Application to amend the Australia New Zealand Food Standards Code to permit 2-methyloxolane as a processing aid

Pennakem Europa
France

2022

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INTRODUCTION

This application requests the Australia New Zealand Food Standards Code (the Code) be amended to permit the use of 2-methyloxolane (2-MeOx) as an extraction solvent processing aid in Australia and New Zealand.

Extraction solvents play a critical role in the processing and manufacture of foods, particularly plant-based foods. Extraction solvents are used to extract and separate components of foods, such as oil and protein from oilseeds. Extraction solvents are also used to extract other components, such as flavours, fragrances and colours.

Pennakem Europa (Pennakem) has developed 2-MeOx as a safe, renewable, biomass derived extraction solvent. 2-MeOx is produced from agricultural by-products such as corn stover, sugarcane bagasse and rice straw, presenting an appealing option for food manufacturers looking to use safe, sustainable chemicals in the production of food.

2-MeOx is an alternative to the widely used, petrochemically derived extraction solvent, hexane. Hexane is the predominant chemical extraction solvent used to produce plant-based food products, flavours, fragrances and colours. Hexane is permitted worldwide to be used as an extraction solvent processing aid, including in Australia and New Zealand. When hexane is used in the production of foods, it is permitted to be present as a residue at up to 20 milligrams per kilogram (mg/kg) in final food products. Extraction plants that currently use hexane as an extraction solvent will therefore be producing food products for sale in Australia and New Zealand that are compliant with the maximum permitted level of hexane of 20 mg/kg.

In order for these extraction plants to be able to transition to the use of 2-MeOx as a substitute for hexane, similar maximum permitted levels will initially be required for foods containing residues of 2-MeOx. That is, a maximum permitted level of 20 mg/kg for 2-MeOx in foods will facilitate the extraction plants transitioning from using hexane to using 2-MeOx as an extraction solvent. It is likely that actual 2-MeOx residue levels in food will be below 20 mg/kg, particularly as uptake in the use of 2-MeOx increases and due to organoleptic reasons described in this application.

Extraction plants that are purpose built to use 2-MeOx as an extraction solvent will result in lower residue levels of 2-MeOx in final food products. However, the cost of constructing a purpose built 2-MeOx extraction plant may initially be difficult to justify for food producers in Australia and New Zealand.

The European Food Safety Authority has recently published a positive opinion on the safety of use of 2-MeOx as an extraction solvent in the European Union.

Pennakem has provided information to support the application in accordance with the requirements of sections 3.1 (General requirements) and 3.3.2 (Processing aids) of the FSANZ Application Handbook.

3.1.1 GENERAL REQUIREMENTS

B Applicant Details

Applicant	Pennakem Europa
Contact	[redacted]
Address	224, avenue de la Dordogne 59640 DUNKERQUE FRANCE
Phone	[redacted]
Email	[redacted]
Nature of applicant's business	Pennakem Europa is a subsidiary of the Minafin Group, which specialises in the production and sales of biobased products derived from furfural and active pharmaceutical ingredients
Details of consultants associated with the application	[redacted]
Primary contact for application	[redacted]

C Purpose of the application

The application requests amendment to section S18—8 of the Australia New Zealand Food Standards Code (the Code) to permit the use of 2-methyloxolane (2-MeOx) as an extraction solvent processing aid in Australia and New Zealand. Pennakem manufactures and markets 2-MeOx under the tradename EcoXtract®.

D Justification for the application

(a) Need for the proposed change

Section 1.1.1—10(6)(c) of the Code prohibits the use of processing aids unless expressly permitted. Section 1.3.3—10 permits substances listed in section S18—8 to be used as a processing aid to perform the technological purpose of an extraction solvent. 2-MeOx is not listed in section S18—8 and is therefore not permitted to be used as an extraction solvent processing aid. Therefore, section S18—8 requires amendment to list 2-MeOx as a permitted extraction solvent processing aid in Australia and New Zealand.

(b) Advantages over status quo

2-MeOx is bio-based, manufactured from by-products of sugar production, and provides an environmentally sustainable alternative to other permitted extraction solvents, many of which are petrochemically derived. Pennakem considers that reducing reliance on fossil fuel products, including petrochemically derived chemicals used in food production, is important for the environment. 2-MeOx provides an effective bio-based alternative to commonly used extraction solvents. 2-MeOx is fully miscible with lipids, making it particularly well adapted for vegetable oil

extraction and other defatting processes, which is a property not often associated with bio-based solvents.

2-MeOx is safe for use as an extraction solvent processing aid at the intended use levels proposed in this application. Pennakem has established a strong evidence base to support the safe use of 2-MeOx as an extraction solvent in food production. The full data package has been supplied with this application. The European Food Safety Authority (EFSA) has assessed the same data package and concluded that the use of 2-MeOx at the intended use levels proposed by Pennakem is safe, establishing a Tolerable Daily Intake (TDI) of 1 mg/kg body weight/day (EFSA 2022). The data package includes toxicological studies on rats (oral administration) compliant with the latest OECD standard (2017) which takes into account an endocrine disruption assessment. In that context, this data package offers an unprecedented level of safety for consumer protection. Some permitted extraction solvents in section S18—8 of the Code have not been subject to extensive safety assessments in some time and/or do not have an extensive amount of quality safety data, particularly relating to oral exposure resulting from residue levels present in foods.

FSANZ recognised this in its assessment of Proposal P277 in 2006, noting that hexane, for example, has very limited animal and human data in relation to metabolism and dietary exposure; insufficient to establish a safe level of exposure (such as an acceptable daily intake). The residues of these chemicals in processed foods, resulting from use as processing aids, has been considered insufficient to warrant any toxicological concern.

However, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) has conducted assessments of hexane and 2-MeOx and classified 2-MeOx in a lower toxicity classification than hexane in the context of use as solvents in the preparation of pharmaceutical products. 2-MeOx is classified as a class 3 solvent, meaning 2-MeOx was considered to have low toxic potential. However, hexane is classified as a class 2 solvent, meaning its use should be limited (class 2 solvents are recommended to be limited in their use due to inherent toxicity), with a Permitted Daily Exposure (PDE) for humans 17 times lower than 2-MeOx. More information on the ICH classifications is included in section B.5.

D.1 Regulatory impact information

D.1.1 Costs and benefits of the application

a) Consumers

Although consumers may not be aware of which processing aids are used in food products (because processing aids generally do not require labelling), consumers will benefit from having access to foods produced using environmentally sustainable inputs, such as 2-MeOx. Pennakem is investigating organic certification in the EU relating to the use of 2-MeOx in the production of food and will also do so in Australia and New Zealand. This process is independent of the FSANZ application process. However, if organic status is considered appropriate for foods produced using 2-MeOx, consumers targeting certified organic foods may benefit from a greater range of foods than currently produced using other extraction solvents.

Another advantage of 2-MeOx for consumers is its safety. 2-MeOx has a recently established, comprehensive body of evidence relating to safety, which as noted above, is not always the case for some alternative extraction solvent processing aids. EFSA has recently provided a positive opinion regarding the safe use of 2-MeOx as a processing aid at the intended levels of use proposed in this application (EFSA 2022). Following EFSA's positive opinion, the European

Commission is preparing a modification of the law (Directive 2009/32/CE) to formally approve the use of 2-MeOx as a processing aid in the European Union early in 2023. The approval of 2-MeOx as a permitted extraction solvent processing aid in Australia and New Zealand will mirror the pending approval in the EU, allowing consumers to have access to food products imported from the EU that have used 2-MeOx during processing.

Products that are manufactured using 2-MeOx may initially be more expensive for consumers than products produced using hexane. Pennakem estimates an additional 10% for the price of oil and meal produced using 2-MeOx compared to oil produced using established extraction solvents, such as hexane. This extra cost will give a payback for the investment the producer will need to do to switch from other extraction solvents to 2-MeOx. It will also cover the extra cost related to the implementation of a new process compared to hexane process, for example, which has been optimised for 70 years. Once the introductory phase is complete, the process cost will be only 2 to 3% above the cost of hexane extraction. However, the use of 2-MeOx will be less expensive than mechanical pressing of oilseeds. Where 2-MeOx is used as an alternative to mechanical pressing, oil prices are likely to be reduced for consumers.

b) Industry

2-MeOx presents an attractive extraction solvent option for producers of plant-based foods and food ingredients, such as oils and particularly plant proteins. The plant protein market has grown considerably in recent years as consumers look for more sustainable and ethical alternatives to animal-based proteins. 2-MeOx is equally effective at extracting oils and proteins from plant-based sources as the commonly used, petrochemically sourced extraction solvent, hexane. 2-MeOx is produced from agricultural by-products, a much more sustainable source than hexane and can assist producers in communicating to consumers the sustainability of foods produced using 2-MeOx. In term of greenhouses gas impact, an average petrochemical solvent has an impact of 2 kg CO_2/kg solvent. The 2-MeOx, thanks to his biobased by-product raw materials has an impact of 0.2 kg CO_2/kg solvent (Slater et al. 2016).

2-MeOx also presents a safer alternative to hexane in the context of workers exposure to chemicals in production plants that use extraction solvents. The safety profile of 2-MeOx is significantly more favourable than hexane, which is increasingly associated with adverse health effects in workers (exposed to hexane). Every year in the world, workers exposed to hexane still develop occupational diseases such as polyneuropathies. 2-MeOx is not neurotoxic and generally not toxic by inhalation. It has a much lower odor threshold than hexane and therefore allows for easy detection of any leakage and for adequate corrective action.

2-MeOx can be used for large scale processes and can substitute hexane in existing plants with limited impact on the cost of extraction. The implementation of 2-MeOx in hexane plants will reduce the environmental impact of said plants. The typical consumption of a hexane plant is around 200 T/year to compensate for losses in the food chain (50 T/year) and in the atmosphere (150 T/year). The substitution of hexane by 2-MeOx will eliminate the release of a neurotoxic volatile and the transfer of fossil carbon from the ground to the air.

c) Government

There should be limited cost impact on government food enforcement agencies if 2-MeOx is permitted to be voluntarily used as an extraction solvent processing aid in the production of food in Australia and New Zealand.

The substitution of hexane with2-MeOx could on the other hand have a positive impact on public health costs because several peer reviewed papers connect hexane (and its toxic metabolite, the 2,5-hexanedione) exposure to cryptogenic polyneuropathies, Parkinson disease, fertility issues for male and female subjects, neuro development issues on pups and endocrine disruption (Salamon et al. 2019, Ruiz-Garcia et al. 2020, EPA 2005).

D.1.2 Impact on international trade

This application is part of a coordinated process of Pennakem seeking approval for the use of 2-MeOx as a processing aid in international jurisdictions, including the European Union and the US. Permission in the Code to use 2-MeOx as a processing aid will facilitate international trade among jurisdictions in which 2-MeOx is already or soon to be permitted.

E Information to support the application

The application contains supporting information in accordance with the Application Handbook's requirements in Guideline 3.3.2 – Processing aids. Safety studies conducted in accordance with OECD guidelines have been conducted by Pennakem. In addition, a literature search for relevant publications was performed using various sites including: toxnet, PubMed, ECHA, EFSA, FDA, CIR and google scholar. Literature references were carefully reviewed for their robustness and compliance with guidelines for data endpoints.

2-MeOx has been evaluated by the European Food Safety Authority's (EFSA's) Scientific Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) with a positive opinion on the safety of 2-MeOx as an extraction solvent being released in March 2022. 2-MeOx has also been evaluated under EC (No) 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). The data from these evaluation forms the basis of this application.

F Assessment procedure

Pennakem considers the application should be assessed under the general procedure, level 1.

G Confidential commercial information (CCI)

The application contains confidential commercial information (CCI) relating to the manufacturing process, and some technical and toxicological studies and methods related to the product. This information is of commercial value to Pennakem and has not been publicly released to date. Public release of this information can reasonably be expected to diminish the commercial value of this information to Pennakem. Non-confidential summaries of this CCI information are provided in the application and the CCI information has been provided separately to FSANZ.

H Other confidential information

No other confidential information is included in this application.

I Exclusive capturable commercial benefit (ECCB)

Permission in Schedule 18 of the Code to use 2-MeOx as an extraction solvent processing aid is not expected to confer an exclusive capturable commercial benefit to Pennakem. The Application Handbook includes a number of factors to assist in considering whether an ECCB is likely to be conferred. Pennakem has addressed these factors below.

Question: Why are you making this application? What are you hoping to get out its approval?
Response: Pennakem is making this application to seek permission in the Australia New Zealand Food Standards Code to use 2-MeOx as a solvent processing aid in Australia and New Zealand.

Question: How will you benefit from the approval of your application?
Response: Pennakem will benefit from selling 2-MeOx and licences related to the 2-MeOx extraction technology to food producers in Australia and New Zealand. Pennakem may also benefit from establishing facilities in Australia and/or New Zealand to produce 2-MeOx locally, rather than importing the processing aid from international production facilities.

Question: Who besides you, will benefit from the approval of your application? How and why will they benefit?
Response: Food producers seeking a 'greener' alternative to hexane will benefit from the availability and effectiveness of 2-MeOx. Producers of industrial chemicals may benefit if they can develop processes to produce 2-MeOx in accordance with approved specifications (in the Code or in reference sources listed in the Code). Hexane is not only used in extraction but also for instance in oil fractioning. If 2-MeOx is approved, food producers will also be able to use 2-MeOx that complies with approved specifications.

Question: If your application is approved, whose permission will be required before anyone can derive a benefit from that approval?
Response: Other parties can manufacture 2-MeOx and can develop processes to use 2-MeOx in the manufacture and processing of food products. Other parties may require permission from Pennakem if intending to use 2-MeOx in the patented context described in the next response.

Question: Who holds the intellectual property in the subject matter of your application?
Response: Pennakem has submitted a patent (WO 2020/128307) in Australia for the extraction of oils rich in polyphenols from a biological substrate, however other food preparation processes could use 2-MeOx and are not part of this patent application.

J International and other standards

J.1 International standards

J.1.1. International Food standards
There are no international standards for processing aids. However, individual jurisdictions have relevant requirements which are outlined below in section J.2.2.

J.1.2 Other international standards

2-MeOx (CAS 96-47-9) under the name 2-methyltetrahydrofuran has been used for more than one decade in pharmaceutical applications. 2-MeOx was officially included by the pharmaceutical experts of the ICH Q3C working group in the low toxicity solvent list in 2021 (ICH Q3C R8 - 2021).

J.2 Other national standards or regulations

J.2.1 Australia and New Zealand

There are no relevant standards in Australia and New Zealand, other than the Code requirements for processing aids identified above.

J.2.2 International

United States

The approval of the 2-methyloxolane for food and feed application is ongoing in the USA. The first contacts with the FDA started in 2019 to define the regulatory path and additional toxicological studies needed. The path is clarified. Pennakem will apply for a Food Contact Substance for food application and for a Feed Additive Petition for the feed application.
The additional studies needed (tests on cow and hen, environmental assessment) were completed in 2020 and 2021. The final reports are pending. The filing is planned for later in 2022. Once filled the Food Contact Substance approval will only take 4 months to be granted. The Food Additive Petition will require 12 months for completion of the assessment.

European Union

Pennakem has submitted an application to the European Union (EU) for assessment of 2-MeOx as a processing aid. The European Food Safety Authority's (EFSA's) Scientific Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) released a positive opinion on the safety of 2-MeOx as an extraction solvent and its maximum residue limits in March 2022. The positive opinion will support the modification of the European law, the Directive 2009/32/CE in the coming 6 months.

Other

Other food and feed approval will be launched in the coming months in other countries.

K Statutory Declaration

To be completed for final version of application.

3.3.2 PROCESSING AID REQUIREMENTS

A Technical information on the processing aid

A.1 Type of processing aid

2-MeOx is a chemical processing aid that functions as an extraction solvent. Extraction solvents are used in a variety of contexts in the production of food ingredients and foods. 2-MeOx is intended to be used to extract and separate oils and proteins from plant-based products, including oilseeds; and to extract other components including flavours, fragrances and colours.

The Code lists permitted extraction solvents in section S18—8. 2-MeOx is suitable for use as an alternative extraction solvent to hexane. Hexane is listed in section S18—8 and is permitted to be used as an extraction solvent in all foods (with a maximum permitted residue level in foods of 20 mg/kg).

A.1.1 Evidence of technological function of 2-MeOx as an extraction solvent

Pennakem has commissioned studies to assess the efficiency of oil and protein extraction and the quality of the resulting product. The quality of the extracted product has been assessed against product extracted by hexane, which is the current standard solvent used for these types of extractions. Key tests and reports are summarised below with additional detail provided in Appendix C. Additional information regarding the efficacy of 2-MeOx as an extraction solvent can be found in the scientific review by Rapinel et al. (2020).

A.1.1.1 Oil extraction

Pennakem commissioned a study to compare extraction and refining of soybean and rapeseed oils using hexane and 2-MeOx (OLEAD 2019 - CCI)). A summary of the study if included below. This study focused on comparing the influence of the solvents on the efficiency of the oil extraction and refining process. Experiments were carried out on two seeds: soybean and rapeseed. Extraction allowed producing four meals: two from whole rapeseed and two from soybean kernels extracted either by hexane or 2-MeOx.

The analysis of meals showed that 2-MeOx extracted slightly more oil than hexane. But these solvents did not significantly modify the protein content (on de-oiled dry matter), the protein solubility, or the glucosinolate content of meals. Oils were distillated, decanted and filtrated before refining. For both seeds, the crude oil extracted by 2-MeOx was darker and slightly more acidic than the one extracted by hexane.

Crude rapeseed oil was prepared by mixing oils from mechanical extraction and from solvent extraction while crude soybean oil was produced only by solvent extraction. Refining operations were similarly conducted for rapeseed and soybean oils. The refining was performed according to the classical alkaline refining method. It allowed reaching a similar oil quality independently of the solvent nature. Oil quality and losses appeared more dependent on the seed type than on the solvent choice.

A.1.1.2 Protein extraction

Pennakem commissioned a study to compare protein extraction and characterization from de-oiled soy meal by hexane and 2-MeOx (Improve 2019 - CCI).

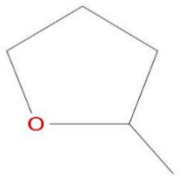
This project compared the 2-MeOx (2-MeTHF) and hexane de-oiled soybean cakes for the following quantitative parameters:

- Comparison of the two de-oiled soy cakes in terms of solubility profiles and Boisen's method digestibility;
- Comparison of the two de-oiled soy cakes in terms of protein extraction by isoelectric precipitation technic;
- Comparison of the two soy protein isolates in terms of technological properties.

The project concluded that the two protein isolates have very high purities, with a higher protein content for the 2-MeOx defatted isolate. Defatting with 2-MeOx does not significantly affect the viscosity of the isolate in solution and the sample retains very good gelling properties. The protein isolate defatted with 2-MeOx keeps correct emulsifying properties, even if they are lower than those obtained after defatting with hexane. Foaming properties are also lower for the 2-MeOx protein isolate. The 2-MeOx protein isolate has a higher water and oil retention capacity than the sample defatted with hexane.

A.2 Identity of the processing aid

Pennakem uses the common name of 2-methyloxolane to describe the processing aid in the context of its regulatory approvals and is abbreviated to 2-MeOx. Pennakem's proprietary name for 2-MeOx is EcoXtract® Food Grade (or EcoXtract®). In some of the data and reports presented in this application, 2-MeOx is referred to by its EC name of Tetrahydro-2-methylfuran, or as the abbreviation 2-MeTHF. However, Pennakem proposes the common name 2-methyloxolane and abbreviation, 2-MeOx be used in future when referring to the processing aid. Additional detail on the identity of 2-MeOx is included below.

EC number:	202-507-4
EC name:	Tetrahydro-2-methylfuran
CAS number (EC inventory):	96-47-9
IUPAC name:	2-methyloxolane
Abbreviation	2-MeOx, 2-MeTHF
Molecular formula:	$C_5H_{10}O$
Molecular weight range:	86.13 g mol^{-1}
Structural formula	
Synonyms/other names	Tetrahydrosylvan Furan, 2-methyl-tetrahydro Tetrahydro-2-methylfuran 2-MethyltetrahydrofuranTetrahydrofuran, 2-methyl 2-Methylfuranidine

A.3 Chemical and physical properties of the processing aid

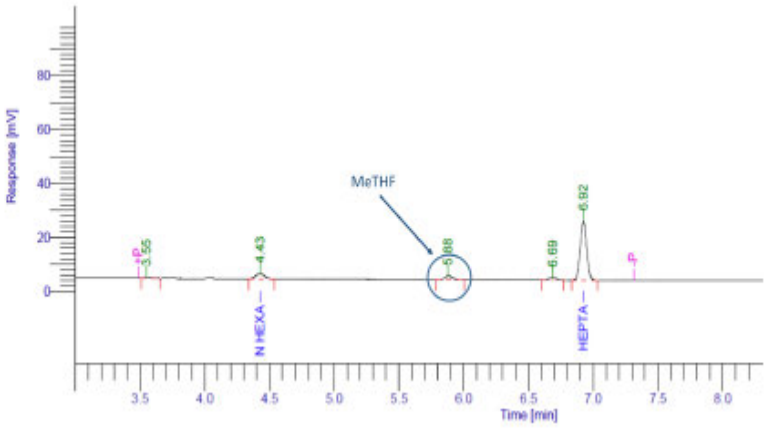
Table A.3-1 gives an overview of the physicochemical properties of the substance in its pure form and the evaluation undertaken for each endpoint. 2-MeOx is a liquid at standard temperature and pressure, with a measured melting point of <-20°C and a measured boiling point of 78°C. It has a measured relative density of 0.855 at 20°C and measured kinematic viscosities of 0.576 mm^2/s and 0.484 mm^2/s at 20°C and 40°C respectively. The substance has a vapour pressure of 102 mmHg at 20°C.

The substance is classified for flammability as a highly flammable liquid in accordance with EC Regulation 1272/2008 on the basis of a measured flash point of -10°C and a measured boiling point of 78°C. It has a measured auto-ignition temperature of 260°C. The substance is not oxidising and neither explosive on the basis of structural examination.

Based on structural examination, 2-MeOx is not susceptible to hydrolytic degradation. It has a predicted log K_{ow} of 1.85 at 25°C, and a reported water solubility of 140 g/l. The substance is not surface active based on structural examination.

A full summary of each study listed in Table A.3-1 is provided in Appendix D.

Table A.3-1. Summary of Physio-chemical properties of 2-MeOx

Appearance and Physical state	Clear colourless liquid at 20°C and 101.3 kPa (Tarran 2012) 2-MeOx is a liquid under standard conditions of temperature and pressure with a freezing point of <-20°C and boiling point of 78°C.
Chromatography	Gas Chromatography: 2-MeOx elutes after 5.88 minutes 
Relative density	Relative density 0.8552 at 20°C CRC Handbook of Chemistry and Physics (Haynes 2017) A relative density of 0.8552 at 20°C was reported for the substance in a handbook or collection of reliable data which has been subject to peer review and in which the original sources are traceable
Refractive index	Not applicable
pH	7.1 +/- 0.2 (Rapinel 2019)
precipitation reaction	Not applicable
colour reaction	Not applicable
Melting / freezing point	Melting point <-20°C (<253 K) (EU Method A.1) (Tarran 2012) A measured freeing point of <-20°C (<253 K) was determined for the substance in accordance with EU Method A.1 and in compliance with GLP.
Viscosity	0.576 mm2/s at 20°C and 0.484 mm2/s at 40°C (OECD 114) (Tarran 2012) Measured kinematic viscosity values of 0.576 mm^2/s at 20°C and 0.484 mm^2/s at 40°C were determined for the substance in accordance with OECD 114 and in compliance with GLP.
Water solubility	140000 mg/l Organic Solvents. CRC Handbook of Chemistry and Physics (Haynes 2017) A water solubility of 1.4E+05 mg/l was reported for the substance in a collection of reliable data which has been subject to peer review and in which the original sources are traceable.
Partition coefficient n-octanol/water (log value)	at 25°C A log K_{ow} of 1.85 was reported for the substance in a collection of reliable data which has been subject to peer review and in which the original sources are traceable. (CRC Handbook of Chemistry and Physics: Haynes 2017)
Boiling point	78°C at 101.3 kPa CRC Handbook of Chemistry and Physics (Haynes 2017)

	A boiling point of 78°C was reported for the substance in a handbook or collection of reliable data which has been subject to peer review and in which the original sources are traceable.
Vapour pressure	In the 2-MeOx product data brochure (Pennakem 2016) Temperature Vapour Pressure (mmHg) -15 °C ..... 18 0 °C .....38 20 °C ..... 102 50 °C ..... 366 CRC Handbook of Chemistry and Physics (Haynes 2017) Temperature Vapour Pressure (mmHg) -20 °C ..... 7.50 19.7 °C .....75.00 79.8 °C 750.06
Flash point	Flash point -10 ± 2°C (Method A9 EC No 440/2008) (White 2013) A measured flash point of -10 ± 2°C was determined for the substance in accordance with ISO 3679 and in compliance with GLP. A flash point of -10°C and a boiling point of 78°C, indicate that the substance should be classified as a highly flammable liquid according to the criteria of EC Regulation 1272/2008.
Autoflammability / self-ignition temperature	Auto flammability 260±5°C (Method A15 EC 440/2008) (White 2013) An auto ignition temperature of 260±5°C at 99.5 - 100.9 kPa was determined for the substance in accordance with EU Method A.15 and in compliance with GLP.
Flammability	Highly flammable. The flammability of liquids is assessed on the basis of flash point. There is no indication on the basis of structure and experience in handling and use that the substance is pyrophoric (flammable in contact with air) or flammable in contact with water.
Explosive properties	Not explosive The molecule has no chemical groups that are associated with explosive properties.
Oxidising properties	Not oxidizing On the basis of structure the substance is expected to be incapable of reacting exothermically with combustible materials

A.3.1 Stability

The 2-MeOx remains stable in a wide temperature and pH range (1 to 14). Like ethers or edible oils, the unstabilised 2-MeOx reacts readily with oxygen, for example on contact with air, to form hydroperoxides (Figure A.3.1-1, Figure A.3.1-2), high boiling point organic acids. The kinetic energy is quite low: it takes at least 4 days for non-stabilised dry 2-MeOx, stirred and exposed to air, to reach detectable levels of peroxides (10 parts per million (ppm) H_2O_2 Eq.).

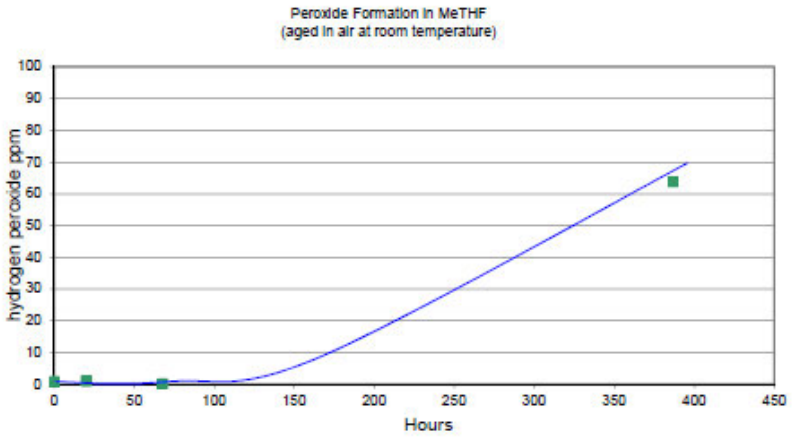


Figure A.3.1-1: Peroxide Formation in 2-MeOx when stored at room temperature with exposure to the air. From Pennakem (2016) Product Data and Handling Guidelines

Pennakem uses an iodine titration method to measure peroxide levels. Details of the method have been provided to FSANZ however the method is commercial in confidence to Pennakem (TA-002-04 - CCI). Peroxides are unstable compounds and decompose continuously into Gamma-valerolactone and 5-Hydroxy-2-pentanone. After a while a dynamic equilibrium is established between reformation and decomposition of the peroxides.

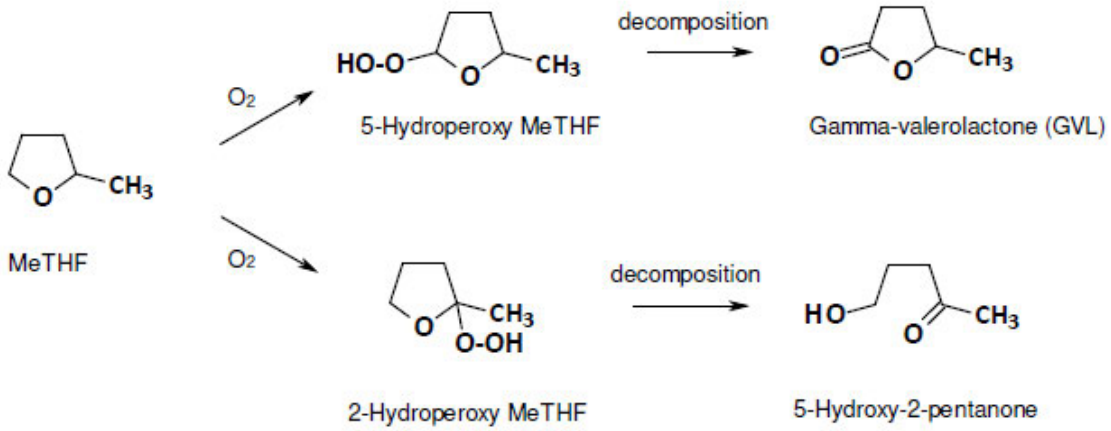


Figure A.3.1-2 the formation of hydroperoxides in contact with O_2

The Gamma-valerolactone (GVL), is a flavour (FEMA[1] GRAS Number 3103) which tastes like herb. The 5-Hydroxy-2-pentanone is not FEMA registered but its close cousin, 3-Hydroxy-2-pentanone (FEMA 3550) tastes like Herb and Truffle. The formation of hydroperoxides can be largely inhibited if the product is in contact with certain stabilizers. Typical stabilizers are BHT (3,5-di-ter-butyl-4-hydroxytoluene), tocopherol (150 ppm) or water (3 to 4 %). To prevent the peroxides development, Pennakem adds 300 ppm of BHT or 150 ppm Tocopherol to its product and packages them under nitrogen. To avoid the risk of peroxide formation Pennakem instructs users to test any aged product for peroxide content and to re-stabilize the product prior to storage.

All the considerations above are related to the use of the 2-MeOx for any application. They fully apply for the use of the 2-MeOx as extraction solvent for food in batch processes. The continuous process used for the oil and protein extraction is particularly well designed to prevent the development of peroxides in the 2-MeOx.

- The presence of natural water and antioxidants (natural polyphenols and tocopherols) in the seeds inhibits the formation of peroxides.

- The solvent water blend/mix is separated from the extract by distillation every two to four hours for re-use in subsequent extractions. The peroxides have no time to reach concentration above 100 ppm in the solvent (more in the application part). Any trace peroxide potentially formed remains with the extract phase and is then removed by subsequent refining operations. In this type of operation, peroxides cannot accumulate in 2-MeOx.

- In this type of process, 2-MeOx is not dried. It is never subject to distillation near dryness.

- For this type of process, in the case of a plant shut down or solvent storage of more than 3 or 4 days, re-stabilization by addition of 150 ppm Tocopherol or storage under nitrogen only is recommended.

- If the product has not been re-stabilized or stored under nitrogen, it is recommended to check peroxide content before reuse and, if that content is more than 100 ppm, it is recommended to add edible oil to the solvent and purify the solvent by distillation. The peroxide will concentrate in the edible oil and can be destroyed later (VR 0023 – peroxides destruction).

- A test was carried out at Dekra (Chilworth) in 2020 to check the safety of 2-MeOx with high peroxide concentration. Dekra exposed the 2-MeOx to air and reached 2000 ppm peroxide. Then they distillated the solvent to reach 5595 ppm peroxides. The DSC result on that 2-MeOx shows that the peroxides degrade at 70°C and that even with 0,5% peroxide, the 2-MeOx never present any safety risk for the user because the maximum heat of decomposition 48,71 J/g remain well below 300 J/g (the limit for the screening of explosive properties) and it is negative to the choc test. The reports from both tests have been provided to FSANZ however these are commercial in confidence to Pennakem (Dekra (2020) DE27587AR and Dekra (2020) DE27587RP - CCI).

In any case, even if the 2-MeOx is not used or stored under nitrogen, storage conditions and use must comply with ATEX environment as it is flammable.

[1] FEMA = Flavor and Extract Manufacturers Association of the United States

The oil extraction industry understands how to deal with peroxides, prevent their development or remove them as part of the oil refining process because peroxides naturally occur in the oils. For instance, olive oil typically should have less than 10 mEqO2/kg oil which is equivalent to less than 5 mmol/kg or less than 160 ppm peroxides (in active oxygen). Stability data for 2-MeOx stabilised with 275 ppm butylated hydroxytoluene (BHT) following storage for 1 year (ambient temperature) and for 2 years (constant temperature) under nitrogen showed no deterioration or increase in peroxide. Similarly, 2-MeOx stabilised with tocopherol for one year showed no deterioration or increase in peroxide.

The data clearly demonstrates that with an appropriate stabiliser there is no degradation of 2-MeOx. For the product EcoXtract® Food Grade, tocopherol is the preferred solution as the stabiliser.

The studies are summarised below.

A.3.1.1 One year stability study (ambient temperature 0-350°C)

Study title: Stability of 2-MeOx
Study Reference: Pennakem internal report
Author: Jackson J
Date: 08/01/2019
GLP: no
Substance: 2-MeOx with 275 ppm butylated hydroxytoluene or with 150 ppm tocopherol
Container: Glass under Nitrogen and Carbon Steel under nitrogen
Temperature: Outside ambient (0 – 35^{0}C)
Duration: 1 year
Method of Analysis: Gas Chromatography

Methods:
The tests were conducted to to determine the stability of 2-MeOx stored at ambient temperature in glass and glass with carbon steel. Studies were performed on both tocopherol and butylated hydroxytoluene (BHT) inhibited materials. Four samples of 2-MeOx were stored under nitrogen in 1 quart glass jars at 20 - 25°C for 54 weeks. The assay of each sample was monitored over the course of storage using methods to detect 2-MeOx (TA-002-01 – CCI), BHT (TA-002-03 – CCI) and peroxide (TA-002-04 – CCI). Each of these methods has been provided to FSANZ however are commercial in confidence to Pennakem.

Table A.3.1.1-1. Storage conditions and sample details for 1 year stability study

Sample ID	Container	Carbon Steel Added	Nitrogen Inerted	Inhibitor
FJ-020-94	1 Quart Glass Jar	No	Yes	BHT
FJ-020-95	1 Quart Glass Jar	Yes (0.25 g)	Yes	BHT
FJ-027-23	1 Quart Glass Jar	No	Yes	Tocopherol
FJ-027-24	1 Quart Glass Jar	Yes (0.25 g)	Yes	Tocopherol

Results:

Table A.3.1.1-2. One Year Storage under nitrogen - % 2-MeOx

Compound	Stored in		0 wk	2 wk	4 wk	6 wk	8 wk	10 wk	14 wk
2-MeOx +276 ppm BHT	glass (ID: FJ-020-94)	GC % assay	99.969	99.962	99.952	99.956	99.939	99.968	99.94
		peroxide (ppm)	0		0				
	carbon steel (ID: FJ-020-95)	GC % assay	99.969	99.964	99.959	99.94	99.939	99.957	99.94
		peroxide (ppm)	0		0				
Compound	Stored in		16 wk	18 wk	20 wk	22 wk	24 wk	26 wk	28 wk
2-MeOx +276 ppm BHT	glass (ID: FJ-020-94)	GC % assay	99.939	99.966	99.937	99.965	99.965	99.963	99.96
		peroxide (ppm)	0		0		0		
	carbon steel (ID: FJ-020-95)	GC % assay	99.939	99.937	99.937	99.942	99.943	99.962	99.929
		peroxide (ppm)	0		0		0		
Compound	Stored in		30 wk	32 wk	34 wk	36 wk	40 wk	42 wk	44 wk
2-MeOx +276 ppm BHT	glass (ID: FJ-020-94)	GC % assay	99.96	99.966	99.946	99.956	99.95	99.937	99.958
		peroxide (ppm)		0		0		0	
	carbon steel (ID: FJ-020-95)	GC % assay	99.962	99.966	99.957	99.959	99.958	99.96	99.961
		peroxide (ppm)		0		0		0	
Compound	Stored in		46 wk	48 wk	52 wk	54 wk		54 wk	
2-MeOx +276 ppm BHT	glass ID: FJ-020-94)	GC % assay	99.955	99.959	99.958	99.949	BHT	249	
		peroxide (ppm)	0		0	0			
	carbon steel (ID: FJ-020-95)	GC % assay	99.96	99.946	99.958	99.955	BHT	250	
		peroxide (ppm)	0			0			
Compound	Stored in		0 wk	12 wk	24 wk	36 wk	54 wk		
2-MeOx +Tocoherol	glass (ID: FJ-027-23)	GC % assay	99.937	99.957	99.942	99.929	99.921		
		peroxide (ppm)	0	0	0	0	0		
	carbon steel (ID: FJ-027-24)	GC % assay	99.937	99.959	99.943	99.93	99.922		
		peroxide (ppm)	0	0	0	0	0		

Conclusion: 2-MeOx inhibited with 275 ppm butylated hydroxytoluene or with 150 ppm tocopherol remains stable at ambient temperature over the period of 1 year when stored in glass or carbon steel.

A.3.1.2 Two-year stability data

Study title: Stability of 2-MeOx
Study Reference: Pennakem internal report
Author: Jackson J
Date: 08/01/2019
GLP:
Substance: 2-MeOx with 150 - 300 ppm butylated hydroxytoluene
Container: Glass under Nitrogen
Temperature: Constant 23^0C
Duration: 2 years, single analysis point
Method of Analysis: Gas Chromatography

Results:

Table A.3.1.2-1. Two Year Storage under nitrogen at 23^0C in glass

Lot no.	original analysis date	% assay	peroxide (ppm)	Reanalysis date	% assay	peroxide (ppm)
2-6A08	Jan. 2016	99.98	0	Mar. 2018	99.97	0
2-6B12	Feb. 2016	99.95	0	Mar. 2018	99.95	0
2-6B26A	Feb. 2016	99.99	0	Mar. 2018	99.98	0
2-6B29	Feb. 2016	99.98	0	Mar. 2018	99.93	0
2-6C23	Mar. 2016	99.95	0	Mar. 2018	99.96	0

Conclusion: 2-MeOx with 250 - 300 ppm butylated hydroxytoluene remains stable at 23˚C temperature over the period of 2 year when stored in glass.

A.3.1.3 Recovery of 2-MeOx

The 2-MeOx can be recovered easily from off-gas streams and miscellas, making it suitable for closed-loop processes designed to save resources and protect the environment. The 2-MeOx has 6 to 20 % solubility in water, depending on water temperature and is therefore compatible with a water scrubber. In case of distillation with water, 2-MeOx forms an azeotrope (10.6 % water). In the condensed azeotrope, the water concentration can be reduced to 4 to 4,5% (depending on the temperature) by simple decantation. The 2-MeOx can be used either in wet (with water > 300 ppm) or dry form for the extraction.

In case of long-term storage of the 2-MeOx between two distillations (> 70 hours) and without contact with natural raw material (which naturally brings antioxidants), or nitrogen blanket, 150 ppm tocopherol should be added to the product within the first 50 hours of storage to prevent the formation of peroxides.

Information on the packaging, transport and storage of 2-MeOx is provided in Appendix E. This information is provided to all Pennakem customers using 2-MeOx and is taken from Pennakem's Product Data and Handling Guidelines (Pennakem 2016 - CCI).

A.3.1.4 Preventing peroxide formation

As discussed in section A.3.1, the 2-MeOx stabilized with BHT or tocopherol can be stored for up to 2 years without significant peroxide formation. To prevent the peroxide development and always keep a high purity product, some simple measures can be taken.

1. Keep the product in its original package as long as you do not need it. Pennakem deliver a stabilized product in Nitrogen blanketed drum with 2 years proven shelf life
2. In case of storage for more than 70 hours, Pennakem recommends adding stabiliser if the product has been distilled previously (which removes the stabiliser).
3. Never store the product in less than half full storage.

In case of storage issue, Pennakem provides to its customers a method to measure the peroxides (Eurofins 2019) and a protocol to remove the peroxides from the product before use (Pennakem 2019). The freshly distilled 2-MeOx is peroxide free as the peroxides and the organic acid have a high boiling point. As the peroxide is not present for up to 100 hours, there is no opportunity for the degradation products to form as the extraction process, whether continuous or batch, requires a distillation every 2 – 3 hours.

Analysis of oils produced from rapeseed and soybean (the same oils as produced for Project number AC-189-050: Comparative tests of extraction and refining of soybean and rapeseed oils depending on solvent: hexane or 2-MeOx. See section 1.3.3.1) were analyzed for GVL. GVL was chosen as an indicator of the presence of peroxide decomposition in the oil because of the low LOQ of its quantification method. Four oils were analysed and all levels were below 1 ppm (0.0001%) (Institut Des Sciences Analytiques, 2019).

A.3.1.5 Fate of residues in food

2-MeOx is not deliberately added to food. All industrial extraction processes are designed to maximise recycling of the extraction solvent. However, some residual substance is still present in the extracted products: refined oils, plant proteins or natural extract (hop extract, carotenoid from algae, chlorophyll). Based on application trials, the residual will be less than one ppm in the refined oil, and in liquid food and beverages, and less than 10 ppm in solid food. Ingested 2-MeOx will be rapidly metabolised and excreted via the kidneys or lungs, according to tests on mice and rats. There is no indication that any bioaccumulation will occur.

The data presented in this dossier confirms that oral exposure to 2-MeOx or its degradation products is not of concern in the low levels present in food.

A.4 Manufacturing process

The manufacturing process for Pennakem's EcoXtract is provided at Appendix F (CCI). A brief, non-confidential summary of the manufacturing process is included here.

2-MeOx is a cyclic ether, issued from carbohydrates derived from lignocellulosic biomass, which represents the most abundant biomass resources on earth. The term lignocellulosic covers a

range of biomass containing cellulose and hemicellulose (polysaccharides), and lignin (aromatic polymer), making a rigid, compact, and complex assembly of polymers naturally recalcitrant to microbial and enzymatic degradation. The content of cellulose is generally in the range of 29–45%- and hemicellulose in the range of 18–30%. Industrially, 2-MeOx is produced from agricultural by-products such as corn stover, sugarcane bagasse and rice straw which could be found mainly in China, South Africa and Dominican Republic. Agricultural by-products such as lignocellulosic biomass are selected because they do not compete with the use of land for the production of food.

First, harsh acidic pre-treatment is required for deconstructing lignocellulose to make polysaccharides into more accessible intermediate sugars for subsequent conversions. After separation of the solid residue (from lignin), the acidic solution containing a mixture of both hemicellulose and cellulose is subject to hydrolysis of the polymers into monomeric C5 (pentoses) and C6 (hexoses) sugars. Then, C6- and C5- monosaccharides undergo multiple acid-catalyzed reactions to give the platform molecules levulinic acid (LA) and furfural (FAL) which are used as the building blocks for the synthesis of 2-MeOx.

A.5 Specification for identity and purity

EcoXtract® Food Grade is composed of 2-MeOx, a single substance with a purity of > 99.9 % v/v; it is stabilised with 150 ppm (0.015%) food grade mixed tocopherols. Other antioxidants have also been used (for example, BHT) and the important criteria is the presence of sufficient antioxidant to ensure shelf-life stability. The applicant would prefer not to state the antioxidant to be used, but to base the specifications on the purity and stability of the product.

2-MeOx is a highly pure product, there are no impurities present at greater than 0.1%. Very low levels of impurities may be present as a result of the manufacturing process. However, these levels are extremely low and do not present any quality or safety concerns. Pennakem has provided a confidential list of potential impurities to FSANZ, including results of batch analyses for these impurities (Appendix G - CCI). Pennakem has asked FSANZ to treat this list as commercial in confidence because the list of impurities may provide competitors with knowledge of elements of the confidential manufacturing process. As noted above, these impurities are present at very low levels and do not present a safety concern to consumers. Pennakem has completed a dietary exposure estimate for these impurities to substantiate that the very low levels do not present a safety concern. The results of the dietary exposure assessment are also included in Appendix G (CCI), but for the reasons explained above, the detail of the assessment is confidential to Pennakem.

To assure quality and safety the following specifications are proposed for 2-MeOx (Table A.5-1). Additional information about the methods is given below the table.

Table A.5-1. Specification for 2-MeOx, analysis conducted on finished product (every 5-tonne batch)

Substance	unit	specification	method	rationale
2-MeOx	% (w/w)	≥ 99.9 %	TA-002-01	confirm purity
Total of impurities	% (w/w)	<0,1%		Control impurities
Moisture	% w/w	<0,03 %	TA-002-02	amount of water present
Peroxides	% w/w active oxygen	< 1ppm	TA-002-04*	storage stability

* some dissolved metals (eg Cu^{+2} and Fe^{+3}) will also give a positive result.

Depending on the antioxidant added, BHT or tocopherol measurement is outlined below.

BHT	ppm	150-400 ppm	TA-002-03	amount of antioxidant present
Tocopherol	ppm	50-150 ppm	TA-002-07	amount of antioxidant present

Additional information about the methods relevant to the specifications for 2-MeOx[2]:

TA-002-01	GC technique using Agilent Technologies Gas Chromatograph equipped with flame ionization detector and Agilent Technologies ChemStation software or equivalent. Column – 60 m x 0.25mm x 1.0µm thickness Liner – Agilent 4 mm ID with glass wool The standard deviation established for this method is ± 0.003% MeTHF based on ten replicates of one sample in a 24-hour period. MeTHF based on a 6-level curve run in triplicate.
TA-002-02	Colourmetric titration method using Combi-coulomat frit The standard deviation established for the precision of this method is ± 12.4-ppm moisture based on ten replicates of one sample in a 24-hour period.
TA-002-03	GC method using a Rxi-5ms 15 m X 320 µm X 0.25 µm column with hydrogen carrier with a 4-minute run time. Concentration determined form chromatogram against a standard calibration curve. The LOQ is 20 ppm and the accuracy is 99.98% +/- 0.57%.
TA-002-04	Titration method using potassium iodide and thiosulfate.
TA-002-07	HPLC equipped with a C8 column and a diode array detector. An external calibration curve is used to determine ppm tocopherol.

Analysis of three batches of 2-MeOx for heavy metals found no notable differences between the three batches (Environmental Testing and Consulting, Inc, Memphis, USA; 2013). Table A.5-2 lists the results of these analyses.

[2] Detail for each of these methods is provided in the relevant references, but is commercial in confidence

Table A.5-2 Heavy Metal analysis

Metal (total amount)	unit	Sample ID		
		D20-South MeTHF	MeTHF 2-2B07N Tocopherol	MeTHF 2-2M27 BHT
Aluminium	µg/kg	<500	<500	<500
Arsenic	µg/kg	<5.00	<5.00	<5.00
Calcium	µg/kg	<500	<500	<500
Cadnium	µg/kg	<5.00	<5.00	<5.00
Chromium	µg/kg	5.53	<5.00	5.50
Cobalt	µg/kg	<5.00	<5.00	<5.00
Copper	µg/kg	<5.00	<5.00	<5.00
iron	µg/kg	<500	<500	<500
Lead	µg/kg	<5.00	<5.00	<5.00
Magnesium	µg/kg	<500	<500	<500
Manganese	µg/kg	<5.00	<5.00	<5.00
Mercury	mg/kg	<0.0133	<0.0133	<0.0133
Nickel	µg/kg	32.5	<5.00	<5.00
Potassium	µg/kg	<500	<500	<500
Silver	µg/kg	<5.0	<5.00	<500
Sodium	µg/kg	<500	<500	<500
Zinc	µg/kg	<25.0	<25.0	<25.0
Lithium	mg/kg	<0.050	<0.050	<0.050

It is concluded that 2-MeOx has very low levels of heavy metals and that as the level of 2-MeOx in food will be <20 ppm then the heavy metal levels will be several orders of magnitude below the maximum levels for metal contaminants listed in section S19—4 of the Code. The use of 2-MeOx as an extraction solvent processing aid will therefore not present a safety concern in relation to heavy metal levels in foods. Additional limits or tests for heavy metals are not proposed by Pennakem.

A.6 Analytical method for detection

2-MeOx will be used to extract lipids from oil rich biomass and defat protein rich biomass. It will also be used to extract natural aroma, flavours and colorants, particularly the lipophilic ones currently extracted with hexane (for example, hop, annatto, carotenoids, chlorophyl).

Although the majority of the product is removed and recovered in the process, there is the potential for residual 2-MeOx to be present in the refined oil, in plant proteins and in food additives. To control the products, we defined and validated different protocols to measure the solvent residue in different matrices:

- In oil or lipophilic liquids (crude oil, refined oil)
- In powders (plant defatted meals, plant protein isolate)

A.6.1 Refined Oils

A method for the analysis of 2-MeOx in refined oils was developed and validated by ITERG, France (ITERG 2019a). The analysis was performed by head space gas chromatography following desorption in a sealed flask by heating at 80 0C. The method was quantified for 1 to 10 mg 2-MeOx/kg of oil. The method was validated as follows (Table A.6.1-1):

Table A.6.1-1. Method validation for detection of 2-MeOx in refined oils

Parameter	Result
Range of application	1 to 10 mg/kg
specificity in refined oil	no interference
linearity 1 – 10 mg/kg	R^2 = 0.9993
Recovery (refined soybean oil) triplicate samples in each of 5 trials at 0, 1, 5 and 10 mg/kg)	81 – 118%
Recovery (refined rapeseed oil) triplicate samples in each of 5 trials at 0, 1, 5 and 10 mg/kg)	85 – 118 %
Overall recovery	within 70 – 120 % validation criteria
Repeatability (refined soybean oil) RSD (%) for 1, 5 and 10 mg/kg	2.5, 2.1, 1.3
Repeatability (refined rapeseed oil)	2.1, 6.9, 1.6
Reproducibility (refined soybean oil)	3.0, 16.3, 11.7
Reproducibility (refined rapeseed oil)	2.0, 8.0, 12.8
Repeatability and reproducibility RSD	values comparable to the precision data given in the standard NF T 60-257

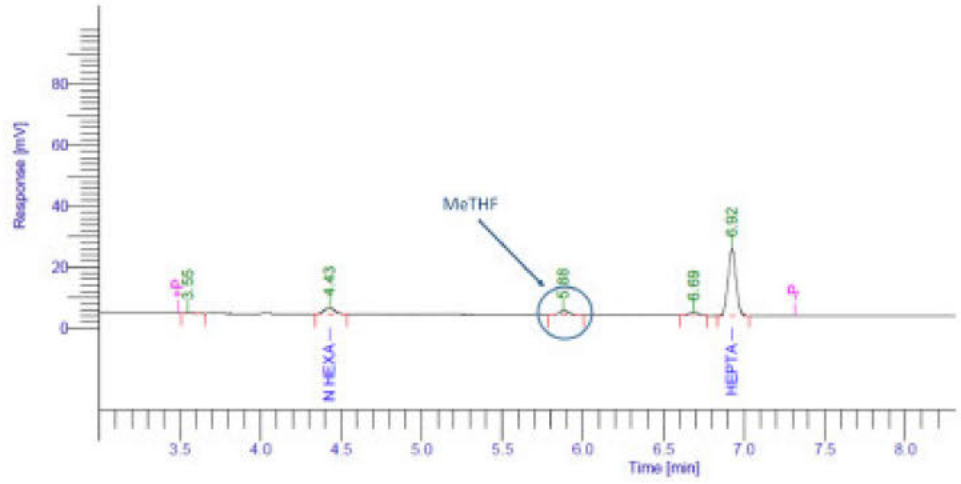


Figure A.6.1-1. Chromatogram showing the specificity of 2-MeOx (MeTHF) in refined oil

A.6.2 Crude oils

A method for the analysis of 2-MeOx in crude vegetable oils was developed and validated by ITERG, France (ITERG 2019b). The analysis was performed by head space gas chromatography following desorption in a sealed flask by heating at 80 ^{0}C. The method was quantified for 50 to 1000 mg 2-MeOx/kg of oil. The method was validated as follows (Table A.6.2-1):

Table A.6.2-1. Method validation for detection of 2-MeOx in crude oils

Parameter	Result
Range of application	50 to 1000 mg/kg
specificity in refined oil	no interference
linearity 1 – 10 mg/kg	R^2 = 0.9994
Recovery (crude rapeseed oil) triplicate samples in each of 5 trials at 0, 50, 200, 500 and 1000 mg/kg)	89.8 – 109.9%
Overall recovery	within 70 – 120 % validation criteria
Repeatability (crude rapeseed oil) RSD (%) for 50, 200, 500 and 1000 mg/kg	1.1, 0.9, 1.1, 1.1
Reproducibility (crude rapeseed oil)	4.1, 5.2, 3.4, 2.9
Repeatability and reproducibility RSD	<20%

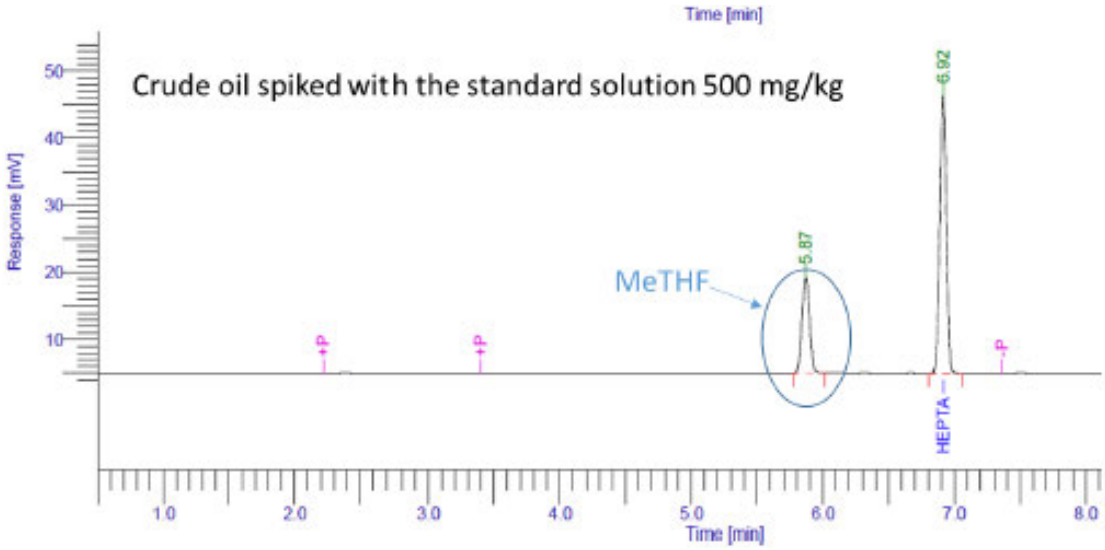


Figure A.6.2-1. Chromatogram showing the specificity of 2-MeOx (MeTHF) in crude oil

A.6.3 Plant defatted meals

The following two methods were used to analyse the amount of residual 2-MeOx remaining in the dried solid residues that are obtained after solvent extraction (defatted vegetable samples). The second method was developed in order to reduce the limit of detection to below 10 ppm (mg/kg).

a) Method on GC-FID

A method for the analysis of 2-MeOx in plant defatted meals was developed and validated by ITERG, France (ITERG 2019c). The analysis was performed by head space gas chromatography following desorption in a sealed flask by heating at 80 ^{0}C. The method was quantified for 200 to 1000 mg 2-MeOx/kg of meal. The method was validated as follows (Table A.6.3-1):

Table A.6.3-1. Method validation for detection of 2-MeOx in plant defatted meals (GC-FID)

Parameter	Result
Range of application	200 to 1000 mg/kg
specificity in refined oil	no interference
linearity 50 – 1000 mg/kg soybean meal	R^2 = 0.9995
linearity 50 – 1000 mg/kg rapeseed meal	R^2 = 0.9998
Recovery (soybean meal) triplicate samples in each of 5 trials at 50, 200, 500 and 1000 mg/kg)	81 – 132% 81 – 102 % excluding 50 mg/kg
Recovery (rapeseed meal) triplicate samples in each of 5 trials at 50, 200, 500 and 1000 mg/kg)	81 – 127 % 81 – 103% excluding 50 mg/kg
Overall recovery	within 70 – 120 % validation criteria for 200 – 1000 mg/kg only
Repeatability (soybean meal) RSD (%) for 50, 200, 500 and 1000 mg/kg	0.9, 0.7, 1,0.7
Repeatability ((rapeseed meal)	3.6, 107, 0.8, 0.9
Reproducibility (soybean meal)	9.4, 5.9, 5.9, 6.9
Reproducibility ((rapeseed meal)	12.8, 7.2, 6.9, 7.3
Repeatability and reproducibility RSD	values comparable to the precision data given in the standard NF EN ISO 8892

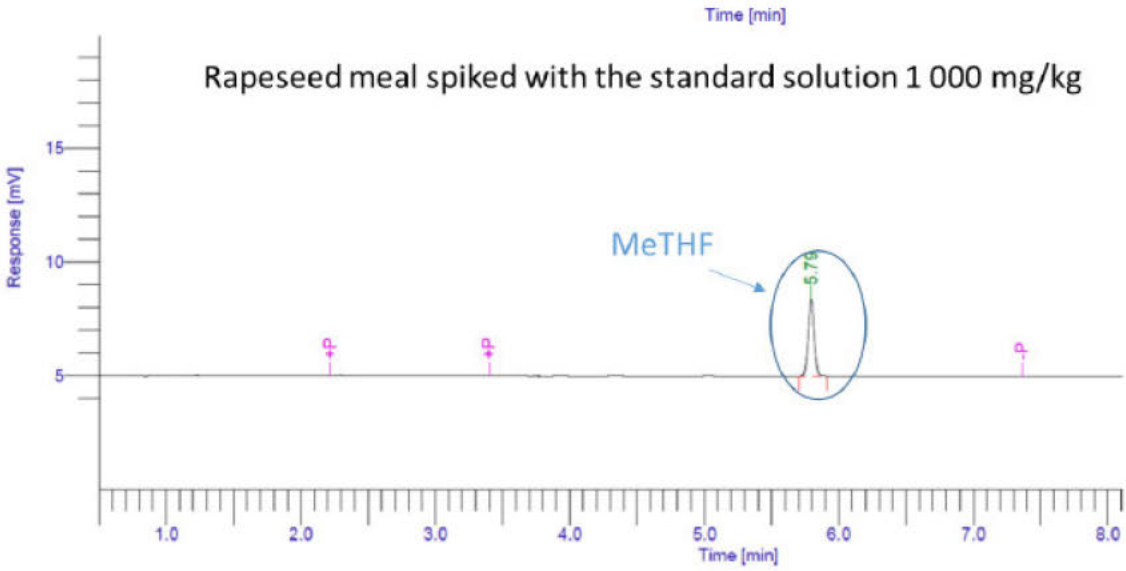


Figure A.6.3-1. Chromatogram showing the specificity of 2-MeOx (MeTHF) in meal

This method is suitable for the analysis of 2-MeOx in vegetable meals.

b) Method on GC-MS-MS

To reach a better sensitivity in the [0 – 200 ppm] range, a method for the analysis of the 2-MeOx in meal was developed using a static headspace-GC-MS method by RIC, Belgium (RIC 2020 - CCI). In this method the column used for analysis was changed and two calibrations runs

(1 - 100 ppm or 50 – 1000 ppm) were used to increase accuracy. It was also confirmed that an aqueous preparation of the dried samples, including ultrasonic treatment to aid dispersion, increased accuracy and recovery. The method was validated as follows (Table A.6.3-2):

Table A.6.3-2. Method validation for detection of 2-MeOx in plant defatted meals (GC-MS-MS)

Parameter	Result
Range of application	1 to 1000 mg/kg
specificity in	no interference
linearity 1 – 100 ppm	R^2 = 0.998
accuracy (2.5 – 100 ppm)	80 – 120 %
Limit of detection	1 ppm
accuracy at 1ppm	70 – 90 %
RSD at 1 ppm	< 20%
Repeatability (2.5 – 20 ppm)	< 10 % RSD
linearity 50 – 1000 ppm	R^2 = 0.998
accuracy (50 – 1000 ppm)	85 – 105 %
Repeatability (50 – 1000 ppm)	< 5 % RSD

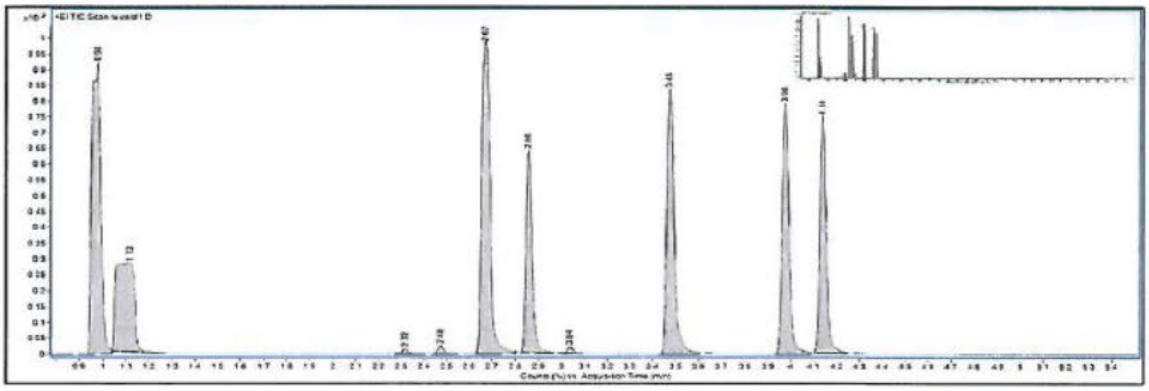


Figure A.6.3-2. Chromatogram of standard mixture on 30 m x 0.25 mm ID x 1 µm HP1-MS.

The identified peaks in Figure A.6.3-2 are listed in Table A.6.3-3.

Table A.6.3-3. Identification of peaks in chromatogram results in Figure A.6.3-2

Peak	RT	identification
1	0.98	air
2	1.12	water
3	2.32	2-methylpentane
4	2.48	3-methylpentane
5	2.67	hexane
6	2.86	THF (=ISTD)
7	3.04	methylcyclopentane
8	3.48	2-methylTHF (=target)
9	3.98	2.5-dimethylTHF
10	4.14	2.5-dimethylTHF

Different samples were prepared with three different matrices (soy, sunflower and canola), with or without traces of 2-MeOx, and with different levels of 2-MeOx to cover the targeted range (Table A.6.3-4). Results from each of the samples is provided in Table A.6.3-5.

Table A.6.3-4. Samples for method validation – detection of 2-MeOx in plant defatted meals

RIC ID	Nature	Defatting solvent	Extraction method	Drying method
Sample 1	Defatted soy flakes	Hexane	Batch (80 kg sample) by OLEAD	Under vacuum – 50 °C
Sample 2	Defatted soy flakes	2-MeOx	Batch (80 kg sample) by OLEAD - 2019	Under vacuum – 50 °C
Sample 3	Defatted canola meal	2-MeOx	Continuous extraction ENAT - 2020	Desolventizer-Toaster – Big bag 25
Sample 4	Defatted canola meal	2-MeOx	Continuous extraction ENAT - 2020	Desolventizer-Toaster – Big bag 35
Sample 5a	Defatted sunflower meal	Hexane	Batch (80 kg sample) by OLEAD - 2019	Under vacuum – 50 °C
Sample 5b	Defatted sunflower meal-grinded	Hexane	Batch (80 kg sample) by OLEAD - 2019	Under vacuum – 50 °C

Table A.6.3-5. Results of 6 samples used for method validation – detection of 2-MeOx in plant defatted meals

Matrix (6 samples of each)	**actual mean concentration of MeTHF (µg/g)**	**repeatability of 20 µg/g sample**	**Mean recovery of 20 µg/g sample**	**repeatability of 200 µg/g sample**	**Mean recovery of 200 µg/g sample**
Sample 1	< 1 ppm	RSD = 4.6	91.6%	RSD – 5.2	95.7 %
Sample 2	658 ppm RSD = 2.2	-	-	-	-
Sample 3	6.7 ppm RSD = 32.2	RSD = 9.5	72.5 %	RSD = 6.6	94.5 %
Sample 4	106.5 ppm RSD = 17.7	RSD = 9.2	139.4 %	RSD = 3.2	106.5 %
Sample 5a	< 1 ppm	RSD = 10.5	97.0 %	RSD = 7.2	91.6 %
Sample 5b	< 1 ppm	RSD = 7.7	92.3 %	RSD = 3.0	93.8 %

The hexane defatted samples were used to produce additional samples by spiking with pure 2-MeOx. The results of the validation work shows that the method has a reliable limit of detection of 1 ppm for all the matrixes. The defatted soy, canola (rapeseed) and sunflower meal are typically used for animal feed. The defatted soy dried without steam ("white flakes") are further processed to produce soy isolates or soy concentrate.

A.6.4 Plant protein (Isolate)

A GC-HS-MS method for the analysis of 2-MeOx in protein isolate was developed and validated by Eurofins (Eurofins 2019); the study was GLP compliant and in accordance with ICH validation guidelines. The method was validated to ≤ 1 ppm. The validation results are described in Table A.6.4-1.

Table A.6.4-1. method validation for detection of 2-MeOx in plant protein isolate

Validation headings	Parameters studied and acceptance criteria	Result obtained
Specificity	No significant interference with the peaks of 2-MeOx Rs peaks of interest / closest peaks ≥ 1.5 in standard and sample solutions.	Blank: no interference Rs ≤ 1.5 for 2-MeOx peak but integration remains appropriate for quantification Method is considered as specific
LOD/LOQ	LOD reported for information LOQ ≤ 1 ppm	LOD = 0.004 ppm LOQ = 0.01 ppm
Linearity of response (response function)	3 series of 5 different concentrations were performed (from 10 to 120 %) the equation of the calibration curve is calculated and r ≥ 0.98.	Y = 63354 + 18306500 X r = 1.00 With Y = Area and X = concentration in µg/mL Intercept ≈ 0 → quantification can be performed with a standard solution at 100%.
Accuracy	At 10 % Level Individual recovery ≥ 50 % Mean recovery ≥ 70 % At 100 and 120 % Level Individual and mean recoveries [70 – 130 %]	At 10 % Level Individual recovery ≥ 60 % Mean recovery = 76 % At 100 and 120 % Level Individual recoveries [70 – 130 %] Mean recovery at 100 % = 83 % Mean recovery at 120 % = 96 %
Repeatability and intermediate precision	RSDr ≤ 15 % RSD_{IP} = NA	RSDr = 12 % RSD_{IP} = 14 %

Due to the variability in repeatability, for routine analysis, three preparations will be analysed.

3.3.2.B Information related to the safety of 2-methyloxalane

B.1 Industrial use of 2-methyloxalane

In the European Union (EU), 2-MeOx has been registered under EC (No) 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). It is registered for annual quantities above 1000 tonnes for its use as a solvent for chemical synthesis including fine chemicals, agrochemicals, and pharmaceuticals. A comprehensive hazard identification and risk assessment has been conducted as part of this process.

The REACH process requires companies registering to conduct hazard identification and risk analysis according to the specified guidelines. The ECHA does not evaluate all REACH dossiers however, as part of their assessment a request was made by the ECHA to conduct additional animal work. In order to fully assess this request, the ECHA will have reviewed the data within the dossier.

The submitted hazard classification, made according to (EC) No 1272/2008) on the classification, labelling and packaging of substances and mixtures (CLP Regulation) was

Flammable liquid category 2	H225: Highly flammable liquid and vapour.
Acute Toxicity category 4	H302: Harmful if swallowed.
Skin Irritant category 2	H315: Causes skin irritation.
Eye Damage category 1	H318: Causes serious eye damage.

The information presented in this dossier agrees with the CLP classification derived from the data submitted for REACH.

The REACH process results in the identification of Derived No Effect Levels (DNEL), for workers and for consumers. The DNEL for oral exposure was calculated to be 1.25 mg/kg/day. The following values were derived; note that REACH is very prescriptive in the use of assessment factors.

Table B.1-1. Hazard conclusions and DNELs for Workers

Route / Type of effect	DNEL derivation	Assessment factors (AF) for DNEL derivation
Inhalation **Systemic effects - Long-term** **200.196 mg/m^3**	**DNEL derivation method:** ECHA REACH Guidance **Dose descriptor starting point:** NOAEC 9960 mg/m^3 adjusted for rate and volume to 5004.9 mg/m^3	AF for difference in duration of exposure: 2 (Default (subchronic to chronic)) AF for other interspecies differences: 2.5 (Default) AF for intraspecies differences: 5 (Default) AF for the quality of the whole database: 1 Overall Assessment Factor: 25
Inhalation **Systemic effects – Acute** **200.196 mg/m^3**	**DNEL derivation method:** ECHA REACH Guidance	Overall Assessment Factor: 25
Dermal **Systemic effects - Long-term** **3.26 mg/kg bw/day**	**DNEL derivation method:** ECHA REACH Guidance **Dose descriptor starting point:** NOAEL 250 mg/kg bw/day	AF for difference in duration of exposure: 2 (Default (subchronic to chronic)) AF for interspecies differences (allometric scaling): 4 (Default (oral rat to dermal human)) AF for other interspecies differences: 2.5 (Default) AF for intraspecies differences: 5 (Default) AF for the quality of the whole database: 1 Overall Assessment Factor: 100
Dermal **Systemic effects – Acute** **3.26 mg/kg bw/day**	**DNEL derivation method:** ECHA REACH Guidance	Overall Assessment Factor: 100

Table B.1-2. Hazard conclusions and DNELs for the general population

Route / Type of effect	DNEL derivation	Assessment factors (AF) for DNEL derivation
Oral **Systemic effects - Long-term** **1.25 mg/kg bw/day**	**DNEL derivation method:** ECHA REACH Guidance **Dose descriptor starting point:** NOAEL 250 mg/kg bw/day	AF for difference in duration of exposure: 2 (Default (subchronic to chronic)) AF for interspecies differences (allometric scaling): 4 (Default (oral rat to dermal human)) AF for other interspecies differences: 2.5 (Default) AF for intraspecies differences: 10 (Default) AF for the quality of the whole database: 1 Overall Assessment Factor: 200
Oral **Systemic effects – Acute** **1.25 mg/kg bw/day**	**DNEL derivation method:** ECHA REACH Guidance	Overall Assessment Factor: 200

B.2 Use of 2-methyloxolane as a processing aid in other countries

Pennakem is seeking authorisation to use 2-MeOx as a processing aid in the European Union (EU) and in the United States (US). The European Food Safety Authority has published a positive opinion on the safe use of 2-MeOx as a processing aid and approval for use in the EU is expected to be made official before the end of 2022.

Approval in the US is ongoing and expected to be released in 2022 for the Food applications (Food Contact Substance) and in early 2023 for the Feed application (Feed Additive Petition).

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), in its Quality Guideline for Residual Solvents (Q3C R8) has adopted a proposal to include 2-MeOx as a low toxicity (Class 3) residual solvent for use in pharmaceutical products; after an April 2021 worldwide call for comments.

In the pharmaceutical industry 2-MeOx has been proposed as a suitable replacement for the solvent tetrahydrofuran. 2-MeOx has been used as a process solvent for the production of pharmaceuticals intermediates since 2007. Not being listed in the Q3C residual solvent guideline until recently, 2-MeOx was used for the production of early intermediates. Inclusion of 2-MeOx as a Class 3, low toxicity residual solvent in the quality guideline facilitates the use of 2-MeOx more broadly in a pharmaceutical context. As part of the ICH's consideration of 2-MeOx, a Permitted Daily Exposure level was calculated. This is described in more detail in section B.5.

B.3 Toxicokinetics and metabolism of 2-methyloxalane

Henderson et. al. (2007) investigated the absorption, metabolism, distribution and elimination (ADME) of single oral and intravenous doses of 2-MeOx, and the disposition of a single intravenous dose of 14C- 2-MeOx was determined in rats and mice. Male Fisher (F344) rats and B6C3F1 mice were administered 1, 10 or 100 mg ^{14}C- 2-MeOx /kg orally or 1 mg ^{14}C- 2-MeOx /kg intravenously. The vehicle for the oral dose was water and 0.9% saline was used for intravenous administration. Four animals/group were used.

Dose levels were chosen to provide a range to include one high dose, which was believed to be above the level able to be completely metabolized by the animals, and sufficient to detect overt toxicity of the compound. It should be noted that no toxicity was observed in the study, including gross tissue examination.

Tissues and body fluids sampled included urine, faeces, blood, expired air, adipose (perirenal, reproductive), muscle (hind leg, trapezius), skin (ears), brain, heart, lung, spleen, kidneys, testes, liver, small intestines and contents, large intestine and contents, stomach and contents, urinary bladder and carcass.

Urine samples were taken 3 (IV only) 6, 12, 24, 48 and 72 hours after dosing. Bladder urine also collected. Faeces samples were taken 12, 24, 48 and 72 hours after dosing. Expired air traps were changed 3 (IV only), 6, 12, 24, 48 or 72 hours after dosing. Metabolite characterisation studies were conducted on individual samples of urine and expired air at the time points given above. Analysis was conducted by HPLC, HPLC-MS-MS, Liquid scintillation counting.

Results

2-MeOx was 93-100% absorbed, and rapidly metabolized in both rats and mice. The major routes of excretion were via urine in mice, and exhalation in rats. The amount of radioactivity remaining in the tissues at the time of euthanasia was less than 8% in mice and from 8 to 22% in rats. In the rat, muscle and skin samples had the highest retained radio activity (3 – 6%) but no internal organ accumulated radioactivity. Table 3.1.B-1 and Table 3.1.B-2 lists the results for recovery of radioactivity from oral and intravenous exposure respectively.

Urinalyses: In mouse urine, the retention times (and percent injected radioactivity) of the three major peaks observed were 3.01 min (6%), 4.02 min (29%), and 5.15 min (59%). In all studies, the measurable radioactivity in the three peaks declined to near baseline by 24 h. In contrast, the 6 h urine samples from rats following a single oral dose of 100 mg/kg showed one prominent peak of approximately 5 min retention time. An additional peak was seen at 3 min, but no clearly defined radioactive chromatographic peaks were observed in urine taken at later time points.

HPLC radio chromatograms of urine samples collected over timed intervals following a single intravenous dose of 1 mg/kg ^{14}C- 2-MeOx to rats and mice were similar to that seen following oral dosing. In mouse 6h urine samples, the observed three major peaks had retention times (and proportion of radioactivity) of 2.73 min (14.7%), 3.70 min (46.4%) and 4.59 min (34.3%). In 6 h urine samples from rats following a single intravenous 1 mg/kg dose, two peaks were observed at retention times of 2.77 and 4.51 min (with injected radioactivity of 35.1% and 60.2%, respectively).

Table B.3.1-1. Recovery of radioactivity following oral exposure

Species	dose (mg/kg)	end of collection period (h)	Cumulative percent of total dose (mean)				
			Urine	Feces	exhaled air		total
					VOC	CO_2	
mice	100	6	35.7	nc	13.5	23.5	72.6
		12	51.1	2.98	13.6	24.8	92.2
		24	52.4	4.68	13.6	25.2	96.1
		48	52.9	4.81	13.6	25.8	96.9
		72	53.0	4.93	13.6	26.2	97.7
mice	10	6	30.3	nc	2.01	27.7	59.9
		12	55.7	0.144	2.05	29.9	87.8
		24	60.0	0.794	2.06	30.9	93.5
mice	1	6	47.5	nc	0.76	28.6	76.8
		12	57.4	1.24	0.79	30.6	90.0
		24	60.4	1.72	0.80	31.5	94.3
rats	100	6	11.4	nc	26.9	28.0	66.3
		12	17.3	0.0454	27.3	36.2	80.7
		24	21.3	0.319	27.4	28.8	87.7
		48	21.	0.648	27.4	40.4	90.0
		72	21.8	0.914	27.4	41.6	91.7
rats	10	6	19.5	nc	3.15	4.9	64.6
		12	24.3	0.465	3.21	48.6	76.7
		24	25.2	1.33	3.25	50.2	79.9
rats	1	6	17.8	nc	1.19	49.	68.5
		12	22.1	0.281	1.23	56.1	79.7
		24	23.4	0.772	1.27	58.5	83.9

nc – not collected, first collection at 12 hours

Table B.3.1-2. Recovery of radioactivity following intravenous exposure

Species	dose (mg/kg)	end of collection period (h)	Cumulative percent of total dose (mean)				
			Urine	Feces	exhaled air		total
					VOC	CO_2	
mice	1	3	42.2	nc	3.95	20.5	66.6
		6	53.0	nc	4.07	25.7	82.8
		12	57.0	0.182	4.10	28.3	89.5
		24	58.4	0.371	4.12	29.8	92.7
rats	1	3	7.90	nc	4.41	22.8	29.1
		6	17.8	nc	4.71	38.0	60.4
		12	2.0	0.285	4.80	43.9	72.1
		24	24.2	1.65	4.83	47.5	78.1

nc – not collected, first collection at 12 hours

Following oral dosing, the major urinary metabolites in mice eluted at approximately 3, 4 and 5 min, while, in rats, only 2 major metabolites were shown, eluting at approximately 3 and 5 min. All peaks in both species eluted much earlier than 2-MeOx, suggesting that they are more polar than the parent compound. HPLC analysis of derivatized urine samples from both species showed no evidence of levulinic acid as a metabolite of 2-MeOx in the urine. Studies in which the urine samples were incubated with beta-glucuronidase indicated that the metabolites were not glucuronides. Because the ^{14}C-label was in the methyl group the polar metabolites are likely due

to the one-carbon unit getting into the metabolic pool and labelling intermediate dietary metabolites or even urea.

Analyses of ^{14}C- 2-MeOx -derived material in expired air: The excretion of exhaled volatile organic compounds (VOC) was dose-dependent in both species; at lower doses exhaled VOC represented 1-5% of dose, but at the highest dose (100 mg/kg) this proportion rose to 14% (mice) and 27% (rats). Analysis of the VOCs exhaled at the high dose indicated that the increase was due to exhalation of the parent compound; suggesting unchanged 2-MeOx is exhaled only when normal metabolism is overwhelmed.

The nature of the ^{14}C- 2-MeOx -derived material captured in the VOC traps was investigated by HPLC and by LC/MS/MS analysis. One major HPLC peak was seen at 13.42 min, which was consistent with the retention time of ^{14}C- 2-MeOx analyzed under identical conditions – this demonstrates that unchanged test material was expelled from the lungs. This analysis was done for rats only as for mice the levels were low and less reliable.

LC/MS/MS analysis revealed one major peak at a mass-to-charge ratio of 87.1 (m/z amu), consistent with 2-MeOx (FW + l), with an intensity of approximately l.7E+06, which is well above background. In the multiple response monitoring mode, a parent-daughter ratio of 87.0/69.21 (Ql/Q3 masses, amu) was observed and was consistent with the ratio obtained with the 2-MeOx standard, confirming the presence of ^{14}C- 2-MeOx in exhaled breath, particularly at 100 mg/kg when normal metabolism may have been overwhelmed.

There were two indications that mice have a higher capacity to metabolise 2-MeOx than rats, (i) at all doses, mice had cleared a slightly higher percentage of the dose than rats by 24 h, and (ii) rats given the high dose cleared a higher dose percentage as exhaled parent compound than did mice.

Conclusion

Based on recovery of radioactivity in excreta (other than faeces) and tissues (other than gastrointestinal tract), absorption or orally administered 2-MeOx was essentially complete (93-100%) and low bioaccumulation potential. There were no overt signs of toxicity observed at any dose. The studies confirm that following oral exposure in the mouse or rat there is almost complete absorption, making both species good models for the assessment of systemic toxicity.

B.4 Toxicity of 2-methyloxolane

Pennakem has commissioned a series of toxicity studies for 2-MeOx and identified other published studies relating to the toxicity of 2-MeOx. The categories of toxicity studies addressed in this application are highlighted below:

Category	Section addressed
Acute toxicity	B.4.1
Short-term / subchronic toxicity	B.4.2
Long-term toxicity and carcinogenicity	Not addressed (see note below table)
Genotoxicity	B.4.3
Reproductive and developmental toxicity	B.4.4
Other studies	B.4.5

Note: Long-term toxicity and carcinogenicity studies are not considered necessary for 2-MeOx, based on a clear no observed effect level (NOEL) being identified from a 90-day study, high dose toxicity being limited to the liver (changes are reversible and most likely associated with increased metabolism), and the substance not being genotoxic.

B.4.1 Acute toxicity

Summary of acute toxicity studies:

End point	Method	Results	Reference
Oral acute toxicity Rat	OECD Guideline 420 (Acute Oral Toxicity - Fixed Dose Method)	LD_{50}: 300-2000 mg/kg bw (female)	Harlan (2013a)
Oral acute toxicity Rat	not current guideline	LD_{50}: 3800 mg/kg bw	Deichmann & Gerarde (1969)
Acute inhalation toxicity rat: vapour	not current guideline	LC_{50} : 22 mg/l air	Deichmann & Gerarde (1969)

B.4.1.1 Acute oral toxicity

Study reference	Tetrahydro-2-methylfuran: Acute oral toxicity in the rat-fixed dose method Report no.: 41205095
Author	Harlan
Date	2013a
GLP	Yes. Conducted at Harlan Laboratories Limited, Derbyshire, UK
Substance	2-MeOx
Analytical purity	99.93%
Lot/batch number	2-2D03S
Expiry	04 March 2015
Methods	Guideline: OECD Guideline 420 (Acute Oral Toxicity - Fixed Dose Method) Test animals Female Wistar rats 8 – 12 weeks old. Group housed in standard laboratory conditions with ad libitum access to food and water with the exception of overnight fasting prior to dose administration.

	Administration / exposures Single bolus oral gavage dose with no vehicle (maximum dose volume was 10 mL/kg) No. of animals per sex per dose: 2000 mg/kg bw, 1 female; 300 mg/kg bw, 5 females Control animals: none Observations Animals were observed at 0.5, 1, 2, 4 hours after dosing and then daily for 14 days. Bodyweight was recorded on days 0, 7, 14 or at death. All animals were subject to macroscopic necropsy; no tissues were retained.

Results

Effect levels (based on 95% CL): Female LD50 >300 mg/kg bw test material

The one animal dosed at the 2000 mg/kg bw dose level was killed due to severity of clinical signs thirty minutes after dosing. These signs included laboured respiration, decreased respiratory rate, hypothermia and pallor of the extremities and the test animal was also comatose. Haemorrhage and epithelial sloughing of the non-glandular epithelium of the stomach and clear fluid present in the stomach were noted at necropsy.

There were no deaths at the 300 mg/kg bw dose level. No signs of systemic toxicity were noted during the observation period.

All animals showed expected gains in bodyweight over the observation period. No abnormalities were noted at necropsy in the animals dosed at 300 mg/kg bw.

Conclusion
The acute oral LD50 was estimated to be in the range of 300 - 2000 mg/kg bodyweight, as determined in a reliable study conducted according to current OECD guideline and in compliance with GLP.

Study reference	Deichmann WB, Gerarde HW, 1969. Toxicology of Drugs and Chemicals
Author	Deichmann WB, Gerarde HW
Date	1969
GLP	No
Substance	2-MeOx
Analytical purity	not specified
Lot/batch number	not specified
Expiry	not specified

This study is cited as a secondary reference, although the limitations of the reporting of the study suggest it is of limited value in the context of evaluating acute oral toxicity of 2-MeOx, particularly given the availability of the Harlan 2013a study. An oral LD_{50} in the rat was found to be 3800 mg/kg bw (based on 95% CL).

The study also identified an inhalation LD_{50} in the rat was found to be 22 mg/L air (based on 95% CL) following 4 hours exposure to the vapour.

B.4.2 Short-term / subchronic toxicity

Summary of short-term oral and inhalation toxicity studies:

End point	Method	Results	Reference
Subchronic toxicity, oral	Similar to OECD 408 rat (Sprague-Dawley) male/female subchronic (oral: gavage) 0, 80, 250, 500 and 1000 mg/kg/day Exposure: 3 months plus 1 month recovery period Similar to OECD 408	NOAEL: 250 mg/kg bw/day (male/female) (increased liver weight and hypertrophy)	Parris P, Duncan NJ, Fleetwood A and Beierschmitt WP (2017)
	rat (Sprague-Dawley) male/female subchronic (oral: gavage) 26 mg/kg bw/day and unspecified lower doses Exposure: "approximately 3 months" (daily) Similar to OECD 408 but limited details of method given in the publication	NOAEL: 26 mg/kg bw/day (male/female) (No toxicity observed)	Antonucci V; Coleman J; Ferry JB; Johnson N; Mathe M; Scott JP (2011)
Subchronic toxicity, inhalation	OECD 413 rat (RccHan™:WIST) male/female subchronic (inhaltion) 0, 2, 4.5 and 10 mg/L Exposure: 3 months (6 hours/day for 5 days/week) Additional investigations added (FOB, estrous cycle monitoring thyroid analysis, sperm analysis)	NOAEC: 10 mg/L (male/female) (some non-adverse transient clinical signs, and minor bwt and food con effects at 10 mg/L)	Envigo (2019a)

The subchronic toxicity of 2-MeOx has been investigated in the rat via the oral and inhalation routes. There are two oral studies. The publication by Antonucci (2011) uses only a high dose level of 26 mg/kg/day and this is also identified as the NOAEL. There is limited information available for this study. The publication by Parris et al. (2017) derives a NOAEL of 250 mg/kg/day. This was a GLP study conducted to support the use of 2-MeOx as a solvent for pharmaceutical manufacture. There is no access to the full report, but detailed group mean data is presented for the parameters assessed in a peer reviewed journal and the results are considered to be very reliable. In addition, this data from the oral study is most relevant to human risk assessment from dietary exposure.

In the oral study (Parris et al. 2017), there were no treatment related mortalities. The 1000 mg/kg/day dose was associated with a slight decrease in male weight gain, and, in both sexes, effects associated with the liver (increased liver weight, minimal/mild centrilobular hypertrophy and increased serum cholesterol). At 500 mg/kg/day there was a slight increase in liver weight but no corresponding pathology. Otherwise, these high dose levels were well

tolerated, and clinical signs were restricted to pre and post dose salivation in limited animals at both dose levels. It is not surprising to see effects in the liver at high doses; it has previously been shown that 93 – 100 % of 2-MeOx is absorbed following oral administration (Henderson et al., 2007) and the effects may be due to first pass metabolism. These findings were not observed at lower doses, in control animals and in the recovery groups.

No significant ophthalmic or haematologic changes were reported. A treatment related increase in serum cholesterol was observed in both sexes at 1000 mg/kg bw/day, with complete reversal reported at the end of the treatment-free period.

250 mg/kg/day was a clear NOAEL for oral exposure.

The oral study was conducted according to pharmaceutical requirements and does not address all of the endpoints given in the revised OECD 408 guideline for oral sub chronic toxicity of chemicals. However, the following endpoints were included in a second sub chronic study (Envigo 2019a) conducted via the inhalation route according to OECD 413 (note the additional endpoints below are not a usually a requirement OECD 413 guideline for sub chronic inhalation toxicity):

- Functional observations battery (FOB)
- Estrous cycle monitoring
- Sperm analysis

A preliminary dose range finding study (Envigo 2018a) demonstrated a dose of 19.7 mg/L induced severe clinical signs justifying early termination of this dose group. The follow-up study (Envigo 2019a) was performed according to OECD TG. The animals (Han Wistar rats) were exposed nose-only for 13 weeks at concentrations of 0, 2, 4.5 and 10 mg/L.

For the highest dose group of 10 mg/L, no mortalities or clinical signs were reported, and there were also no histopathological changes in the CNS or peripheral nerves. Transient lower body weight gains were noted for males exposed to 10 mg/L, but terminal weights were within 5% of control values. Irregular oestrus cycles were seen in all treated groups and a shift in cycle length from 4 to 5 days was observed in females exposed to the highest concentration. There were no treatment related histopathology findings, including in the thymus. In bronchoalveolar lavages, the cell counts, total protein and lactate dehydrogenase activities were not changed. The analysis of thyroid hormones (T_3 and T_4) was unchanged.

The 10 mg/L dose was associated with some effects that were considered to be non-adverse and included transient unsteady gait and excessive salivation which were observed at routine observations; during the FOB in week 13 abnormal gait/posture was also noted in females at this dose level. Liver weight was also slightly elevated at 4.5 and 10 mg/L, but there was no corresponding histopathology, and the observation was again considered to be non-adverse. Although it was not quantified, inhalation exposure may increase the systemic exposure to the parent compound, 2-MeOx, compared to oral exposure where the majority of the parent compound is subject to first pass metabolism. As clinical signs following oral exposure to 1000 mg/kg/day were restricted to excessive salvation, the observation of unsteady gait following inhalation exposure is considered to be without relevance when considering oral exposure in humans.

The inhalation study did include sperm analysis, but the percentage of normal sperm was lower in all males, including controls. Microscopically, atrophy of the testis was noted in control and

treated animals and has previously been attributed to increased body temperatures and thermal stress during inhalation administration (Lee et al 1993); there was also no change in testes weight in the 13-week study by Parris (2017). There were no clear test material effects on sperm analysis. At 10 mg/L, estrous cycle monitoring found a slight increase in irregular cycling in the treated females compared to controls, and a shift in the regular cycle length, from 4 to 5 days was noted in the high dose group. As all females continued to cycle these findings were considered not to be adverse. There was also a slight increase in bodyweight gain in this female dose group.

Taking into consideration all studies, the oral NOEL for repeat dose exposure is considered to be 250 mg/kg/day.

Toxicokinetic assessment confirms that 2-MeOx is readily absorbed from the gastrointestinal tract and therefore the effects of unabsorbed material do not need to be investigated.

Greater detail on these studies is provided in Appendix G.

B.4.3 Genotoxicity

Summary of genotoxicity studies:

End point	Method	Results	Reference
in vitro bacterial mutation	Assay: bacterial reverse mutation assay (e.g. Ames test) (gene mutation) *S. typhimurium* TA 1535, TA 1537, TA 98, TA 100 and TA 102 (met. act.: with and without) Guideline: equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Evaluation of results: negative (with and without metabolic activation) Test concentrations: 10-10000 µg/plate	Seifried, H.E. *et al* (2006) NTP data summary
	Assay: bacterial reverse mutation assay (e.g. Ames test) (gene mutation) *S. typhimurium* TA 1535, TA 1537, TA 98, TA 100 and *E. coli* WP2 (met. act.: with and without) Guideline: OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Evaluation of results: negative (with and without metabolic activation) Test concentrations: up to 5490 µg/plate	Antonucci V; Coleman J; Ferry JB; Johnson N; Mathe M; Scott JP (2011)
in vitro mammalian mutation	Assay: mammalian cell gene mutation assay (gene mutation) mouse lymphoma L5178Y cells (met. act.: with and without) Guideline: equivalent or similar to OECD Guideline 476 (*In vitro* Mammalian Cell Gene Mutation Test)	Evaluation of results: negative (with and without metabolic activation) Test concentrations: 63.75 - 1020 µg/ml	Harlan 2013e

End point	Method	Results	Reference
	Assay: mammalian cell gene mutation assay (gene mutation) mouse lymphoma L5178Y cells (met. act.: with and without) Guideline: equivalent or similar to OECD Guideline 476 (*In vitro* Mammalian Cell Gene Mutation Test)	Evaluation of results: negative (with and without metabolic activation) Test concentrations: 1500-5000 µg/ml	Seifried, H.E. *et al* (2006)
in vitro mammalian micronucleus	Assay: human lymphocytes (chromosome aberration) (met. act.: with and without) Guideline: OECD Guideline 487 (Micronucleus Test in Human Lymphocytes in vitro)	Evaluation of results: negative (with and without metabolic activation) Test concentrations: up to 10 mM	Envigo, 2019b
in vitro mammalian cytogenicity	Assay: *in vitro* mammalian chromosome aberration test (chromosome aberration) lymphocytes: peripheral human (met. act.: with and without) Guideline: OECD Guideline 473 (*In vitro* Mammalian Chromosome Aberration Test)	Evaluation of results: negative (with and without metabolic activation) Test concentrations: up to 10.7 mM	Antonucci V; Coleman J; Ferry JB; Johnson N; Mathe M; Scott JP (2011)
in-vivo micronucleus	Assay: micronucleus assay (chromosome aberration) rat male/female Guideline: OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	Evaluation of results: negative Oral dose: gavage up to 26 mg/kg/day	Antonucci V; Coleman J; Ferry JB; Johnson N; Mathe M; Scott JP (2011)

The genotoxicity of 2-MeOx has been investigated by the National Toxicology Programme (NTP) and by the pharmaceutical company Merck.

Seifried et al. (2006) presents a review of historical genotoxicity data conducted by the NTP; only summary results are presented in the paper, but these confirm that 2-MeOx was negative in the Ames test for bacterial mutagenicity and the Mouse Lymphoma Assay for mammalian mutagenicity. The actual study data for the Ames test is also available on the NTP web site for independent review. The NTP works to standard protocols and the data generated is considered to be suitable for risk assessment, so these data are considered reliable and demonstrate a lack of mutagenicity in bacterial and mammalian cell systems.

Antonucci (2011) summarised the comprehensive investigation of genotoxicity conducted by Merck. These studies were done to GLP and to OECD guidelines, they were conducted to support the use of 2-MeOx as a greener solvent for pharmaceutical manufacture. Unfortunately, the individual data is not presented for review in the publication, but they concluded that 2-MeOx was negative in the Ames test, did not induce cytogenicity in human lymphocytes, and did not increase

the incidence of micronuclei in an in vivo test in rats. The individual data cannot be reviewed independently, but the conclusion supports that of Seifried (2006) that 2-MeOx is not genotoxic.

Gentoxicity studies are summarised briefly below, with additional detail provided in Appendix G.

B.4.3.1 In vitro bacterial mutation

Briefly, Seifried describes an Ames test (pre-incubation method) conducted using S. typhimurium TA 1535, TA 97, TA 98 and TA 100 with and without metabolic activation (Aroclor induced rat liver S9 or Hamster S9 at 10 and/or 30%) at doses of 10-10000 µg/plate. The vehicle was water. A maximum dose of 10,000 µg/plate was used, and the test was not limited by cytotoxicity. All results were negative (with and without metabolic activation). Antonucci et al. (2011).

Antonucci et al. (2011) describes an Ames test conducted using S. typhimurium strains TA 1535, TA 1537, TA 98, TA 100 and E. coli WP2uvrA at up to 5490 µg/plate. The plate incorporation method was used with and without S-9 metabolic activation; sue to the volatility of the test item plates were sealed in air-tight bags during incubation. The sensitivity of the system and activity of the S9 mix was demonstrated by the results of positive controls. No substantial increases in the revertant colony counts were observed and there was no apparent toxicity to the bacteria. There was no evidence of mutagenic activity in this in vitro guideline test bacterial mutations.

B.4.3.2 Mammalian gene mutation test

The mutagenic potential of 2-methyloxolane (purity 99.5%) was investigated in the TK+/TK- gene mutation assay in L5178Y mouse lymphoma cells. The study (Harlan 2013e) was designed to be compatible with the OECD TG 476 (OECD, 1997b) and GLP. Two experiments were performed using the same protocol (same dose range in the presence and absence of metabolic activation), with the exception of the amount of rat liver S9-mix (1% vs. 2%) and the increased time of incubation (4 vs. 24 h) in the absence of metabolic activation in the second experiment. The doses of the test item (63.75, 127.5, 255, 510, 765, 1,020 µg/mL) were chosen based on the results of a preliminary cytotoxicity test.

No precipitation of the test item was observed at any dose level. No evidence of marked toxicity, as measured by relative suspension growth (% RSG) and relative total growth (% RTG) was observed in any experimental condition. 2-Methyloxolane did not induce any increase in the mutation frequency at any tested dose with or without metabolic activation in either experiment. No variation in the percentage of large and small colonies was observed in any experimental condition.

The test item did not induce any increase in mutation frequency at the TK+/TK- locus in L5178Y cells under the experimental conditions employed in this study. That is, there was no evidence of mutagenicity in mammalian cells at concentrations up to the limit of 10 mM (1020 µg/ml).

B.4.3.3 In vitro micronucleus test

Envigo (2019b) describes an in vitro micronucleus assay carried out in human peripheral blood lymphocytes according to OECD TG 487 (OECD, 2016) and following GLP. In a preliminary cytotoxicity test, the highest concentration recommended by the OECD guideline (10 mmol/L, equivalent to 860 lg/mL) did not induce relevant toxic effects. 2-Methyloxolane was then tested at

0, 26.88, 53.75, 107.5, 215, 430 and 860 lg/mL. No precipitation of the test item was observed in the cultures at the end of the exposure at any dose level in any exposure group.

Three exposure conditions were used in two experiments: (1) 4 h without metabolic activation (S9-mix); (2) 24 h without S9-mix; (3) 4 h with 2% S9-mix. To induce binucleated cells, cytochalasin B (4 µg/mL) was added during the recovery period (24 h in all the exposure conditions). No toxicity was observed in any experimental condition. No increase in the micronucleus frequency was observed after treatment with 2-methyloxolane in any experimental condition.

The test item did not induce any increase in micronuclei in cultured human peripheral blood lymphocytes under the experimental conditions employed in this study.

B.4.3.4 Other tests

Antonucci et al. (2011) describes two studies performed by Merck assessing effects of 2-MeOx on chromosomal aberration in human peripheral blood lymphocytes and an in vivo micronucleus assay conducted on rat bone marrow. No evidence of toxicity and no statistically significant increases in the proportion of cells with chromosome aberrations were found in human blood lymphocytes that were stimulated into division in culture and treated with 2-MeOx at up to 1.7 mM. 2-MeOx was also found to not induce micronuclei in rat bone marrow after 3 months treatment at up to 25 mg/kg/day

B.4.3.5 Genotoxicity conclusion

2-MeOx did not induce gene mutations in bacteria either in the presence or the absence of metabolic activation (Ames test). Negative results were also reported in two well-conducted in vitro studies in mammalian cells: an in vitro micronucleus assay carried out in human peripheral blood lymphocytes and a gene mutation assay at the TK+/- locus in L5178Y mouse lymphoma cells. 2-MeOx also returned negative results for chromosomal aberrations in human peripheral blood lymphocytes and did not induce micronuclei in rat bone marrow.

Pennakem considers 2-MeOx does not raise a concern for genotoxicity. This view has been supported in EFSA's assessment (EFSA 2022).

B.4.4 Reproductive and developmental toxicity

In the oral 90-day study in the rat (Parris et al. 2017) there was no reported effect on the weight of the reproductive organs, or histopathological changes in either sex at a dose of 1000 mg/kg/day.

In the 13-week inhalation study (Envigo 2019a), atrophy of the testis was noted in control and treated animals and sperm analysis found the number of normal sperm to be lower in all groups, including the controls. Testicular atrophy has previously been attributed to increased body temperatures and thermal stress during inhalation administration (Lee et al 1993). There were no clear test material effects on sperm analysis. At 10 mg/L, estrous cycle monitoring found a slight increase in irregular cycling in the treated females compared to controls, and a shift in the regular cycle length, from 4 to 5 days was noted in the high dose group. As all females continued to cycle these findings were considered not to be adverse. There was also a slight increase in bodyweight gain in this female dose group.

2-MeOx is readily absorbed from the gastro-intestinal tract and so Tier 2 reproductive and developmental testing should be considered. The usual strategy for this assessment is a prenatal developmental toxicity study (OECD 414) in the rabbit and an Extended One-Generation Reproductive study (EOGRTS) (OECD 443) in the rat. As part of the testing required for Regulation (EC) No 1907/2006 a prenatal developmental study (OECD 414) in the rat has been conducted. An EOGRTS, including all 3 optional cohorts, has also recently been completed.

Table B.4.4-1. Summary of Reproductive toxicity studies

End point	Method	Results	Reference
Preliminary Developmental toxicity Oral	Range-finding study rat (Sprague-Dawley Crl:CD (SD) IGS BR) female (oral gavage) 0, 250, 500 and 1000 mg/kg/day Exposure: Gestation Days 3 to 19	Maternal NOAEL: 1000 mg/kg/day Foetal NOEL: 500 mg/kg/day (reduced foetal weight)	Envigo (2018b)
Developmental toxicity Oral	OECD 414 rat (Sprague-Dawley Crl:CD (SD) IGS BR) female subchronic (oral gavage) 0, 100, 300 and 1000 mg/kg/day Exposure: Gestation Days 3 to 19	Maternal NOAEL: 1000 mg/kg/day (transient bwt reduction) Fetal NOAEL: 1000 mg/kg/day (slight reduction in fetal bwt) Fetal NOEL: 300 mg/kg/day	Envigo (2018c)
Preliminary Reproduction and Fertility	Range-finding study rat (Sprague-Dawley RjHand:SD) male and female (oral gavage) 0, 100, 300 and 1000 mg/kg/day Exposure: 2 weeks prior to mating and then continuously until termination of parental generation on day 21 post-partum and F1 offspring on Days 22 – 28.	NOAEL:100 mg/kg/day (pup survival, clinical effects and reduced pup bodyweight, minor effects at 300 g/kg/day)	Charles River (2020a)
Reproduction and Fertility (EOGRTS)	OECD 443 including all cohorts rat (Sprague-Dawley RjHand:SD) male and female (oral gavage) 0, 100, 250 and 625 mg/kg/day Exposure: 2 weeks prior to mating and then continuously until termination of parental generation, 1st generation or 2nd generation.	Systemic NOAEL males: 250 mg/kg/day (clinical signs at 625 mg/kg/day) Systemic NOAEL females: 625 mg/kg/day (no effects prior to parturition) Reproductive/developmental NOAEL: 100 mg/kg/day based on lower live birth index in parental generation, Developmental neurotoxicity testing NOAEL: 250mg/kg/day (highest dose fully tested) Developmental immunotoxicity testing NOAEL: 250mg/kg/day (highest dose fully tested)	Charles River (2020b)

EOGRTS: Extended One-Generation Reproductive Toxicity Study

Developmental toxicity was investigated in a GLP OECD 414 study in the rat. A clear NOAEL of 300 mg/kg/day for the survival, growth and development of the conceptus was identified. Observations at 1000 mg/kg/day were limited to a transient decrease in maternal bodyweight gain

(which was insufficient to exclude this dose level form being the maternal NOAEL) and a very slight reduction in foetal growth as evidenced by a 4 % decrease in mean foetal weight. Most importantly there were no internal or external effects on foetal development.

An EOGRTS, with all 3 possible cohorts, assigned a NOAEL of 100 mg/kg/day for reproduction and development; there were no effects on developmental neurotoxicity or immunotoxicity. Interpretation of the study was made more difficult because of the high prevalence of dystocia (difficult labour) which was noted in all groups including the controls. This is a very uncommon finding and was not a usual background observation in the laboratory; however, it was noted in a number of studies conducted in the same time period as the EOGRTS.

Although there were fewer pups at the 625 mg/kg/day dose level to fully investigate all 3 cohorts, there were sufficient at 250 and 100 mg/kg/day to fulfil the study requirements. The NOAEL of 100 mg/kg/day is based on the reduced live birth index observed at 250 mg/kg/day in the parental generation, this finding was not repeated at 250 mg/kg/day when the F1 generation gave birth. Hence a NOAEL of 100 mg/kg/day was considered a conservative value as the possible influence of the dystocia could not be determined.

B.4.4.1 Reproductive and developmental toxicity conclusions

Table B. 4.4.1-1 summarises the NOAELs, based on the EOGRT study described above. EFSA supported these NOAELs in its recently published opinion on 2-MeOx (EFSA 2022).

Table B.4.4.1-1. NOAELs identified by endpoint in the EOGRT study

Endpoint	NOAEL (mg/kg bw per day)	Clinical observations
Systemic toxicity	250 (in males)	Hypoactivity, staggering gait, sudden startle and/or tremors at 625 mg/kg bw per day
	625 (in females)	No effects found before parturition
Reproductive and developmental toxicity	100	A decrease in female fertility index in cohorts 1A and 1B at 250 mg/kg bw per day
Developmental neurotoxicity	250	No effects (highest dose tested in cohort 2B)
Developmental immunotoxicity	250	No effects (highest dose tested in cohort 3)

NOAEL: no observed adverse effect level; EOGRT: extended one-generation reproductive toxicity; bw: body weight.

Additional detail on reproductive and developmental toxicity studies is described in Appendix G.

B.4.5 Other toxicity studies

A variety of other toxicity studies have been conducted on 2-MeOx, primarily in the context of its use as an industrial chemical by workers. Studies investigating acute, in vitro and in vivo dermal toxicity, in vitro eye irritation and skin sensitisation do not raise concerns in the context of oral exposure to 2-MeOx, however details of these studies is included in Appendix H.

B.4.6 Overall conclusion on the safety of proposed uses of 2-MeOx

The data generated from animal models indicate that following oral exposure we can expect that 2-MeOx is rapidly, and almost completely, absorbed from the gastrointestinal tract. Excretion is via urine or as exhaled carbon dioxide; unchanged 2-MeOx is eliminated from the lungs only following exposure at very high doses (over 100 mg/kg/day in the rat), when metabolism is overwhelmed.

Acute oral and dermal toxicity is low and 2-MeOx was found to be irritating to the skin and eyes, but is not a skin sensitiser.

2-MeOx was negative in a range in vitro mutagenicity tests (bacterial and mammalian cells) and in an in vivo micronucleus test.

A comprehensive 13 weeks oral (gavage) study in the rat was completed to support the use of 2-MeOx for pharmaceutical manufacture using dose levels of 0, 80, 250, 500 and 1000 mg/kg/day. There was a slight decrease in male weight gain at 1000 mg/kg/day and the following reversible changes, associated with the liver were noted: increased serum cholesterol, increased liver weight and centrilobular hypertrophy. At 500 mg/kg/day changes were limited to increased liver weight. It is likely that these reversible changes are adaptive and associated with metabolism at high doses, but a conservative No Observed Effect Level of 250 mg/kg/day was established.

A nose-only inhalation study was also conducted and 10.0 mg/L was well tolerated and considered to be the NOAEC. This dose level was associated with some effects that were considered to be none adverse and included transient unsteady gait and excessive salivation which were observed at routine observations; during the FOB in week 13 abnormal gait/posture was also noted in females at this dose level. Liver weight was also slightly elevated at 4.5 and 10 mg/L, but there was no corresponding histopathology, and the observation was again considered to be non-adverse. Although it was not quantified, inhalation exposure may increase the systemic exposure to the parent compound, 2-MeOx, compared to oral exposure where the majority of the parent compound is subject to first pass metabolism. As clinical signs following oral exposure to 1000 mg/kg/day were restricted to excessive salvation, the observation of unsteady gait following inhalation exposure is considered to be without relevance when considering oral exposure in man.

In neither the 13-week oral nor inhalation studies were there any pathological changes in the reproductive organs. Sperm analysis was conducted in the 13-week inhalation study but the percentage of normal sperm was lower in all dose groups, including the controls and this has previously been attributed to increased body temperatures and thermal stress during inhalation administration (Lee et al 1993). In an oral (gavage) developmental toxicity study conducted in the rat there were no external, visceral or skeletal changes at doses up to 1000 mg/kg/day associated with 2-MeOx exposure. The NOAEL for the females was considered to be 300 mg/kg/day due to a slight decrease in weight gain at 1000 mg/kg/day; at 1000 mg/kg/day this resulted in a slight reduction in foetal weight but without an effect on survival or development. The results of an Extended One Generation Reproductive Toxicity Study support the NOAEL of 250 mg/kg/day for systemic toxicity; a high dose of 625 mg/kg/day was associated with some clinical signs.

The study included an assessment of developmental neurotoxicity and immunotoxicity where no signs were observed at 250 mg/kg/day; this was set as the NOAEL because not all investigations could be conducted at the higher dose of 625 mg/kg/day. The assessment of reproductive toxicity was confounded by a high background incidence of dystocia which was concluded to be a new genetic trend in the strain used as it also occurred in control animals. At 250 and 625 mg/kg/day there was a reduction in live birth index for the Parental generation; this was not repeated in the F1 generation and there were no other adverse effects on reproductive performance at 250 mg/kg/day. Consequently, a conservative NOEL of 100 mg/kg/day was selected. Therefore, Pennakem considers that 100 mg/kg/day provides a robust overall NOAEL for risk assessment from dietary exposure.

Pennakem has derived an acceptable daily intake (ADI) of 1.0 mg/kg/day (taking into account an uncertainty factor of 100 (x10 animal-to-human based on robust data base and x10 intra species sensitivity)).

B.5 Safety assessment reports by other agencies

European Food Safety Authority

The European Food Safety Authority (EFSA) Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) published a safety assessment of Pennakem's 2-MeOx early in 2022 (EFSA 2022). The CEP assessed the safety of 2-MeOx as an extraction solvent at the following intended uses and proposed maximum residue limits:

Name	Conditions of use (summary description of extraction)	MRLs in the extracted foodstuff or food ingredient
2-methyloxolane	Production or fractionation of fat, oil or butter	1 mg/kg in the fat, oil or butter
	Preparation of defatted protein product, defatted flour, and other defatted solid ingredients	10 mg/kg in the food containing the defatted protein product, defatted flour or other defatted solid ingredients
	Defatted protein product, defatted flour, and other defatted solid ingredients for use in category 13 products[(a)]	1 mg/kg in the food of the category 13 products containing the defatted protein product, defatted flour or other defatted solid ingredients

These limits are similar to the permitted residue limits for hexane in the EU and recognise that the intended use of 2-MeOx is in processes that currently use hexane.

The CEP concluded that 2-MeOx is rapidly metabolised with a low bioaccumulation potential and does not raise genotoxicity concern. The CEP set a tolerable daily intake (TDI) of 1 mg/kg bw/day, based on the lowest identified NOAEL (100 mg/kg bw/day) for reproductive and developmental toxicity in oral toxicity studies. The TDI was not exceeded by any population groups at mean and 95th percentile dietary exposure. The CEP concluded that 2-MeOx does not raise a safety concern when used according to the intended uses and at the proposed MRLs in extracted foods and food ingredients.

In this application, Pennakem is requesting that FSANZ establish residue limits for 2-MeOx that correspond to the limits for hexane in section S18—8 of the Code; that is, 20 mg/kg. This is higher than the residue levels assessed by the CEP and is only requested by Pennakem because hexane is permitted to be present as a residue in foods at a higher level than in the EU. As noted in section 3.3.2.F of this application, a residue limit of 20 mg/kg in foods for 2-MeOx will facilitate the transition from hexane to 2-MeOx in existing extraction plants in Australia and New Zealand. Pennakem has conducted additional dietary modelling at the 20 mg/kg residue level to demonstrate that dietary exposure is still below the TDI of 1 mg/kg (more detail on dietary modelling assumption and results is provided in section F below).

International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) has conducted an assessment on 2-MeOx in the context of the use of 2-MeOx as a solvent in the preparation of pharmaceutical products. The ICH publishes a document titled, QC3(R8) 'Impurities: Guideline for residual solvents'. The ICH has recently (May 2021) updated the guideline to list 2-MeOx as a class 3 solvent (solvents with low toxic potential). The ICH uses a risk assessment process to categorise solvents used in the production of pharmaceutical products. As part of the ICH's consideration of classifying 2-MeOx, a Permitted Daily Exposure (PDE) was calculated (Parris et al. 2017). To enable the calculation of a PDE a GLP compliant 3-month repeat-dose toxicity study in rats (with a 1-month recovery period) was conducted using dose levels of 0, 80, 250, 500 and 1000 mg/kg/day (Parris *et al*. 2017). Administration of doses up to 1000 mg/kg/day was well tolerated and based upon minimal observed effects of hepatocellular centrilobular hypertrophy in the liver at 500 mg/kg/day and above, the NOAEL was considered to be 250 mg/kg/day. Using a safety factor of 250 a PDE of 50 mg/day (for an average 50 kg human) was derived to support usage in the pharmaceutical industry. This value was endorsed in the final ICH guideline (which concluded that the PDE of 50 mg/kg/day was applicable and placed the 2-MeOx into Class 3 'Solvents with low toxic potential'.

The pharmaceutical PDE value was calculated in the absence of any reproductive toxicity data. This application takes into account the possible effect on reproduction and has derived a lower NOEL of 100 mg/kg/day. Using this NOEL value, the PDE is re-calculated as follows, based on ICH Topic Q3C (R4) Impurities: Guideline for Residual Solvents (ICH 2009):

$$\text{PDE} = \frac{100 \text{ mg/kg/day} \times 50 \text{ Kg}}{5 \times 10 \times 1 \times 1 \times 1} = 100 \text{ mg/day}$$

F1 = 5 to account for extrapolation from rats to humans
F2 = 10 to account for differences between individual humans
F3 = 1 for reproductive studies in which the whole period of organogenesis is covered.
F4 = 1 because no severe effects were observed
F5 = 1 because a NOEL was established

In the original calculation an F3 value of 5 was used, to reflect that the NOEL was based only on 3-month data. The re-calculated value of 100 mg/day is higher than 50 mg/day and confirms that 2-MeOx should be in Class 3 'Solvents with low toxic potential' (all the solvents having a PDE >= 50 mg/day).

The study by Parris et al. (2017) is described in more detail in sections B.4.2 and B.4.4 above. No other regulatory evaluations or limits have been identified.

3.3.2.F INFORMATION RELATED TO THE DIETARY EXPOSURE TO THE PROCESSING AID

2-MeOx is a suitable replacement for the use of hexane in oil and protein extraction from vegetative sources (for example, corn, sunflower, rapeseed, soy etc). 2-MeOx could also be used to replace hexane and other solvents in food additives extraction (flavours, colours, antioxidants). 2-MeOx is not intentionally added to any food, but consideration must be given to the possible residues in processed foods. Extraction plants that currently use hexane as an extraction solvent will be producing food products that are compliant with the maximum permitted level of hexane, which is 20 mg/kg.

In order for these extraction plants to be able to transition to the use of 2-MeOx as a substitute for hexane, similar maximum permitted levels will initially be required. That is, a maximum permitted level of 20 mg/kg for 2-MeOx in foods will facilitate transition for extraction plants from using hexane to using 2-MeOx as an extraction solvent.

This strategy is inspired by the European Commission feedback to Pennakem's approval request for 2-MeOx in food. In the current discussions for the modification of the European law (Directive 2009/32/CE), the European authorities proposes for the 2-methyloxolane, the same Maximum Residue Limits (MRL) than the one in force for hexane.

It is likely that actual 2-MeOx residue levels in food will be below 20 mg/kg, particularly as uptake in the use of 2-MeOx increases and due to organoleptic reasons described below.

Extraction plants that are purpose built to use 2-MeOx as an extraction solvent will result in lower residue levels of 2-MeOx in final food products. However, the cost of constructing a purpose built 2-MeOx extraction plant may initially be prohibitive for in Australia and New Zealand.

In practice, for organoleptic reasons, in beverage or liquid food, food producers may have to limit the quantity of 2-MeOx residue to less than 1 mg/kg. For fats and oils, organoleptic tests have shown that levels as low as 5 mg/kg are detectable and associated with a poor taste. Sensory tests show that, unlike hexane, which is known to have a high smell and taste threshold, traces of 2-MeOx can be detected as low as 5 mg/kg. Two sensory tests were conducted, according to ISO 11035, using standard edible soy oil and rapeseed oil spiked with known quantities 2-MeOx (0, 0.1, 0.25, 0.5, 1 and 5 mg/kg) (ITERG 2018a, ITERG 2018b). The only residue associated with poor taste was 5 mg/kg. Level of residues below 1 mg/kg can be reached without any difficulties in liquids by a standard refining process and delivers oils with good organoleptic properties.

The smell and taste thresholds are higher in solid food than in liquid. The threshold depends on the composition of the food, but it is always lower than for hexane.

From a public health point of view, unlike hexane which cannot be detected in an oil even at level as high as 25 mg/kg, the low taste threshold for 2-MeOx brings additional safety to prevent producers from selling oils from which EcoXtract® has not been properly removed. The taste threshold has not been fully characterised in solid food, but it is well known for aroma that the taste threshold in solid food is higher than in liquid food. Moreover, the smell of the 2-MeOx is described in the same report as strong and unpleasant. The medium ODT combined with the bad taste will represent a strong incentive to remove properly the 2-MeOx from the liquid and solid extraction products.

Pennakem has conducted conservative dietary exposure estimates, based on dietary intake data in the EU and on the presence of 2-MeOx in foods at 20 mg/kg and compared these estimates to the TDI in humans of 1 mg/kg bw/day (set in the EFSA CEP's assessment (EFSA 2022)). Pennakem expects these estimates to be similar for Australian and New Zealand populations but does not have access to similarly detailed dietary intake data for local populations. Pennakem's initial dietary exposure estimates identified that the presence of 2-MeOx in infant formula and foods for infants may present a scenario where high consumers are above the TDI. Reducing the presence of 2-MeOx in infant formula products and foods for infants to 5 mg/kg reduced the estimate of dietary exposure to below the ADI for high consumers (up to 0.8 mg/kg bw/day at the 95th percentile for infants).

2-MeOx is not likely to be used in all foods because the production of food does not always require the use of extraction solvents, such as 2-MeOx. Based on available market data, the likely use pattern of food subject to this type of processing and the proposed limits defined above, Pennakem considers the food categories and limits described in section F.1 are most likely representative of intended future uses of 2-MeOx in Australia and New Zealand (food categories are based on the food classification system for food additives described in Schedule 15 of the Code). Table F.1-1 lists the food groups and maximum residue levels of 2-MeOx in these foods. This list provides a more complete picture of potential uses and residues of 2-MeOx in foods for use by FSANZ in its dietary exposure assessment (and reflects the levels used in Pennakem's dietary exposure assessment using EU dietary intake data).

Despite the conservative nature of the dietary exposure assumptions, the presence of 2-MeOx in foods at 20 mg/kg does not present a risk to consumers.

F.1 A list of foods or food groups likely to contain the processing aid

Pennakem is requesting a residue limit for 2-MeOx in foods of 20 mg/kg, with the exception of infant formula and foods for infants, which are requested to have a lower residue limit of 5 mg/kg. For the purposes of dietary modelling, Pennakem has prepared a list of food categories that are most likely to be subject to the use of 2-MeOx as an extraction solvent (Table F.1-1).

Table F.1-1. Representative intended maximum use levels of 2-MeOx in food categories

Number	Name	Proposed limit (ppm)
01.6	Cheese and cheese products	20
2	Edible oils and fat and oil emulsions	20
3	Ice cream and edible ices	20
4.3	Processed fruits and vegetables	20
5.1	Chocolate and cocoa products	20
	Cocoa butter only	20
5.2	Sugar confectionery	20
5.4	Icings and frostings	20
6.3	Processed cereal and meal products	20
6.4	Flour products – pasta (potato gnocchi)	20
6.4	Flour products – noodles	20
7.1	Bread and related products	20
7.2	Biscuits, cakes and pastries	20
8.1	Raw meat, poultry and game	20
8.2	Processed meat, poultry and game products in whole cuts or pieces	20
8.3	Processed comminuted meat, poultry and game products (other than sausage and sausage meat containing raw, unprocessed meat)	20
8.4	Edible casings	20
8.5	Animal protein products	20
9.2	Processed fish and fish products (including molluscs and crustaceans)	20
12	Salts and condiments - mustard	20
12.9	Protein products, excluding products covered in category 1.8	20
13.1	Infant formula products	5
13.2	Foods for infants	5
13.3	Formulated meal replacements and formulated supplementary foods (except food for infants)	20
13.5	Food for special medical purposes	20
14.1.5.1	Coffee, coffee substitutes, tea, herbal infusions and similar products	20
14.2.1	Beer and related products	20
14.2.4	Mead	20
20	Foods not included in items 0 to 14 – potato-, cereal-, flour- or starch-based snacks	20
20	Foods not included in items 0 to 14 – processed nuts	20

F.2 The levels of residues of the processing aid for each food or food group

Information on levels of residues of 2-MeOx in foods is included in section F.1 above.

F.3 Likely level of consumption of foods not listed in ANZ NNSs

Demand for plant-based, or alternative, proteins as substitutes for traditional animal-based proteins has increased recently worldwide. However, the market is still considered small domestically, particularly compared to the US (Admassu et al. 2020). According to a report from Admassu et al. (2020), the largest market share for alternative proteins is dairy milk analogues (9% in Australia). While consumption is reported in Australian and New Zealand national nutrition surveys for products such as nut milks, it is possible that increased consumption of some more contemporary foods based on plant proteins is not reflected in this consumption data. However, on a population level, Pennakem does not expect the impact of this to be significant in the context of dietary exposure calculations for 2-MeOx (or other extraction solvents) at the present time.

F.4 Percentage of food group in which 2-MeOx is likely to be used

Pennakem's production capacity is currently only a very small fraction of the production of hexane around the world. Approximately 1.1 million tonnes of hexane is produced worldwide per year. Pennakem currently produces 5000 tonnes of 2-MeOx per year. Pennakem has projected less than 3% market share of the chemical extraction market on a global scale, based mainly on the production capacity of 2-MeOx. Pennakem considers the market situation in Australia and New Zealand is likely to mirror the global situation, suggesting that market penetration of the use of 2-MeOx as a processing aid in comparison to hexane is unlikely to exceed 3% of the market in the short to medium term. As production capacity increases, market share could also increase proportionately.

F.5 Information relating to levels of residues in foods in other countries

It is important to note that in Europe, the Maximum Residue Levels- (MRLs) authorised for hexane are much lower than the one in force in Australia and New Zealand. These hexane MRLs are reported in the 2009/32/CE Directive as follows:

In Annex I to Directive 2009/32/EC:

(a) In Part II, the following entry is found:

Hexane	Production or fractionation of fats and oils and production of cocoa butter	1 mg/kg in the fat or oil or cocoa butter
	Preparation of defatted protein products and defatted flours	10 mg/kg in the food containing the defatted protein products and the defatted flours
		30 mg/kg in the defatted soya products as sold to the final consumer
	Preparation of defatted cereal germs	5 mg/kg in the defatted cereal germs

(b) In Part III, hexane is inserted in the following table:

Name	Maximum residue limits in the foodstuff due to the use of extraction solvents in the preparation of flavourings from natural flavouring materials
Hexane	1 mg/kg

Based on the March 2022 EFSA positive opinion and the Tolerable Daily Intake already mentioned, the European Authority proposes to modify the law as follows:

Annex I to Directive 2009/32/EC is amended as follows:

(a) In Part II, the following new entry is inserted after the entry for hexane:

,

2-methyloxolane	*Production or fractionation of fats and oils and production of cocoa butter*	*1 mg/kg in the fat or oil or cocoa butter*
	Preparation of defatted protein products and defatted flours	*10 mg/kg in the food containing the defatted protein products and the defatted flours*
		30 mg/kg in the defatted soya products as sold to the final consumer
	Preparation of defatted cereal germs	*5 mg/kg in the defatted cereal germs*

(b) In Part III, the following new entry is inserted after the entry for hexane:

2-methyloxolane	*1 mg/kg*

The amended law should be published at the beginning of 2023.

F.6 Information on likely levels of consumption of foods for which consumption has changed in recent years

Section F.3 has addressed this in the context of increasing popularity of plant-based foods.

F.7 Additional dietary exposure information

As noted in the introduction to section 7, Pennakem has completed dietary exposure calculations for 2-MeOx based on the food categories and residues listed in Table F.1-1. A complete list the food categories and residue levels used in Pennakem's dietary exposure calculations are provided below in Table F.7-2 and is based on EFSA's food additive intake model (FAIM).

The dietary exposure estimates are listed in Table F.7-1. The results are presented as minimum and maximum mean and minimum and maximum 95th percentile values for a range of population groups.

All average exposure levels are less than 50% of the TDI of 1 mg/kg bw/day. The max 95th percentile estimate of exposure for infants is the highest (0.839 mg/kg bw/day) but still below the TDI. Given the conservative nature of the assumptions used in the calculation of dietary exposure estimates, actual exposure to 2-MeOx from its use as an extraction solvent in the preparation of foods is likely to be significantly lower than presented in Table F.7-1, which reinforces the safety of use of 2-MeOx at the intended use levels of 5 mg/kg in infant formula products and infant foods and 20 mg/kg in other foods.

Table F.7-1. Minimum and maximum mean and 95th percentile dietary exposure estimates per population group (mg/kg bw/day)

Population Group	Min Average	Max Average	Min 95th	Max 95th
Infants	0.199	0.490	0.496	0.839
Toddlers	0.236	0.428	0.418	0.723
Other children	0.203	0.300	0.354	0.531
Adolescents	0.110	0.196	0.197	0.358
Adults	0.094	0.249	0.167	0.525
Elderly and very elderly	0.078	0.273	0.150	0.532

Notes on assumptions:

These dietary exposure estimates are likely to be overestimates of actual dietary exposure to residues of 2-MeOx. The estimates make a number of conservative assumptions:

- 2-MeOx is used at a level resulting in residues of 20 mg/kg in all foods (and 5 mg/kg in infant formula products and foods for infants).
    - actual use levels are likely to result in much lower residue levels; and
    - not all foods in each category will use 2-MeOx as an extraction solvent.
- Consumers only consume foods containing 2-MeOx residues at the maximum levels
    - As noted above, not all foods will be produced using 2-MeOx as an extraction solvent because other extraction solvents are permitted and available to food producers.
- The EU food consumption data is based on one or two days of consumption which can result overestimates of the habitual consumption of foods.

The food categories listed in Table F.7-2 are based on the EU food categorisation system. The food categories listed in Table F.1-1 have been adapted from the EU categorisation system to reflect the categories used in Australia and New Zealand.

Table F.7-2. Occurrence level per food category (mg/kg)

Food Category		Occurrence level (mg/kg)
01.1	Unflavoured pasteurised and sterilised (including UHT) milk	0.00
01.2	Unflavoured fermented milk products, including natural unflavoured buttermilk (excluding sterilised buttermilk) non heat-treated after fermentation	0.00
01.4	Flavoured fermented milk products including heat-treated products	0.00
01.5	Dehydrated milk as defined by Directive 2001/114/EC	0.00
01.6	Cream and cream powder	
01.6.1	Unflavoured pasteurised cream (excluding reduced fat creams)	0.00
01.6.2	Unflavoured live fermented cream products and substitute products with a fat content of less than 20%	0.00
01.6.3	Other creams	0.00
01.7	Cheese and cheese products	0.00
01.7.1	Unripened cheese excluding products falling in category 16	0.00
01.7.2	Ripened cheese	0.00
01.7.4	Whey cheese	0.00
01.7.5	Processed cheese	20.00
01.7.6	Cheese products (excluding products falling in category 16)	0.00
01.8	Dairy analogues, including beverage whiteners	20.00
02.1	Fats and oils essentially free from water (excluding anhydrous milkfat)	20.00
02.2	Fat and oil emulsions mainly of type water-in-oil	20.00
02.2.1	Butter and concentrated butter and butter oil and anhydrous milkfat	20.00
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions	20.00
03	Edible ices	20.00
04.1	Unprocessed fruit and vegetables	0.00
04.1.1	Entire fresh fruit and vegetables	0.00
04.1.2	Peeled, cut and shredded fruit and vegetables	0.00
04.1.3	Frozen fruit and vegetables	0.00
04.2	Processed fruit and vegetables	20.00
04.2.1	Dried fruit and vegetables	0.00
04.2.2	Fruit and vegetables in vinegar, oil, or brine	20.00
04.2.3	Canned or bottled fruit and vegetables	0.00
04.2.4.1	Fruit and vegetable preparations excluding compote	0.00
04.2.4.2	Compote, excluding products covered by category 16	0.00
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EC	0.00
04.2.5.3	Other similar fruit or vegetable spreads	0.00
04.2.5.4	Nut butters and nut spreads	20.00
04.2.6	Processed potato products	20.00
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	20.00
05.2	Other confectionery including breath freshening microsweets	20.00
05.2.1	Other confectionery with added sugar	20.00
05.2.2	Other confectionery without added sugar	20.00
05.3	Chewing gum	0.00
05.3.1	Chewing gum with added sugar	0.00
05.3.2	Chewing gum without added sugar	0.00

Food Category		Occurrence level (mg/kg)
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	20.00
06.1	Whole, broken, or flaked grain	0.00
06.2	Flours and other milled products and starches	
06.2.1	Flours	20.00
06.2.2	Starches	0.00
06.3	Breakfast cereals	20.00
06.4	Pasta	20.00
06.4.1	Fresh pasta	20.00
06.4.2	Dry pasta	20.00
06.4.4	Potato Gnocchi	20.00
06.5	Noodles	20.00
06.6	Batters	20.00
06.7	Pre-cooked or processed cereals	20.00
07.1	Bread and rolls	20.00
07.2	Fine bakery wares	20.00
08.1	Fresh meat, excluding meat preparations as defined by Regulation (EC) No 853/2004	0.00
08.2	Meat preparations as defined by Regulation (EC) No 853/2004	20.00
08.3	Meat products	20.00
08.3.1	Non-heat-treated meat products	0.00
08.3.2	Heat–treated meat products	0.00
09.1.1	Unprocessed fish	0.00
09.1.2	Unprocessed molluscs and crustaceans	0.00
09.2	Processed fish and fishery products including molluscs and crustaceans	20.00
09.3	Fish roe	0.00
10.1	Unprocessed eggs	0.00
10.2	Processed eggs and egg products	0.00
11.1	Sugars and syrups as defined by Directive 2001/111/EC	0.00
11.2	Other sugars and syrups	0.00
11.3	Honey as defined in Directive 2001/110/EC	0.00
11.4	Table Top Sweeteners	0.00
11.4.1	Table Top Sweeteners in liquid form	0.00
11.4.2	Table Top Sweeteners in powder form	0.00
11.4.3	Table Top Sweeteners in tablets	0.00
12.1	Salt and salt substitutes	
12.1.1	Salt	0.00
12.2	Herbs, spices, seasonings	
12.2.1	Herbs and spices	0.00
12.2.2	Seasonings and condiments	0.00
12.3	Vinegars	0.00
12.4	Mustard	20.00
12.5	Soups and broths	0.00
12.6	Sauces	20.00
12.7	Salads and savoury based sandwich spreads	20.00
12.8	Yeast and yeast products	0.00
12.9	Protein products, excluding products covered in category 1.8	20.00

Food Category		Occurrence level (mg/kg)
13.1.1	Infant formulae as defined by Directive 2006/141/EC	5.00
13.1.2	Follow-on formulae as defined by Directive 2006/141/EC	5.00
13.1.3	Processed cereal-based foods and baby foods for infants and young children as defined by Directive 2006/125/EC	5.00
13.1.4	Other foods for young children	5.00
13.1.5.2	Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC	5.00
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	20.00
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	20.00
14.1.1	Water, including natural mineral water as defined in Directive 2009/54/EC and spring water and all other bottled or packed waters	0.00
14.1.2	Fruit and vegetable juices	0.00
14.1.2.1	Fruit juices as defined by Directive 2001/112/EC	0.00
14.1.2.2	Vegetable juices	0.00
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products	0.00
14.1.4	Flavoured drinks	0.00
14.1.4.1	Flavoured drinks with sugar	0.00
14.1.4.2	Flavoured drinks with sweetener	0.00
14.1.5	Coffee, tea, herbal and fruit infusions, chicory; tea, herbal and fruit infusions and chicory extracts; tea, plant, fruit and cereal preparations for infusions, as well as mixes and instant mixes of these products	
14.1.5.1	Coffee, coffee extracts	20.00
14.1.5.2	Other	0.00
14.2.1	Beer and malt beverages	0.00
14.2.2	Wine and other products defined by Regulation (EC) No 1234/2007, and alcohol free counterparts	0.00
14.2.3	Cider and perry	0.00
14.2.4	Fruit wine and made wine	0.00
14.2.5	Mead	
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	0.00
14.2.7.1	Aromatised wines	0.00
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol	0.00
15.1	Potato-, cereal-, flour- or starch-based snacks	20.00
15.2	Processed nuts	20.00
16	Desserts excluding products covered in category 1, 3 and 4	0.00
17	Food supplements as defined in Directive 2002/46/EC	0.00
17.1	Food supplements supplied in a solid form, excluding food supplements for infants and young children	20.00
17.2	Food supplements supplied in a liquid form, excluding food supplements for infants and young children	20.00

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