Australian Government
Department of Health
Australian Industrial Chemicals Introduction Scheme

2,5,8,11,14-Pentaoxapentadecane

Evaluation statement

28 January 2022

Draft

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AICIS evaluation statement

Subject of the evaluation

2,5,8,11,14-Pentaoxapentadecane (tetraglyme)

Chemical in this evaluation

Name	CAS registry number
2,5,8,11,14-Pentaoxapentadecane	143-24-8

Reason for the evaluation

The Evaluation Selection Analysis indicated a potential risk to human health.

Parameters of evaluation

The chemical is listed on the Australian Inventory of Industrial Chemicals (the Inventory). This evaluation is a human health risk evaluation for all identified industrial uses of the chemical.

Summary of evaluation

Summary of introduction, use and end use

There is currently no Australian specific information about the introduction and industrial use of tetraglyme.

Based on international use information, the chemical is used as solvent in synthesis reactions, as a functional fluid, as a lubricant and additive and in printing and publishing.

Although some possible domestic uses of the chemical in cleaning products, adhesives, paints and air fresheners have been reported internationally, based on available information these uses are not expected to be widespread.

Human health

Summary of health hazards

The critical health effects for risk characterisation include:

- effects on fertility and development following repeated oral and inhalation exposures.

The chemical is one member of a family of ethylene glycol ethers, known as “glymes”. It is water soluble and, based on the read across approach, it is expected to be well absorbed following oral and inhalation exposure and rapidly metabolised and excreted in urine. Due to the observed extensive metabolism, the bioaccumulation potential is estimated to be negligible. The metabolic pathway is expected to be similar to other glymes, leading to formation of 2-methoxyacetic acid and 2-methoxyethanol.

Based on the available data, the chemical is expected to cause specific adverse effects on fertility and development. Similar effects have been seen in studies with the other glymes.

Reproductive/developmental toxicity studies and a 28 day repeated dose study show adverse effects of tetraglyme on the testes (decreased testes weight and seminiferous tubular degeneration) and fertility (decreased sperm count increase mating time and decreased implantation sites). Small but significant effects occurred at 500 mg/kg bw/day in one reproductive toxicity study and fertility related findings were evident in 2/10 pairs at 300 mg/kg bw/day in another study A no observed adverse effect level (NOAEL) of 250 mg/kg bw/day was established in a 28 day repeated dose study in rats. Effects, mainly on the male reproductive organs and the haematopoietic system, were only observed in the 1000 mg/kg bw/day.

A reproductive/developmental toxicity and a prenatal developmental toxicity dose range finding study show adverse effects of tetraglyme on development in rats (increased post-implantation loss at $\geq$500 mg/kg bw/day and increased soft tissue and skeletal malformations at $\geq$250 mg/kg bw/day) in the absence of any significant maternal toxicity.

The chemical has low acute oral toxicity. Acute dermal and inhalation toxicity studies for tetraglyme are not available. However, based on acute toxicity data on structurally similar chemicals, triglyme and diglyme, the chemical can be expected to have low acute dermal and inhalation toxicity. Based on the available data, the chemical is not considered to be a skin or eye irritant. Using read across information on analogues of tetraglyme, it is not considered to be a skin sensitiser.

Based on the read across information from the analogues, the chemical is not considered to have genotoxic potential.

Health hazard classification

The chemical satisfies the criteria for classification according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (UNECE, 2017) for hazard classes relevant for work health and safety as follows. This does not consider classification of physical hazards and environmental hazards.

Health hazards	Hazard category	Hazard statement
Reproductive toxicity	Repr. 1B	H360FD: May damage fertility. May damage the unborn child.

Summary of health risk

Public

Based on its international use patterns, limited consumer use of the chemical is expected in Australia. Therefore there are no identified risks to the public that require management.

Workers

During product formulation and packaging, dermal and inhalation exposure might occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. Worker exposure to the chemical at lower concentrations could also occur while using formulated products containing the chemical. The level and route of exposure will vary depending on the method of application and work practices employed.

Given the critical systemic long term health effects, the chemical could pose a risk to workers. Control measures to minimise dermal and inhalation exposure are needed to manage the risk to workers (see **Proposed means for managing risk** section).

Proposed means for managing risk

Workers

Recommendation to Safe Work Australia

It is recommended that Safe Work Australia (SWA) update the Hazardous Chemical Information System (HCIS) to include classifications relevant to work health and safety.

Information relating to safe introduction and use

The information in this statement including recommended hazard classifications, should be used by a person conducting a business or undertaking (PCBU) at a workplace (such as an employer) to determine the appropriate controls under the relevant jurisdiction Work Health and Safety laws.

Control measures that could be implemented to manage the risk arising from exposure to the chemical include, but are not limited to:

- using closed systems or isolating operations
- using local exhaust ventilation to prevent the chemical from entering the breathing zone of any worker

- minimising manual processes and work tasks through automating processes
- work procedures that minimise splashes and spills
- regularly cleaning equipment and work areas
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemical.

Measures required to eliminate or manage risk arising from storing, handling and using a hazardous chemical depends on the physical form and the manner in which the chemical is used.

These control measures should be supplemented with:

- conducting health monitoring for any worker who is at significant risk of exposure to the chemical, if valid techniques are available to monitor the effect on the worker's health.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

Conclusions

The conclusions of this evaluation are based on the information described in this Evaluation Statement.

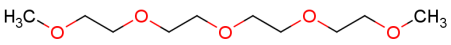
Considering the proposed means of managing risks, the Executive Director is satisfied that the identified human health risks can be managed within existing risk management frameworks. This is provided that all requirements are met under environmental, workplace health and safety and poisons legislation as adopted by the relevant state or territory and the proposed means of managing the risks identified during this evaluation are implemented.

Note: Obligations to report additional information about hazards under *Section 100 of the Industrial Chemicals Act 2019* apply.

Supporting information

Chemical identity

Chemical name	2,5,8,11,14-Pentaoxapentadecane
CAS No.	143-24-8

Synonyms	bis[2-(2-methoxyethoxy)ethyl tetraethylene glycol, dimethyl ether tetraglyme dimethoxytetraethylene glycol
Structural formula	
Molecular formula	C10H22O5
Molecular weight (g/mol)	222.3
SMILES	COCCOCCOCCOCCOC
Chemical description	Tetraglyme belongs to the group of polyglycol ethers, end capped with a methyl-, ethyl-, butyl- group (glymes). The lack of reactive functional groups makes glymes inert chemically; hence, they are often used in chemical synthesis applications.

Relevant physical and chemical properties

Physical form	Liquid, at room temperature
Melting point	-30 °C
Boiling point	275.3 °C (Chemwatch)
Vapour pressure	0.1 Pa (20 °C) OECD Test Guideline 104.
Water solubility	Completely miscible in water (20 °C)
Density	1.01 - 1.02 g/cm^3 (20 °C)
log K_{ow}	-0.84 at 23 °C (ECHA)

Introduction and use

Australia

There is currently no specific information about the introduction, use and end use of the chemical in Australia.

International

The following international uses have been identified through the European Union Registration, Evaluation and Authorisation of Chemicals (EU REACH); ECHA Annex XV report, the Substances in Preparations in Nordic countries (SPIN) database and the United States Environmental Protection Agency (US EPA) Chemical and Product Categories database (CPCat).

The chemical has reported commercial uses, including:

- as a solvent in synthesis reactions
- as an extraction agent
- as a functional fluid
- as a lubricant additive
- as a formulation component
- in printing and publishing
- in fabrics, textiles and apparel manufacture
- in cleaning/washing products
- in automotive care products
- in paints and coating products
- in paint strippers and adhesives removers
- in inks, toners and colourant products
- in lubricant and greases.

Some of these commercial uses may also be have domestic applications. No consumer uses are registered under REACH. The majority of uses reported under the US Chemical Data Reporting (CDR) under the *Toxic Substances Control Act* (US EPA 2016) were commercial, but a single potential consumer use in inks, toners and colourant products was identified. There are no identified uses of the chemical in a North American consumer product database (DeLima Associates).

The chemical has reported domestic use in the Substances and Preparations in Nordic countries (SPIN) database. However, it should be noted that SPIN does not distinguish between direct use of the chemical and use of the materials that are produced from chemical reactions involving the chemical.

The chemical has been detected as a contaminant in e-cigarettes (NICNAS 2019).

The substance has reported site-limited uses including:

- use in lithium-ion battery technology
- in manufacture of soldering fluxes
- in adsorption liquid and gas scrubbing
- in manufacture of computer, electronic and optical products
- inert additive for the fixation of methylated methylolmelamine resins in durable-press cotton and cellulosic fabrics
- as an HFC/CFC lubricant (automotive air conditioning (A/C) compressors).

Existing Australian regulatory controls

AICIS

No specific controls are currently available for the chemical.

Public

No specific controls are currently available for the chemical.

Workers

The chemical is not listed on the Hazardous Chemicals Information System (HCIS) and no exposure standards are available in Australia (SWA).

International regulatory status

Canada

Tetraglyme is included in the *Canada Identification of Risk Assessment Priorities (IRAP) - Appendix C*. Substances identified for data gathering.

European Union

European Chemicals Agency (ECHA) has classified the chemical as: Toxic for reproduction Category 1B (H360FD: May damage fertility. May damage the unborn child) in accordance with *Article 57 (c) of the REACH Regulation*.

The chemical is listed on the Candidate List of Substances of Very High Concern (SVHC) for eventual inclusion in Annex XIV (ECHA 2020). The reason for inclusion in the list is the chemical is considered 'Toxic for reproduction (Article 57c)'. In the European Union (EU), the inclusion in the Candidate List brings immediate obligations for suppliers of the substance, such as:

- supplying a safety data sheet
- communicating safe use
- responding to consumer requests within 45 days
- notifying ECHA if the article they produce contains an SVHC in quantities above one tonne per producer/importer per year and if the substance is present in those articles above a concentration of 0.1% (w/w).

The chemical is listed in Regulation (European Commission (EC)) 1223/2009 on cosmetic products, Annex II – List of substances prohibited in cosmetic products (EC).

USA

In the Federal Register of July 12, 2011 the EPA proposed a significant new use rule (SNUR) under the for 14 ethylene glycol ethers including tetraglyme. However, the EPA (2014) decided not to apply the final SNUR to seven of the 14 ethylene glycol ethers including tetraglyme because the Agency believes that these chemicals are not sufficiently similar to the seven chemicals subject to this SNUR and; therefore, do not raise the same concern for potential exposure to these chemicals.

Exposure standards

No specific exposure standards were identified (Chemwatch).

Health hazard information

Toxicokinetics

Tetraglyme belongs to the homologous series of glymes, where there is an incremental increase in the number of CH_2CH_2O units. Therefore, it can be assumed that tetraglyme and other glyme members (mono-, di-, and triglyme) share similar toxicokinetic properties and toxic mode of action. Increasing chain lengths could slightly slow down bioavailability or metabolism leading to reduced potency of higher chain variants (ECHA 2017).

Based on the read across approach using diglyme as the source chemical, tetraglyme is expected to be rapidly metabolised and excreted in urine. The main pathway of biotransformation of diglyme involves cleavage of the central ether bond which result in formation of 2-(2-methoxyethoxy)ethanol, which is further metabolised to 2-methoxyethanol (2-ME) and subsequently oxidised to methoxyacetic acid (MAA). Due to the observed extensive metabolism, the bioaccumulation potential is estimated to be negligible (NICNAS 2014; ECHA 2017; REACH).

The OECD QSAR Toolbox (rat liver S9 metabolism simulator) predicted the formation of MAA as well as other metabolites (OECD, 2021). This is the same for other glymes (ECHA, 2018). Primary hepatocytes isolated from male Sprague Dawley rats were cultured as monolayers and incubated with [^{14}C]-diglyme at 1, 10, 30 and 50 µM for up to 48 hours. The principal metabolite from primary rat hepatocytes and in the urine was (2 -methoxyethoxy)acetic acid (MEAA) (approx. 67% of the administered dose after 48 hours). Other prominent metabolites included 2-(2 -methoxyethoxy)ethanol (MEE), methoxyacetic acid (MAA), 2-methoxyethanol (2-ME) and diglycolic acid. Diglyme was demonstrated to be not cytotoxic to rat hepatocytes (REACH).

The formation of the metabolite 2-methoxyacetic acid is also identified by the OECD QSAR Toolbox.

Owing to its close structural similarity with diglyme, similar skin penetration behaviour is expected. As the molecular weight of tetraglyme is higher than that of diglyme, the substance is expected to be absorbed by the skin at a lower rate compared with diglyme.

Glycol ethers are typically readily distributed throughout the body and eliminated in urine (ECETOC 2016).

Acute toxicity

Oral

Tetraglyme has low oral acute toxicity based on results from animal tests following oral exposure.

In an acute oral toxicity study conducted in accordance with OECD TG 401, female Wistar rats (10/dose) were given single doses of 2500, 3150, 3550, 3750, 4000 or 5000 mg/kg bw tetraglyme by gavage. Five out of ten animals died in the 3750 mg/kg bw group. The median lethal dose (LD50) was 3750 mg/kg bw. Reported sublethal signs of toxicity included hyperactivity, ataxia, squatting position, prone position, dorsal position, constriction of pupil, nasal discharge, skin redness, and noisy breathing (REACH).

Dermal

Based on an acute dermal toxicity study with triglyme, tetraglyme is expected to have low acute dermal toxicity.

The LD50 value for triglyme was >6900 mg/kg bw from an acute dermal study in male Wistar rats conducted according to OECD guidelines TG 402 (NICNAS 2016).

Inhalation

Based on an acute inhalation study with diglyme, the chemical is expected to have low acute inhalational toxicity.

Diglyme was assessed in an inhalation toxicity study conducted according to OECD TG 403 (acute inhalation toxicity). Male and female Wistar rats (six animals/sex) were exposed to the aerosolised chemical at a concentration of 11.1 mg/L for a period of seven hours (nose-only system). No mortality occurred during the study. No irreversible clinical signs were noted and no macroscopic lesions were observed at necropsy. On the basis of this study a median lethal concentration (LC50) of >11.1 mg/L was determined for diglyme (NICNAS 2016).

Corrosion/Irritation

Skin irritation

In a skin irritation study conducted in accordance with OECD TG 404, 3 New Zealand White (NZW) rabbits (sex not specified) were treated with the chemical for 4 hours under occluded conditions. Observations were recorded at 1, 24, 48, 72 hours after patch removal. The following mean scores for individual animals were reported for observations at 24, 48 and 72

hours: 1.3, 1.0, 0 for erythema and 0, 0, 0, for oedema. Signs of irritation include very slight to well defined erythema. The erythema was reversible in all animals (NICNAS 2016).

The chemical does not warrant classification based on the available data.

Eye irritation

In an eye irritation study conducted in accordance with OECD TG 405, the chemical was instilled into one eye each of three NZW rabbits (sex not specified). The eyes were washed out after 24 hours, and observed at 1, 24, 48, 72 hours. All mean scores in all animals were zero, except in 1 rabbit in which clear colourless discharge and conjunctival hyperaemic blood vessel were observed (mean score 0.3) (REACH).

Based on the available data, the chemical does not warrant classification.

Sensitisation

Skin sensitisation

There are no skin sensitisation studies currently available for tetraglyme. Based on the weight of evidence the chemical is not considered to be a skin sensitiser.

The chemical's sensitisation potential was assessed based on the read across approach, using sensitisation studies with diethylene glycol methyl ethyl ether (diethylene glycol ethyl methyl ether, CAS No. 1002-67-1).

In a local lymph node assay (LLNA) performed in accordance with OECD TG 429, 4 female CBA mice received topical applications of 25, 50 and 100% of diethylene glycol methyl ethyl ether in acetone/olive oil (4:1 v/v). The reported stimulation indices (SI) were 0.73, 1.03 and 0.69 for concentrations of 25, 50 and 100%, respectively. The EC3 value could not be calculated, since every S.I. is below 3 (NICNAS 2015; REACH).

The chemical was reported to have no structural alerts for protein binding based on the mechanistic profiling functionality of the OECD QSAR Application Toolbox (OECD 2021).

Repeat dose toxicity

Oral

In a 28 day study, conducted in accordance with OECD TG 407, Wistar rats (5/sex/dose) were administered tetraglyme (purity not specified) by gavage at 62.5, 250 and 1000 mg/kg bw/day.

At the highest dose (1000 mg/kg bw/day), the absolute and relative thymus weights were decreased in male and female animals and absolute and relative testes weights were decreased in the males. Haematological analysis showed that the thrombocyte counts were slightly decreased in females of the high dose group. Histopathology investigation showed

narrowed and loosened cortex with high reduction of lymphocytes in the thymus of 4 males and 2 females. In testes of 2 males, degradation of germinal epithelium and increased single cell necrosis were found. In these animals, the number of matured sperm cells was significantly reduced.

Based on the effects on organ weights and histopathology, the no observed adverse effect level (NOAEL) for tetraglyme was determined to be 250 mg/kg bw/day for male and female rats (REACH).

In a similar 28 day repeated oral dose study, conducted in accordance with OECD TG 407, Wistar rats (5/sex/dose) were administered triglyme (purity not specified) by gavage at 62.5, 250 and 1000 mg/kg bw/day. The absolute and relative thymus weight was significantly decreased in all animals of the 1000 mg/kg bw/day group. Female rats of the 250 mg/kg bw/day group had decreased thymus weights. The size of the testes was reduced in male rats of the 1000 mg/kg bw/day dose group. No microscopic changes occurred at any dose level with the exception of changes of the testes, epididymides and spermatogenesis in male rats of the 1000 mg/kg bw/day dose group, resulting in oligo- and aspermia. In both male and female rats, involution of the thymus was observed in the high dose group. The NOAEL was determined to be 250 mg/kg bw/day, although slightly reduced thymus weights were observed at this level (NICNAS 2016; REACH).

At 1000 mg/kg bw/day, comparable findings were obtained for tri- and tetraglymes for all rats. The degree of testes and thymus effects and thrombocyte reduction was higher for triglyme.

Dermal

There is currently no information available about the dermal effects of this chemical.

Inhalation

Studies on repeated inhalation toxicity with tetraglyme are not available. Limited data are available for diglyme. In the 14 day subacute inhalation toxicity study, similar to OECD TG 412, CRL:CD BR rats (20 males and 10 females per dose group) were exposed nose only to concentrations of 110, 370 or 1100 ppm diglyme in air, 6 hours/day, 5 days/week for 2 weeks. Rats were sacrificed immediately following exposure, after 14, 42 or 84 days of recovery period.

Exposure to diglyme produced a variety of concentration related changes. The most striking effect produced in all test groups was cellular injury involving the testes, seminal vesicles, epididymides and prostate. Mean absolute and relative weights of male reproductive organs were significantly lower in diglyme exposed rats compared with the control group. The magnitude of the weight effects was concentration related, with the most severe effects observed in the 1100 ppm group. Concentration related reductions in mean absolute prostate and seminal vesicle weights were seen in the 370 ppm and 1100 ppm groups. Although these effects were more severe at the higher concentrations, partial or complete recovery was seen by 84 days post exposure. The prostate, seminal vesicles and thymus appeared normal at necropsy by 42 days of recovery. The small size of the testes and epididymides persisted through the recovery period. Overall, the gross lesions in male rats were consistent with the microscopic observations. Stage specific germ cell damage occurred at all concentrations and the effects were both concentration and time dependent. A no observed adverse effect level (NOAEL) could not be established in this study for males, and the lowest

observed adverse effect level (LOAEL) was reported to be 110 ppm. No compound related gross lesions were noted at necropsy in female rats.

Changes in the haematopoietic system occurred in both sexes and involved the bone marrow, spleen, thymus, leucocytes and erythrocytes. Minimal to severe bone marrow hyperplasia and lymphoid tissue atrophy of the spleen and thymus were apparent in both male and female rats exposed to 1100 ppm diglyme. While the atrophic changes in the haematopoietic tissues of rats from this group had resolved after 14 days of recovery, extramedullary haematopoietic foci were evident in the liver of rats from both sexes and in the spleen of males. Evidence of haematopoietic effects in males was essentially absent after 42 days of recovery (NICNAS 2014; REACH).

Genotoxicity

In the absence of sufficient data to assess the genotoxicity of tetraglyme, studies on other glyme analogues were used to assess its genotoxicity potential. Based on available data, the chemical is not considered to have genotoxic potential in vitro or in vivo.

In vitro

In vitro studies with tetraglyme and its analogues gave negative results:

- Negative results were reported with tetraglyme in a bacterial reverse mutation assay (OECD TG 471) in *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538 and *Escherichia coli* WP2 *uvr*A, with and without rat liver S9 mixes, at concentrations of 4−5000 µg/plate (REACH).
- Negative results were reported with the analogue, triglyme (CAS No. 112-49-2), in a bacterial reverse mutation assay (OECD TG 471) in *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538, with and without rat liver S9 mixes. The concentrations of the chemical tested were 4−10000 µg/plate (NICNAS 2016).

In vitro genotoxicity studies (bacterial reverse mutation assays in *S. typhimurium*, DNA damage and repair assay in human embryonic intestinal cells) conducted with diglyme were reported to be negative (NICNAS 2014).

In vivo

In vivo studies on tetraglyme genotoxicity are not available. Relevant in vivo studies with its analogues were reported to be negative.

- In a mammalian erythrocyte micronucleus test conducted in accordance with OECD TG 474, NMRI mice (n=5/sex/dose) were treated with monoglyme by gavage at up to 2000 mg/kg bw/day (2 applications, one daily). The reported incidence of micronuclei in bone marrow polychromatic erythrocytes did not increase in any of the treated groups, indicating a lack of clastogenicity.
- In a mammalian bone marrow chromosomal aberration test, conducted in accordance with OECD TG 475, Chinese hamsters (n=5/sex/dose) were administered monoglyme by gavage at doses of 2000 mg/kg bw/day. The reported incidence of chromosome aberrations in bone marrow did not increase in any of the treated groups, indicating a lack of clastogenicity.

- In vivo tests (mammalian bone marrow chromosome aberration assays in rats) conducted with diglyme were reported to have negative results (NICNAS 2014).

In a dominant lethal assay conducted in accordance with OECD TG 478, male Crj: CD(SD) rats (n=10/dose) were exposed to diglyme atmospheres for 7 hours/day for 5 days. Multiple dosed rodents received 100 mg/kg bw/day for 5 days while single dosed rats received 250 mg/kg bw/day. A significant increase in preimplantation loss and reduction in implantation were observed in weeks 6 to 7 after exposure of the male to 1000 ppm diglyme. Recovery from this influence was complete in week 10. Considering the known effects of the chemical on fertility, the report concluded that reduced fertility was responsible for these effects, rather than genetic damage (NICNAS 2014).

Carcinogenicity

There are no data currently vailable for tetraglyme. A range of related compounds, including glyme, diglyme, triglyme and related monomethyl esters (cellosolves) are not considered to be carcinogens (NICNAS 2016).

Reproductive and development toxicity

Very little information is available on the reproductive and developmental toxicity of tetraglyme. Based on the limited data on tetraglyme and available studies on its analogues in rabbits and rodents, the chemical is expected to cause specific adverse effects on fertility and development following oral and inhalation exposure. Information on reproduction and development effects from dermal exposure is currently not available.

Glymes, which consist of repetitive ethylene glycol units, are known to cause testes toxicity and developmental toxicity. Mono-, di- and triglyme, as well as 2-ME and MAA all have a harmonised classifications (ECHA 2018).

Reproductive toxicity

In a combined reproduction/developmental toxicity screening test similar to the OECD TG 421, (only 3 rats/sex/dose were used), Wistar rats were administered 250, 500 or 1000 mg/kg bw/day tetraglyme by gavage, from 28 days premating period through to the postnatal day 4 (PND4) (28 days for males; 54 days for females).

No significant clinical signs of toxicity were observed in treated animals. In males, the body weights were comparable to control animals, but in females, during the gestation period, body weight gain was reduced to 30% of the control value for animals in the 1000 mg/kg bw/day group. In the lactation phase the body weight gain reduced dose dependently for treated females. No changes were noted in haematology or clinical biochemistry in either males or females.

All males of the highest dose group (1000 mg/kg bw/day) showed reduced organ weight for testes (mean value reduced to 60% of controls) and epididymides (mean value reduced to 70% of controls). At this dose it was reported that no live pups were delivered. At 500 mg/kg

bw/day, the number of live pups per litter reported was reduced compared to the controls (8 vs 11). The body weight development of live born pups from treated animals was reported to be comparable to those from the controls. No effects were seen at 250 mg/kg bw/day.

Due to the low number of rats used in each dose group, a NOAEL could not be established. However, the study demonstrated that tetraglyme induces male reproductive and developmental toxicity.

ECHA reported the following effects from a combined repeated dose toxicity study with reproductive and developmental toxicity screening (OECD 422) (ECHA, 2018):

- significantly reduced testes weights (-50%) and epididymis weights (- 30%) at 1000 mg/kg bw/day
- marked bilateral seminiferous tubular degeneration (with associated depletion of germ cells) was reported in the testes and, in the epididymides, slight to moderate hypospermia at 1000 mg/kg bw
- at 300 mg/kg bw/day 2/10 males showed abnormalities in their sexual organs and the females mating with these males did not give birth to any pups
- decreased numbers of corpora lutea and implantation sites were observed in females at 300 and 1000 mg/kg bw/day.

Developmental toxicity

Developmental toxicity studies with tetraglyme and its analogues in rabbits and rats are summarised below.

Tetraglyme – Rat

In a prenatal developmental toxicity study, conducted in accordance with OECD TG 414, pregnant Wistar rats (8/dose) were administered 250, 500 or 1000 mg/kg bw/day of tetraglyme by gavage, on gestation days (GD) 5-19. Dams were sacrificed on GD19 and the uterine contents were examined for embryonic or foetal mortalities, and the number of viable foetuses.

At 1000 mg/kg bw/day, only 3 living pups were found in 8 dams and all these pups were visibly abnormal. Post implantation loss of 14.5 % was observed at 500 mg/kg bw/day. At all doses nearly all foetuses exhibited abnormalities, most notably being skeletal malformations of the paw, absent phalanges and absent 4th metacarpal. Incidences of absent sternebrum, absent hyoid and absent xiphoid were also significantly increased. Increased incidence of incomplete ossification in the skull was seen. Evaluation of pups from the highest dose group (1000 mg/kg bw/day) was not meaningful due to the low number of live pups and their gross external abnormalities.

In the combined reproduction/developmental toxicity screening study (similar to OECD TG 421) described above, increased post implantation loss occurred at 500 and 1000 mg/kg bw/day.

Triglyme – Rabbit

In a prenatal developmental toxicity study, conducted in accordance with OECD TG 414, pregnant NZW rabbits (25/dose) were administered 75, 125, 175, or 250 mg/kg bw triglyme by gavage, once daily on GD 6–19. Dams were sacrificed on GD 30 and the foetuses were examined.

On GD19, maternal body weight was reported to be significantly low in the 250 mg/kg bw/day group, and body weight gain was decreased at 175 mg/kg bw/day and above. In dams there was a dose related decrease in gravid uterine weight and an increase in absolute liver weight reported. Significantly increased post implantation losses were observed in treated animals. The number of does with entirely resorbed litters and no live foetuses was reported as 1, 1, 2, 2, and 7 for the 0, 75, 125, 175, and 250 mg/kg bw/day groups, respectively. The reported percentage of resorptions per litter, percentage of non-live implants, and percentage of adversely affected implants each increased in a dose dependent manner.

There was no significant difference reported among the treated groups in the number of corpora lutea per doe, or the number of implantation sites per litter. The percentage of preimplantation loss; however, exhibited a significant increasing trend and was significantly high in the highest dose animals. The percentage of resorptions per litter, the percentage of non-live and adversely affected implants increased in a dose dependent manner from 125 mg/kg bw/day onwards. The percentage of litters with non−live implants exhibited a significant treatment effect in the 175 and 250 mg/kg bw/day groups. The number of live foetuses/litter exhibited a decreasing trend and was significantly low in the 125, 175, and 250 mg/kg bw/day dose groups.

Triglyme treatment was reported to cause an increased trend in the percentage of foetuses per litter having one or more malformations, with the 175 and 250 mg/kg bw/day dose groups significantly increased over controls. The percentage of litters with one or more malformed foetuses exhibited a significant dose effect at 175 and 250 mg/kg bw/day. Malformations observed most frequently included missing toenails in foetuses of normal size with no digital abnormalities, abnormally small spleen, and hydronephrosis. Variations that were noted as unusual were an abnormal number of papillary muscles, clubbed limbs without bone change, and small cysts on the reproductive organs of both sexes.

A NOAEL for developmental toxicity was set at 75 mg/kg bw/day, based on the reduced number of live foetuses/litter at 125 mg/kg bw/day and above (NICNAS 2016).

Triglyme – Mouse

In a study conducted according to OECD TG 414, female CD-1 mice (28-32/dose) were administered triglyme at 250, 500 or 1000 mg/kg bw/day by gavage on GD 6-15. In treated mice, gravid uterine weight decreased in a dose related manner, indicating compromised pregnancy status, and relative maternal liver weight was significantly increased over controls at doses of 500 mg/kg bw/day and above. The number of live foetuses per litter was unaffected by treatment, although average foetal body weight per litter was significantly reduced at doses of 500 mg/kg bw/day and above. The percentage of adversely affected conceptuses per litter (post-implantation loss + malformed live foetuses) exhibited a dose related increase at 500 and 1000 mg/kg bw/day. The percentage of malformed live foetuses per litter (0.3, 0, 0.8 and 11.1% at 0, 250, 500 and 1000 mg/kg bw/day, respectively), and percentage of litters with one or more malformed foetuses were significantly increased at 1000 mg/kg bw/day, as a result of a significant increase in both external and skeletal malformations. Malformations in animals observed at external examination included primarily

neural tube closure defects and cleft palate. Those animal malformations observed in stained carcasses solely affected the axial region (fused ribs, fused sternebrae).

A NOAEL of 250 mg/kg bw/day was reported for developmental toxicity which was established based on the effect on conceptuses at 500 mg/kg bw/day and above (NICNAS 2016).

Similar findings were reported for rabbits and mice treated with diglyme at lower dose levels (NICNAS 2014).

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