results of these studies, the NOAEC was determined to be 20,000 ppm or 37,685 mg/m^3, the highest concentration tested. In addition, a 13-week study was identified whereby Syrian Golden hamsters were exposed to dimethyl ether at concentrations of 0, 5,000,

10,000, or 20,000 ppm, equivalent to 0, 9,421, 18,842, or 37,685 mg/m^3 (CIVO, 1983). A significant increase in blood cell count was noted in males of the 5,000 ppm and 10,000 ppm groups at day 56, and a significant decrease in blood cell count was observed in males of the 10,000 ppm group at the end of the study period. Based on these findings, the authors proposed a no-observed-effects concentration (NOEC) of 5,000 ppm (9,421 mg/m^3) in hamsters.

A 2-year Good Laboratory Practice (GLP) inhalation study was also reviewed by the CEF Panel whereby male and female Crl:CD(SD)BR rats were exposed to concentrations of 0, 2,000, 10,000, or 25,000 ppm, equivalent to 0, 3,768, 18,842, or 47,106 mg/m^3, respectively (DuPont Co., 1986). No significant changes in hematological and serum biochemistry parameters were noted between the treatment groups and control group. Similarly, no specific tissue damage was observed upon histopathological examination. A non-significant slight decrease in survival time was observed in animals of the 10,000 ppm and 25,000 ppm groups (no further details provided). Similarly, no evidence of increased cancer incidences in any tissue or organ was noted in any treatment group. Based on the slight decrease in survival time, the authors determined the NOEC to be 2,000 ppm, equivalent to 3,768 mg/m^3, in Crl:CD(SD)BR rats.

The reproductive and developmental toxicity of dimethyl ether was considered in 2 studies conducted in rats (CIVO, 1981; Haskell, 1981). In the first study, pregnant albino Wistar rats were exposed to dimethyl ether in the atmosphere at concentrations up to 2.8%, equivalent to 28,000 ppm or 52,759 mg/m^3, from gestation day (GD) 6 to GD 16 (CIVO, 1981). No significant changes in body weights, food consumption and food efficiency were observed. At necropsy, no abnormal morphological findings were observed. In addition, no teratogenic effects or adverse changes in reproductive parameters (organ weights, number of corpora lutea, implantation sites, and number of dead and alive fetuses *per* litter) were observed. Supernumerary lumbar ribs and variations in degree of ossifications were observed in the 2.0 and 2.8% group, however, these effects were not dose-related and changes in ossifications were considered normal variations in this species strain. Based on the results of this study, the authors determined a NOEL of 2.8% or 52,759 mg/m^3.

In the second study, pregnant female Crl:CD(SD)BR rats (27/group) were exposed to dimethyl ether (99.9% purity in the atmosphere at concentrations of 0, 0.125, 0.5, or 2.0%, equivalent to 0, 1,250, 5,000, or 20,000 ppm or 0, 2,355, 9,421, or 37,685 mg/m^3, respectively, for 6 hours/day on GD 6 to GD 15 (DuPont Co., 1981; Haskell, 1981). This study was performed in accordance with GLP (no further details provided). Food and water were available *ad libitum* except during the exposure period. Females were mated to mature males of the same strain and mating was verified by detection of spermatozoa in the vaginal lavage following cohabitation. Mated females were housed individually. Body weights and food consumption were measured throughout gestation, and animals were observed daily for clinical signs of

toxicity and behavior until termination. Dams were examined for gross pathologic changes, liver and uterine weights, and reproductive parameters. The following parameters were measured: copora lutea, implantation sites, live and dead fetuses, resorptions, fetal weight, and number and position of all live, dead, and resorbed fetuses. All live and dead fetuses were weighed and sexed externally and internally. Live fetuses were examined for external changes.

The pregnancy ratios were 25/27, 24/27, 27/27, and 25/27 for the 0, 1,250, 5,000, and 20,000 ppm groups, respectively. No significant changes were observed in maternal body weight and organs, gross morphological examinations, and reproductive parameters (corpora lutea, implantations, number of resorptions, and total number of dead and live fetuses) in any treatment group. Similarly, the number of corpora lutea was 16.7, 16.3, 15.2, and 15.7 for the 0, 1,250, 5,000, and 20,000 ppm groups, respectively. The number of implantations was 14.0, 15.3, 14.7, and 14.9, respectively. Significantly decreased fetal body weight and increased incidence of skeletal variation were noted in the 5,000 ppm group. The total number of live fetuses was increased in the 20,000 ppm group compared to the control (14.0 *versus* 13.0), however, the statistical significance was not reported. Based on the results of this study, the authors determined a NOAEC of 1,250 ppm or 2,355 mg/m^3 in rats. Upon review, the CEF Panel calculated an internal dose of 518 mg/kg body weight/day[3] based on the NOAEC determined from this study (EFSA, 2015).

No mutagenic or genotoxic effects were observed in a series of GLP-conducted studies including an *in vitro* bacterial reverse mutation assay in *Salmonella typhimurium* (*S. typhimurium*) TA98, TA100, TA1535, TA1537, and TA1538 and *Escherichia coli* (*E. coli*) WP2, *in vitro/in vivo* host-mediated assay with *E. coli* K12 and *S. typhimurium* TA1538 in N:NIH mice, and in *in vivo* sex-linked recessive lethal test in *Drosophila melanogaster* at concentrations up to 120,000 ppm (226,110 mg/m^3), 20,000 ppm (37,685 mg/m^3), and 28,000 ppm (52,759 mg/m^3), respectively.

The toxicology dataset reviewed by the CEF Panel suggest that dimethyl ether is of low toxicity and does not have genotoxic or mutagenic potential. Based on the proposed residual limit of 9 µg/kg in extracted animal proteins, the exposure to dimethyl ether was estimated as 0.18 µg/person/day (0.003 µg/kg body weight/day for a 60 kg individual)[4], providing a margin of safety in the order of 10^8 times lower than the internal dose calculated from the Haskell (1981) study. As a result, the CEF Panel concluded that dimethyl ether is not of safety concern when used as a solvent at a level of up to 2% of the final food product with a maximum residual level of 9 µg/kg in extracted animal protein (EFSA, 2009).

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[3] Internal dose (mg/kg body weight/day) calculated as: (absorption efficiency x inhalation rate x duration of exposure x concentration) / body weight. Where the absorption efficiency is 75%, inhalation rate and mean body weights for rats is 240 mL/min and 0.25 kg, respectively, and the exposure duration is 6 h/day (EFSA, 2015).

[4] Exposure estimate calculated as a worst-case scenario where a 60 kg individual consumes 1 kg of meat containing 2% of dimethyl ether-extracted animal protein daily.

The CEF Panel re-evaluated the safety of dimethyl ether as an extraction solvent in the processing of animal protein products such as collagen and collagen derivatives, and under a new intended condition of use, gelatin, and established maximum residual limits in each proposed use (EFSA, 2015). In their evaluation, the CEF Panel reviewed previous safety data in addition to *in vitro* genotoxicity studies [unscheduled DNA synthesis (UDS) assay in primary rat liver cells, hypoxanthine-guanine phosphoribosyl transferase (HGPRT) forward mutation assay in V79 Chinese hamster cells, and chromosome aberration test in human peripheral lymphocytes] that became available since the CEF Panel's last review. The additional genotoxicity studies were consistent with previous negative genotoxicity and mutagenicity results (EFSA, 2009). The existing maximum residual level of 9 µg/kg in the extracted animal protein was increased to 3 mg/kg in the latest review and the use level was increased from 2.0 to 3.5%. Based on the new proposed residual limit, the exposure of dimethyl ether was estimated to be 2.0 µg/kg body weight/day[5]. In addition, a maximum residual limit of 9 µg/kg in gelatin was proposed, corresponding to an estimated exposure level of 0.014 µg/kg body weight/day[6] for a 60 kg individual. The margin of exposure was calculated to be approximately $2x10^5$, providing a large margin of safety for the use of dimethyl ether as an extraction solvent in the preparation of animal protein materials. As a result, the CEF Panel concluded that the proposed residual limits for defatted animal protein products (3 mg/kg) and gelatin (0.009 mg/kg) are of no safety concern.

6.4.2 Food Standards Australia New Zealand

In their review, FSANZ evaluated a similar dataset as the CEF Panel (EFSA, 2009, 2015) that was submitted as part of the applications to support the use of dimethyl ether as a processing aid in the production of dairy and non-dairy food ingredients and products (FSANZ, 2011a,b). Due to the low levels of use and the inherent chemical properties of dimethyl ether (*e.g.*, low boiling point), exposure to dimethyl ether was considered to be negligible due to rapid evaporation of the solvent following processing. Based on the available scientific information, FSANZ concluded that the use of dimethyl ether as an extraction solvent processing aid "*does not pose any public health and safety risk*". As such, dimethyl ether is permitted for use as a processing aid for all foods, including dairy ingredients and dairy products, with maximum residual levels of 2 mg/kg (FSANZ, 2011a,b).

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[5] Exposure estimated calculated conservatively where 3.5% of the animal protein product (*e.g.,* collagen) is added to food, and a 60 kg individual consumes 1 kg of food per day.
[6] Exposure estimated calculated conservatively where 9% of gelatin is added to food, and a 60 kg individual consumes 1 kg of food per day.

6.5 Overall Conclusions Related to Safety

The available pharmacokinetic and metabolic fate data indicate that inhaled dimethyl ether is handled very similarly to ingested dimethyl ether as it is rapidly absorbed, distributed, and eliminated (Kemper and Eckard, 1978; Eckard and Kemper, 1979; Daly and Kennedy, 1987). Moreover, dimethyl ether is not metabolized, is unreactive, and is eliminated *via* exhaled air as an unchanged compound and does not cause any site of contact toxic effects (EFSA, 2009, 2015). These dimethyl ether characteristics meet the criteria described by Rennen *et al.* (2004) for supporting reliable route-to-route extrapolation of inhalation toxicity data to the oral route. As such, studies conducted *via* the inhalation route were considered applicable to evaluate the oral safety of dimethyl ether from its use as an extraction solvent of food products. Two inhalation toxicology studies conducted for 13 and 30 weeks with dimethyl ether were identified in a comprehensive search of the published literature. In the 30-week study, significant changes were limited to an increase in ALT levels in the high-dose group, an increase in AST level in males of the mid-dose group, and a decrease in relative-to-body liver weight in males of the high-dose group (Collins *et al.,* 1978). The increase in ALT level was observed in males and females treated with 6,048 or 6,720 mg/kg body weight/day, respectively, and was accompanied by a decrease in absolute and relative liver weight in males only. It should be noted that similar findings were not observed in other 13-week or 2-year studies conducted in rats at similar or greater dose levels of dimethyl ether (CIVO, 1978, 1983; DuPont Co., 1986). A slight but significant increase in AST levels in males of the mid-dose group was also observed (Collins *et al.,* 1978). This effect was considered sporadic and not related to treatment with dimethyl ether as it was observed in males only and was not observed at higher doses. Therefore, the true NOAEL from this study may be the mid-dose (631 or 701 mg/kg body weight/day for males and females, respectively) rather than the reported low-dose (63 or 70 mg/kg body weight/day for males and females, respectively).

When considering a worst-case exposure scenario where the residual level of dimethyl ether in the food ingredient is equivalent to the limit of detection (LOD) of the method of analysis (*i.e.,* LOD 2 mg/kg), and the dimethyl ether-extracted ingredient is used at 100% of a final food product (*i.e.,* the dimethyl ether-extracted ingredient containing 2 mg/kg of the solvent is consumed), a margin of safety[7] of ~1.9×10^4 to 2.1×10^4 can be calculated. These large margins of safety in terms of 10^4 indicate no safety concerns of dimethyl ether as an extraction solvent at the proposed levels of use. It should be noted, that this margin of safety is likely greater on the basis that the extracted food ingredient will be used at lower levels in final food products (*i.e.,* less than 100%) and does not account for further volatilization of the solvent during formulation of the final food product.

[7] Margin of safety calculated as (NOAEL/exposure), where exposure is calculated with a maximum residue level of 2 mg/kg, and it is assumed that a 60 kg individual consumes 1 kg of meat per day.

The NOAEL determined from the study by Collins *et al.* (1978) is greater than the internal dose (~518 mg/kg body weight/day) estimated by EFSA based on the results of the developmental study, in which a margin of safety of $2x10^5$ was calculated (EFSA, 2015). In addition, the reported NOAEC from the 2-year GLP inhalation study was 2,000 ppm, equivalent to 642 or 714 mg/kg body weight/day in male and female animals, respectively (DuPont Co., 1986). The margin of safety based on the NOAEC from the 2-year GLP inhalation study was also calculated (~$1.9x10^4$ to $2.1x10^4$). Overall, the results of these unpublished toxicology studies demonstrate a large margin of safety with the use of dimethyl ether as an extraction solvent.

6.6 Expert Panel Evaluation

Callaghan Innovation has concluded that the dimethyl ether, meeting appropriate food-grade specifications, is GRAS for use as an extraction solvent in the processing of various food products, as described in Part 1.3, on the basis of scientific procedures. Callaghan Innovation's conclusion on the GRAS status of dimethyl ether under the conditions of its intended use is based on data generally available in the public domain supporting the safety of the extraction solvent.

A Panel of Experts (the Expert Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients unanimously concluded on the GRAS status of dimethyl ether under conditions of its intended use. The Expert Panel consisted of the following qualified scientific experts: Dr. I. Glenn Sipes (University of Arizona), Dr. David Brusick (Toxicology Consultant), and Dr. Stanley Tarka Jr. (The Tarka Group Inc., and The Pennsylvania State University, College of Medicine).[8]

The Expert Panel, convened by Callaghan Innovation, independently and critically evaluated all data and information presented herein and concluded that dimethyl ether, meeting appropriate food-grade specifications, is safe for use as an extraction solvent in the processing of various food products, as described in Part 1.3, and is GRAS based on scientific procedures. A summary of data and information reviewed by the Expert Panel, and evaluation of such data as it pertains to the proposed GRAS uses of the dimethyl ether is presented in Appendix A.

[8] The panelists participated in their individual capacities. Institutional affiliations are provided for identification purposes only.

6.7 Conclusion

Based on the above data and information presented herein, Callaghan Innovation has concluded that the intended uses of dimethyl ether as an extraction solvent, as described in Part 1.3, are GRAS based on scientific procedures. The GRAS status of dimethyl ether is further supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training to evaluate the safety of food ingredients, who concluded that the intended use of dimethyl ether as an extraction solvent in the processing of food products, as described herein, is GRAS. Furthermore, the GRAS status of dimethyl ether is corroborated through the independent safety evaluations conducted by EFSA and FSANZ who likewise approved the use of dimethyl ether as an extraction solvent.

Dimethyl ether, therefore, may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21, Section 170.3 of the Code of Federal Regulations.

Part 7. §170.255 List of Supporting Data and Information

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Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Dimethyl Ether for Use as an Extraction Solvent in the United States

December 28, 2016

Introduction

Callaghan Innovation ("Callaghan") convened a panel of independent scientists (the "Expert Panel"), qualified by their scientific training and relevant national and international experiences on the safety evaluation of food ingredients, to conduct a critical and comprehensive evaluation of the available data and information on dimethyl ether in order to determine whether its intended conditions of use as an extraction solvent for the processing of various food products would be Generally Recognized as Safe (GRAS) based on scientific procedures.

The Expert Panel consisted of the below-signed qualified scientific experts: Dr. I. Glenn Sipes (University of Arizona), Dr. David Brusick (Toxicology Consultant), and Dr. Stanley Tarka Jr. (The Tarka Group Inc., and The Pennsylvania State University, College of Medicine).

The Expert Panel, independently and collectively, critically evaluated a comprehensive package of scientific information and data compiled from the literature and provided by Callaghan. This information was presented in a dossier titled ["Documentation Supporting the Evaluation of Dimethyl Ether as Generally Recognized as Safe (GRAS) for Use as an Extraction Solvent in the United States" dated December 28, 2016], and which included a review of all publically available scientific data and information, both favorable and unfavorable, relevant to the safety of the intended food uses of Callaghan's dimethyl ether-extracted food products. This information was prepared in part from a comprehensive search of the scientific literature performed through December 2016 and included information characterizing the identity and purity of the solvent, manufacture of the solvent, product specifications, supporting analytical data, intended conditions of use, and estimated exposure under the intended uses. Also reviewed were safety studies characterizing the toxicity of the ingredients that have been previously reviewed by authoritative bodies such as the European Food Safety Authority (EFSA) and Food Standards Australia New Zealand (FSANZ). In addition, the Expert Panel evaluated other information deemed appropriate or necessary.

Following its independent evaluation, the Expert Panel unanimously concluded that the intended uses described herein of dimethyl ether, meeting appropriate food-grade specifications, are GRAS based on scientific procedures. A summary of the basis for the Expert Panel's conclusion is presented below.

Summary and Basis for GRAS

Description

Dimethyl ether is a colorless gas at room temperature, and can be readily liquefied by compression. Dimethyl ether is soluble in methanol, ethanol, isopropyl alcohol, chlorinated hydrocarbons, and toluene. Dimethyl ether is a powerful polar solvent when pressurized and heated as it has the solute-carrying power of liquids, diffusivity between gases and liquids, and similar viscosity to gases. As such, it can be used effectively as a solvent for the extraction of both polar and non-polar lipid components from source materials. In addition, dimethyl ether is an inert compound and therefore can be used to extract lipids without denaturation of residual proteins. Callaghan intends to market various food product ingredients, such as marine lipids, egg phospholipids, bacteria-derived lipids, algae-derived lipids, plant lipids, egg proteins, plant proteins, meat proteins, and fruit sugars that had been prepared via dimethyl ether extraction.

Manufacturing, Specifications and Batch Analyses

Callaghan uses dimethyl ether that is manufactured and supplied by Akzo Nobel. The production process of dimethyl ether is briefly described herein. Dimethyl ether is produced by catalytic dehydration of methanol under high temperature and pressure conditions. Methanol is passed through a heat recovery unit and vaporized in a pre-heater and then reacted in a fixed-bed reactor in the presence of a catalyst to yield dimethyl ether and water. Other dimethyl ether manufacturing process reports the use of a solid-acid catalyst, such as γ-Al_2O_3 (Bai *et al.,* 2013). The reaction products (*i.e.,* methanol, dimethyl ether, and water) are then passed to a dimethyl ether distillation column *via* a heat recovery unit where dimethyl ether is separated from methanol and water. Dimethyl ether is passed through a condenser for storage, whereas methanol and water are passed into a recovery distillation column where they are separated and methanol can be recycled for further processing. The dimethyl ether is stored and transported in food-grade dedicated gas cylinders or stainless steel tanks in a well-ventilated area and kept away from heat, possible ignition sources, oxygen cylinders and other oxidizing materials. Dimethyl ether is analyzed to ensure compliance with the proposed product specifications.

Food-grade specifications have been established for dimethyl ether, and include limits for purity, color, and residual methanol, water, and C1-C4 hydrocarbons. A typical lot of dimethyl ether has a purity ≥99.99%. The product specifications for the dimethyl ether used by Callaghan are consistent with specifications proposed by EFSA and FSANZ following their review of the use of dimethyl ether as an extraction solvent in the preparation of animal protein products, including gelatin, at maximum residual levels of 0.009 mg/kg and in collagen and collagen derivatives, except gelatin, at maximum residual levels of 3 mg/kg, and for use as an extraction solvent in non-dairy and dairy ingredients and products at permitted residual levels of 2 mg/kg (EFSA, 2009, 2015; FSANZ, 2011a,b). Callaghan provided product analysis for 5 non-consecutive

batches of dimethyl ether from their supplier demonstrating that the manufacturing process produces a consistent product in compliance with the established product specifications.

Dimethyl ether is an extremely stable material due to the presence of alpha-hydrogens that protect the compound against oxidation that can cause the formation of peroxides (Naito *et al.,* 2005; Sakuth *et al.,* 2012). When heated up to 353 K (~80°C), dimethyl ether was shown to be chemically stable and did not form any peroxides. Dimethyl ether is not expected to undergo autooxidation. Dimethyl ether, manufactured as described above, is produced in a closed environment, limiting the presence of oxygen and, ultimately, the formation of peroxides. In addition, the solvent is stored in dedicated gas cylinders or tanks in well-ventilated areas away from sources of heat, ignition, and oxidizing compounds. Analytical data on 5 non-consecutive batches of dimethyl ether show the absence of C1-C4 hydrocarbons, methanol, and water, suggesting that dimethyl ether does not degrade or form reaction byproducts.

Intended Food Uses and Proposed Use Levels

Callaghan intends to use dimethyl ether as an extraction solvent in the processing of various food products, such as in the preparation of lipid extracts and defatted (non-lipid) products derived from marine and animal products, plants, fruits, seeds, and micro-organisms. After processing, these food ingredients are intended for use in the production of finished food products. Examples of lipid extract products include marine lipids, egg phospholipids, bacteria-derived lipids, algae-derived lipids, and plant lipids. Examples of defatted (non-lipid) products include egg proteins, plant proteins, meat proteins, and fruit sugars.

The proposed use level of dimethyl ether in the production process is 5 to 6 volumes of dimethyl ether per unit volume of starting material. Dimethyl ether is recycled internally and recovered; as such, the net use of dimethyl ether is approximately 2 to 5% of the amount of the processed food ingredient. The dimethyl ether-extracted food ingredient may then be used in the production of final food products. The final food products contain approximately 0.5 to 10% of a dimethyl ether-extracted ingredient, with some dehydrated or defatted foods potentially containing higher levels of the dimethyl ether-processed ingredients.

Several dimethyl ether-processed products were analyzed for residual levels of dimethyl ether at different time periods (*e.g.,* immediately after processing, 24 hours after processing, after spray drying, or after rotary evaporation of water). Residual levels of dimethyl ether were below the limit of detection (2 ppm).

Information to Establish Safety

Safety Evaluations by Authoritative Bodies

European Food Safety Authority (EFSA)

EFSA reviewed the safety of use of dimethyl ether as an extraction solvent in the processing of animal proteins, specifically collagen, with a proposed residual limit of 9 µg/kg (EFSA, 2009). In that review, it was noted that toxicology studies conducted *via* the oral route were not available, and their conclusions were based on results from inhalation exposure studies. Findings from inhalation toxicology studies were available and considered applicable to the safety evaluation of dimethyl ether through oral exposure as the compound is rapidly absorbed and distributed to the systemic system following inhalation exposure, similar to absorption that may occur from the gastrointestinal tract following ingestion (Kemper and Eckard, 1978; Eckard and Kemper, 1979). Organ distribution of dimethyl ether has been reported to be directly proportional to the concentrations of exposure. Following exposure to concentrations up to 1,000 ppm, equivalent to 1,884 mg/m^3, for 60 minutes, levels of dimethyl ether were detected in muscle (14 mg/kg), lungs (15 mg/kg), liver (15 mg/kg), spleen (16 mg/kg), kidney (17 mg/kg), heart (17 mg/kg), brain (18 mg/kg), blood (19 mg/kg), and fat tissue (22 mg/kg) (Kemper and Eckard, 1978). In another inhalation study by Eckard and Kemper (1979), rats were exposed to dimethyl ether at concentrations up to 2,000 ppm, equivalent to 3,769 mg/m^3, for 180 minutes. In all tissues and organs (blood, heart, lungs, liver, spleen, kidneys, fat, muscle, and brain), levels of dimethyl ether were 16.4±1.4 mg/kg, except for muscles, where concentrations of dimethyl ether did not exceed 8 mg/kg. Steady-state levels of dimethyl ether were reached within 30 minutes of exposure (Kemper and Eckard, 1978; Eckard and Kemper, 1979). The results showed that unchanged dimethyl ether is rapidly excreted *via* exhaled air in a biphasic manner, with levels returning to background levels within 90 minutes (Kemper and Eckard, 1978). The $t_{1/2\alpha}$ and $t_{1/2\beta}$ were reported to be 10 and 90 minutes, respectively. Due to the absence of reactive functional groups, dimethyl ether is not metabolized and is excreted as an unchanged molecule.

The reproductive and developmental toxicity of dimethyl ether was considered in two studies conducted in rats (CIVO, 1981; Haskell, 1981). The study by Haskell (1981) performed in accordance with Good Laboratory Practices (GLP), was considered to be the pivotal study in which EFSA based its conclusions. In this study, Crl:CD(SD)BR rats (27/group) were exposed to dimethyl ether (99.9% purity) in the atmosphere at concentrations of 0, 0.125, 0.5, or 2.0%, equivalent to 0, 1,250, 5,000, or 20,000 ppm or 0, 2,355, 9,421, or 37,685 mg/m^3, respectively, for 6 hours/day on gestational day (GD) 6 to GD 15 (DuPont Co., 1981; Haskell, 1981). Food and water were available *ad libitum* except during the exposure period. Females were mated to mature males of the same strain and mating was verified by detection of spermatozoa in the vaginal lavage following cohabitation. Mated females were housed individually. Body weights and food consumption were measured throughout gestation, and animals were observed daily for clinical signs of toxicity and behavior until termination. At necropsy, dams were examined for

gross pathological changes in tissues and organs, liver and uterine weights, and reproductive parameters. The following reproductive parameters were measured: copora lutea, implantation sites, live and dead fetuses, resorptions, fetal weight, and number and position of all live, dead, and resorbed fetuses. All live and dead fetuses were weighed and sexed externally and internally. Live fetuses were examined for external changes.

The pregnancy ratios were 25/27, 24/27, 27/27, and 25/27 for the 0, 1,250, 5,000, and 20,000 ppm groups, respectively. No significant changes were observed in any treatment group for the following parameters: maternal body weight and organs, gross observations, and reproductive parameters (corpora lutea, implantations, number of resorptions, and total number of dead and live fetuses). Similarly, the number of corpora lutea was 16.7, 16.3, 15.2, and 15.7 for the 0, 1,250, 5,000, and 20,000 ppm groups, respectively. The numbers for implantations were 14.0, 15.3, 14.7, and 14.9, respectively. Significantly decreased fetal body weight and increased incidence of skeletal variation were noted in the 5,000 ppm group. The total number of live fetuses was increased in the 20,000 ppm group compared to the control (14.0 *versus* 13.0), however, the statistical significance was not reported. Based on the results of this study, the authors determined a no-observed-effect level of 1,250 ppm or 2,355 mg/m^3 in rats. Upon review, EFSA calculated an internal dose of 518 mg/kg body weight/day[1] based on the no-observed-effect concentration determined from this study (EFSA, 2015).

No mutagenic or genotoxic effects were observed in a series of GLP-conducted studies including an *in vitro* bacterial reverse mutation assay in *Salmonella typhimurium* (*S. typhimurium*) strains TA98, TA100, TA1535, TA1537, TA1538 and *Escherichia coli* (*E. coli*) WP2, an *in vitro/in vivo* host-mediated assay with *E. coli* K12 and *S. typhimurium* TA1538 in N:NIH mice, and an *in vivo* sex-linked recessive lethal test in *Drosophila melanogaster* at concentrations up to 120,000 ppm (226,110 mg/m^3), 20,000 ppm (37,685 mg/m^3), and 28,000 ppm (52,759 mg/m^3), respectively (CIVO, 1978; RIVM, 1981).

Based on the proposed residual limit of 9 µg/kg in extracted animal proteins, the exposure to dimethyl ether was estimated as 0.18 µg/person/day (0.003 µg/kg body weight/day for a 60 kg individual)[2], providing a margin of safety in the order of 10^8 times lower than the internal dose calculated from the Haskell (1981) study. As such, due to the large margin of safety, the EFSA Panel concluded that dimethyl ether is not of safety concern when used as a solvent at a level of up to 2% of the final food product with a maximum residual level of 9 µg/kg in extracted animal protein (EFSA, 2009).

[1] Internal dose (mg/kg body weight/day) calculated as: (absorption efficiency x inhalation rate x duration of exposure x concentration)/body weight. Where the absorption efficiency is 75%, inhalation rate and mean body weights for rats is 240 mL/minute and 0.25 kg, respectively, and the exposure duration is 6 hours/day (EFSA, 2015).
[2] Exposure estimate calculated as a worst-case scenario where a 60 kg individual consumes 1 kg of meat containing 2% of dimethyl ether-extracted animal protein daily.

EFSA re-evaluated the safety of dimethyl ether as an extraction solvent in the processing of defatted animal protein products under a new maximum residue level (3 mg/kg) and in the processing of animal protein products such as gelatin at a maximum residue level of 9 µg/kg (EFSA, 2015). In addition to their evaluation of previously reviewed safety data, an *in vitro* chromosomal aberration test in human peripheral lymphocytes, an unscheduled DNA synthesis (UDS) assay in rat primary hepatocytes and a HGPRT mutation assay published since EFSA's last review were also evaluated. The results of these studies were consistent with previous negative genotoxicity and mutagenicity results (U.S. EPA, 2000). Based on the absence of a safety concern, the existing maximum residual level of 9 µg/kg for defatted animal protein was increased to 3 mg/kg and the use level was increased from 2.0 to 3.5%. The exposure of dimethyl ether was estimated to be 2.0 µg/kg body weight/day[3] based on the new maximum residue level. A maximum residual limit of 9 µg/kg in gelatin was also proposed, corresponding to an estimated exposure level of 0.014 µg/kg body weight/day[4] for a 60 kg individual. The margin of exposure was calculated to be approximately $2x10^5$, corresponding to a large margin of safety of the use of dimethyl ether as an extraction solvent in the preparation of animal protein materials. EFSA concluded that the proposed residual limits for defatted animal protein products (3 mg/kg) and gelatin (0.009 mg/kg) are of no safety concern.

Food Standards Australia and New Zealand (FSANZ)

FSANZ reviewed a similar unpublished dataset as EFSA that was submitted as part of applications supporting the use of dimethyl ether as a processing aid in the production of dairy and non-dairy food ingredients and products (FSANZ, 2011a,b). Due to the low levels of use and the inherent chemical properties of dimethyl ether (*e.g.*, low boiling point), exposure to dimethyl ether was considered to be negligible due to rapid evaporation of the solvent following processing. Based on the available scientific information, FSANZ concluded that the use of dimethyl ether as an extraction solvent processing aid "*does not pose any public health and safety risk*". As a result, dimethyl ether is permitted for use as a processing aid for all foods, including dairy ingredients and dairy products, with maximum residual levels of 2 mg/kg (FSANZ, 2011a,b).

Published Toxicology Studies

Two published toxicology studies were identified in the literature (Collins *et al.,* 1978; Reuzel *et al.,* 1981) and were included in the summary of data by the U.S. EPA (2000), which was subsequently reviewed by EFSA in their evaluation of the safety of using dimethyl ether as an extraction solvent (EFSA, 2009, 2015). It should be noted that these studies were determined by the U.S. EPA to be "not substantially additive to the database" (U.S. EPA, 2000). In the

[3] Exposure estimated calculated conservatively where 3.5% of the animal protein product (*e.g.,* collagen) is added to food, and a 60 kg individual consumes 1 kg of food per day.
[4] Exposure estimated calculated conservatively where 9% of gelatin is added to food, and a 60 kg individual consumes 1 kg of food per day.

30-week study by Collins *et al.* (1978) whereby Wistar rats were exposed to dimethyl ether in chambers at concentrations of 0, 0.02, 0.2, or 2%, equivalent to internal doses of 0, 96, 960, and 9,197 mg/kg body weight/day, respectively, no significant changes in clinical observations, body weight, food consumption, ophthalmologic examination, hematology, urinalysis, or histopathology were observed in any group. A significant increase in ALT levels was observed in the high-dose group, an increase in AST levels in mid-dose males, and a decrease in relative-to-body liver weight in mid-dose males. No corresponding histopathological finding was noted which might suggest a hepatotoxic effect of dimethyl ether. Similarly, the change in AST level was considered sporadic and not treatment-related as it was observed only in males and at the mid-dose group. Therefore, the true no-observed-adverse-effect level (NOAEL) from this study may be ~960 mg/kg body weight/day rather than the reported 96 mg/kg body weight/day (Collins *et al.,* 1978). In the 13-week inhalation study by Reuzel *et al.* (1981), CPB Wistar rats were exposed to dimethyl ether in chambers containing concentrations of 0, 2,000, 10,000, or 20,000 ppm, equivalent to internal doses of 0, 821, 4,072, or 8,143 mg/kg body weight/day, respectively. No significant changes were observed in any study parameter, and the authors determined a no-observed-adverse-effect concentration (NOAEC) of 20,000 ppm, the highest concentration tested, under the conditions of this study. The results of these studies were consistent with the results of other unpublished toxicology studies on dimethyl ether.

Overall Conclusions Related to Safety

In their review, EFSA and FSANZ considered studies conducted *via* the inhalation route applicable to evaluate the oral safety of dimethyl ether as the absorption and distribution kinetics, including the absence of metabolism and site of contact toxicity, suggest that inhaled dimethyl ether will act similar to ingested dimethyl ether. EFSA determined an internal dose of ~518 mg/kg body weight/day based on the NOAEC from the Haskell (1981) developmental study. In addition, an internal dose of 821 mg/kg body weight/day was determined based on the NOAEC of 2,000 ppm reported in the 2-year GLP toxicity study in rats (DuPont Co., 1986). These doses can be used to determine a margin of safety as they were conducted according to GLP, the study endpoints are sensitive, and the results provide the lowest reported NOAEC. When considering a worst-case scenario where the residual level of dimethyl ether is equivalent to the detection limit of the method of analysis (2 mg/kg), and if the food ingredient was used at 100% of the final food product, a margin of safety[5] of ~1.6×10^4 to 2.5×10^4 can be calculated, suggesting no safety concerns with the proposed use of dimethyl ether as an extraction solvent at the proposed levels of use. It should be noted that this margin of safety is greater as it is expected that the processed food ingredient will be further diluted when used in the processing of final food products.

[5] Margin of safety calculated as (NOAEL/exposure), where exposure is calculated with a maximum residue level of 2 mg/kg, and it is assumed that a 60 kg individual consumes 1 kg of meat per day.

Conclusion

We, the members of the Expert Panel, have independently and collectively critically evaluated the information summarized above and conclude that dimethyl ether, meeting appropriate food-grade specifications, is safe for use as an extraction solvent in the processing of various food products. Based on our review, we the members of the Expert Panel, also unanimously conclude that dimethyl ether meeting appropriate food-grade specifications, is Generally Recognized as Safe (GRAS), based on scientific procedures.

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

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