

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name:

Tetraglyme

EC Number: 205-594-7

CAS Number: 143-24-8

Index Number: -

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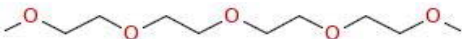
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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table A.1.1.: Substance identity

Substance name:	EC name: bis(2-(2-methoxyethoxy)ethyl) ether IUPAC name: bis(2-(2-methoxyethoxy)ethyl) ether; tetraglyme Synonym: Tetraglyme
EC number:	205-594-7
CAS number:	143-24-8
Structure	
Annex VI Index number:	

For impurities see confidential Annex.

1.2 Harmonised classification and labelling proposal

Table A.1.2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	No classification
Current proposal for consideration by RAC	Repr. 1B, H360
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Repr. 1B, H360

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table A.1.3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification ¹⁾	Reason for no classification ²⁾
2.1.	Explosives	none	none	none	Not evaluated
2.2.	Flammable gases	none	none	none	Not evaluated
2.3.	Flammable aerosols	none	none	none	Not evaluated
2.4.	Oxidising gases	none	none	none	Not evaluated
2.5.	Gases under pressure	none	none	none	Not evaluated
2.6.	Flammable liquids	none	none	none	Not evaluated
2.7.	Flammable solids	none	none	none	Not evaluated
2.8.	Self-reactive substances and mixtures	none	none	none	Not evaluated
2.9.	Pyrophoric liquids	none	none	none	Not evaluated
2.10.	Pyrophoric solids	none	none	none	Not evaluated
2.11.	Self-heating substances and mixtures	none	none	none	Not evaluated
2.12.	Substances and mixtures which in contact with water emit flammable gases	none	none	none	Not evaluated
2.13.	Oxidising liquids	none	none	none	Not evaluated
2.14.	Oxidising solids	none	none	none	Not evaluated
2.15.	Organic peroxides	none	none	none	Not evaluated
2.16.	Substance and mixtures corrosive to metals	none	none	none	Not evaluated
3.1.	Acute toxicity - oral	none	none	none	Not relevant for this dossier, classification proposal focuses on reproductive toxicity only
	Acute toxicity - dermal	none	none	none	Not evaluated
	Acute toxicity - inhalation	none	none	none	Not evaluated
3.2.	Skin corrosion / irritation	none	none	none	Not evaluated
3.3.	Serious eye damage / eye irritation	none	none	none	Not evaluated
3.4.	Respiratory sensitisation	none	none	none	Not evaluated
3.4.	Skin sensitisation	none	none	none	Not evaluated
3.5.	Germ cell mutagenicity	none	none	none	Not relevant for this dossier, classification

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					proposal focuses on reproductive toxicity only
3.6.	Carcinogenicity	none	none	none	Not evaluated
3.7.	Reproductive toxicity	Repr. 1B	none	none	-
3.8.	Specific target organ toxicity –single exposure	none	none	none	Not relevant for this dossier, classification proposal focuses on reproductive toxicity only
3.9.	Specific target organ toxicity – repeated exposure	none	none	none	Not relevant for this dossier, classification proposal focuses on reproductive toxicity only
3.10.	Aspiration hazard	none	none	none	Not evaluated
4.1.	Hazardous to the aquatic environment	none	none	none	Not evaluated
5.1.	Hazardous to the ozone layer	none	none	none	Not evaluated

¹⁾ Including specific concentration limits (SCLs) and M-factors

²⁾ Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Signal word:

Danger

Pictogram:



Hazard statements:

H360: May damage fertility or the unborn child

Precautionary statements:

P201: Obtain special instructions before use.

P202: Do not handle until all safety precautions have been read and understood.

P280: Wear protective gloves/protective clothing/eye protection/face protection.

P308+P313: IF exposed or concerned: Get medical advice/attention.

P405: Store locked up.

P501: Dispose of contents/container to... (in accordance with local regulation.)

Proposed notes assigned to an entry:

-

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

The substance was never classified and labelled via an internationally harmonized procedure, no history available.

2.2 Short summary of the scientific justification for the CLH proposal

Within one sub-acute oral rat study and within a dose range finding study for a rat reproduction developmental toxicity screening study and within a dose range finding study for a rat developmental toxicity study clear effects on **fertility** were observed for tetraglyme in terms of adverse effects on weight and histopathology of testis, histopathology of epididymis, spermatogenesis, gestation length and on **development** in terms of absence of or reduced number of live pups or post-implantation loss as well as malformations. All these effects were observed in the absence of severe general parental toxicity and at or below maximal standard testing doses of 1000 mg/kg bw day.

Similar effects on reproductive organs and developmental toxicity were observed for the category of glymes (triglyme, diglyme, monglyme) and the presumed common metabolites 2-Methoxyethanol (2-ME) and Methoxyacetic acid (MAA) and all of these 5 source substances are classified for reproductive toxicity category 1B. Structural similarity of this chain-length category as well as similarity of the experimental toxicological and physchem data matrix support the read across of the reproductive toxicity data.

The read-across argumentation is presented in chapter 4.10.3. (Other relevant information) and should be consulted before reading the rest of the CLH report.

Please note that no detailed re-evaluation of the studies for source substances in the category of glymes and metabolites is presented, since a harmonised classification is available for these substances. The studies from the source substances are described detailed enough in order to allow a conclusion on read-across. The level of detail is sufficient to conclude that the toxicological profile is similar for tetraglyme and the other glyme category members.

2.3 Current harmonised classification and labelling

Currently no harmonized classification and labelling.

2.4 Current self-classification and labelling

Currently self-classification and labelling in the C&L inventory and in the registration dossier identical with the current proposal for harmonized classification, i.e. reproductive toxicity category 1B.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

It is proposed to classify tetraglyme for Reproductive Toxicity, Category 1B, H360. Harmonised classification and labelling for CMR and respiratory sensitisation is a Community-wide action under article 115 of REACH and article 36(1) of CLP. Tetraglyme is currently not classified according to Annex VI of CLP.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

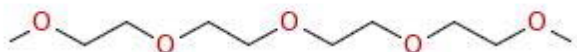
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table B 1.1.: Substance identity

EC number:	205-594-7
EC name:	bis(2-(2-methoxyethoxy)ethyl) ether
Synonym	Tetraglyme
CAS number (EC inventory):	143-24-8
CAS number:	
CAS name:	2,5,8,11,14-Pentaoxapentadecane
IUPAC name:	bis(2-(2-methoxyethoxy)ethyl) ether; tetraglyme
CLP Annex VI Index number:	
Molecular formula:	C ₁₀ H ₂₂ O ₅
Molecular weight range:	222.2787

Structural formula:



1.2 Composition of the substance

See confidential Annex

1.3 Composition of test material

Similar to registered substance, see confidential Annex.

1.4 Physico-chemical properties

Table B.1.4.: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Tetraglyme is a colourless homogeneous liquid at 20°C and 101.3 kPa.	CSR+IUCLID	Not assessed for CLH dossier
Melting/freezing point	-30°C (handbook)	CSR+IUCLID	Not assessed for CLH dossier
Boiling point	275 ± 4 °C (OECD 103) 273.5 °C (handbook)	CSR+IUCLID	Not assessed for CLH dossier
Relative density	1.0121 g/cm ³ (20°C, pycnometer method) 1.0114 g/cm ³ (20 °C, handbook)	CSR+IUCLID	Not assessed for CLH dossier
Vapour pressure	0.099 Pa at 20°C 0.25 Pa at 25°C 16 Pa at 50°C	CSR+IUCLID	Not assessed for CLH dossier
Surface tension	34.16 dyn/cm at 20°C and 100 vol.-% (capillary rise method). 66.7 ± 0.5 mN/m at a concentration of 1 g/L and 20 °C (plate method).	CSR+IUCLID	Not assessed for CLH dossier
Water solubility	miscible with water at 20°C.	CSR+IUCLID	Not assessed for CLH dossier
Partition coefficient n-octanol/water	Partition coefficient n-octanol/water log Pow = -0.84 at 23°C.	CSR+IUCLID	Not assessed for CLH dossier
Flash point	Flash point: 136 ±2°C at 100.35 kPa	CSR+IUCLID	Not assessed for CLH dossier
Flammability	Flash point: 136 °C @ 100.35 kPa. No pyrophoricity. No flammability on contact with water.	CSR+IUCLID	Not assessed for CLH dossier
Explosive properties	not explosive under influence of a flame not more sensitive to shock than m-dinitrobenzene	CSR+IUCLID	Not assessed for CLH dossier
Self-ignition temperature	Self-ignition temperature: 270 °C at 1016 hPa.	CSR+IUCLID	Not assessed for CLH dossier
Oxidising properties	non oxidising	CSR+IUCLID	Not assessed for CLH dossier

Granulometry	Substance is a liquid under ambient conditions.	CSR+IUCLID	Not assessed for CLH dossier
Stability in organic solvents and identity of relevant degradation products	Stability in organic solvents is not considered to be critical.	CSR+IUCLID	Not assessed for CLH dossier
Dissociation constant	no dissociating groups present in the molecule	CSR+IUCLID	Not assessed for CLH dossier
Viscosity	at 20 °C: kinematic viscosity: 3.69 ± 0.01 mm ² /s dynamic viscosity: 3.73 ± 0.02 mPa.s at 40 °C: kinematic viscosity: 2.39 ± 0.01 mm ² /s dynamic viscosity: 2.37 ± 0.02 mPa.s	CSR+IUCLID	Not assessed for CLH dossier

2 MANUFACTURE AND USES

2.1 Manufacture

Manufacture

PROC 1: Use in closed process, no likelihood of exposure

PROC 3: Use in closed batch process (synthesis or formulation)

PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises

PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities

PROC 15: Use as laboratory reagent

Environmental release category 1: Manufacture of substances

Formulation of preparations

PROC 1: Use in closed process, no likelihood of exposure

PROC 2: Use in closed, continuous process with occasional controlled exposure

PROC 3: Use in closed batch process (synthesis or formulation)

PROC 5: Mixing or blending in batch processes for formulation of preparations and articles (multistage and/or significant contact)

ERC 2: Formulation of preparations

2.2 Identified uses

Industrial use of Tetraglyme as solvent in synthesis reactions or as extracting agent

Industrial use of Tetraglyme as gas absorption liquid

Industrial use

Use in industrial chemical processes

Use in Functional Fluids

Charging and discharging of substances and mixtures

Agent absorbing gases

Use in Functional Fluids, professional

Use in laboratories, professional

Charging and discharging of substances and mixtures, professional

Use of ink by professionals

Service life of printed paper articles

Information on uses advised against:

Consumer products

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated.

4 HUMAN HEALTH HAZARD ASSESSMENT

The human health hazard assessment is based on data for tetraglyme and data read across from source substances monoglyme, diglyme and triglyme, further supported by assumed common metabolites 2-methoxyethanol and methoxyacetic acid. For description of the source substances and explanation and assessment of the read across approach see chapter 4.10.3. This chapter should be consulted before reading the rest of the CLH report.

Please note that no detailed re-evaluation of the studies for the source substances in the category of glymes and metabolites is presented, since a harmonised classification is available for these substances. The studies from the source substances are described detailed enough in order to allow a conclusion on read-across. The level of detail is sufficient to conclude that the toxicological profile is similar for tetraglyme and the other glyme category members.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non Human information

See Annex 8.2.

4.1.2 Human information

See Annex 8.2.

4.1.3 Summary and discussion on toxicokinetics

Within metabolism studies with Diglyme including in vitro studies with rat and human microsomes and primary rat hepatocytes as well as in vivo studies with rat and pregnant mouse i.a. 2-methoxyacetic acid was identified as metabolite. This metabolite appears to induce malformations in a mouse developmental toxicity study (see section 4.10). Considering structural similarity also tetraglyme is expected to be metabolized to this toxic degradation product.

Dermal absorption of diglyme appears high in an in vitro study with human skin (flux about 3.4 mg/cm²/h at steady state). Considering structural and physicochemical similarity (see chapter 4.10.3) also tetraglyme is expected to be easily taken up via skin.

For a justification for read across and an explanation of the relevance of these toxicokinetic studies carried out with diglyme and monoglyme see section 4.10.3.

4.2 Acute toxicity

Table B.4.2. Acute oral toxicity:

Method	Results	Remarks	Reference
rat (Wistar) female oral: gavage equivalent or similar to OECD Guideline 401 (Acute Oral Toxicity)	LD50: 3850 mg/kg bw (female)	2 (reliable with restrictions) key study Test material: Tetraglyme	Hoechst (1982)
rat (Wistar) male oral: gavage equivalent or similar to OECD Guideline 401 (Acute Oral Toxicity)	LD50: 5140 mg/kg bw (male) based.	4 (not assignable) supporting study Test material: Tetraglyme	Smyth, H. F. jr., Seaton, J., Fischer, L. (1941)

Acute toxicity inhalation: not evaluated

Acute toxicity dermal: not evaluated

4.2.1 Non-human information

Oral:

- female rats, LD50: 3850 mg/kg bw
- female mice, LD50: 5140 mg/kg bw (supporting data)

Further information available, see Annex 8.2.

Oral:

- female rats, LD50 (triglyme): 5390 mg/kg bw (supporting data)
- female rats, LD50 (diglyme): 4760 mg/kg bw (supporting data)
- female rats, LD50 (monoglyme): 5370 mg/kg bw (supporting data)

Inhalation:

- male/female rats, LC0 (diglyme; 7h exposure): 11 mg/L -> 19.3 mg/L (4h calculated exposure)
- male/female rats, LC0 (monoglyme; 1h exposure): 240 mg/L -> 60 mg/L (4h calculated exposure)
- rats, LC50 (monoglyme; 6h exposure): > 20 mg/L

Dermal:

- male rats, LD50 (triglyme): > 6900 mg/kg bw

4.2.2 Human information

No data.

4.2.3 Summary and discussion of acute toxicity

It can be concluded that tetraglyme and source substances relevant for read across to tetraglyme have low acute toxicity.

4.2.4 Comparison with criteria

These data are just presented to support the read across between the category of glymes and related metabolites (see chapter 4.10.3), i.e. the similar toxicological profile of these category members.

Oral LD50 value = 3850 mg/kg bw for tetraglyme is above 2000 mg/kg bw.

4.2.5 Conclusions on classification and labelling

The data support a similar toxicological profile of the category of glymes.

The classification proposal focuses on reproductive toxicity only.

4.3 Specific target organ toxicity – single exposure (STOT SE)

Not evaluated.

4.4 Irritation

Not evaluated.

4.5 Corrosivity

Not evaluated.

4.6 Sensitisation

Not evaluated.

4.7 Repeated dose toxicity

Table B. 4.7. Repeated dose toxicity

Method	Results	Remarks	Reference
<p>rat (Wistar) male/female subacute (oral: gavage) 0, 62.5, 250, 1000 mg/kg bw (actual ingested) Exposure: 28 days (once per day) OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents)</p>	<p>LOAEL: 1000 mg/kg bw/day (actual dose received) (male/female) based on thrombocyte counts↓, alkaline phosphatase ↓, thymus and testis weight↓, histopath. thymus and testes ↑, mature sperm ↓</p>	<p>2 (reliable with restrictions) key study experimental result Test material : Tetraglyme</p>	<p>Hoechst AG (1992a)</p>

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Hoechst AG 1992a reports an oral gavage 28 day study in rats. **Tetraglyme** was administered daily to male and female Wistar rats at dose levels of 62.5, 250 and 1000 mg/kg body weight/day. A control group was treated similarly with the vehicle - water only.

In all the groups, the behaviour, clinical signs, body weight, food consumption and water consumption were recorded. The Haematology and clinical chemistry analyses, and urinalysis tests were performed at the end of the treatment. All animals were killed, necropsied and examined post mortem. Histological examinations were performed on organs and tissues.

All animals survived during the treatment period. No clinical signs, body weight, food and water consumption changes were noted. Haematological analysis showed that the thrombocyte counts were slightly decreased in females of the high dose group. The clinical chemistry test showed that decreased AP activity and elevated creatinine were found in both males and females at 1000 mg/kg bw. The urinalysis showed no compound related toxicity. In male and female animals of the high dose group, the absolute thymus weights were decreased (males -8%, females -19% relative to control), also the absolute testis weights were decreased (-13% relative to control). Furthermore the relative thymus weights were decreased (-9% relative to control) as well as the relative testis weights (-10% relative to control). According to the table in the study report absolute organ weight changes were not statistically significant and relative organ weights were not statistically evaluated.

Histopathology investigation showed several treatment related effects in animals of the high dose group. In the thymus of 4 (from total 5) males and 2 (from total 5) females, narrowed and loosened cortex with high reduction of lymphocytes were observed. In testes of 2 (from total 5) males, degradation of germinal epithelium and increased single cell necrosis were found. The number of matured sperm cells was significantly reduced. More details as far as available from the study report are presented in the confidential Annex.

Based on the effects of clinical chemistry, organ weight and histopathology, the no observed effect level (NOEL) of the test item is 250 mg/kg bw/day for male and female rats.

For more details see confidential Annex.

4.7.1.2 Repeated dose toxicity: inhalation

No data.

4.7.1.3 Repeated dose toxicity: dermal

No data.

4.7.1.4 Repeated dose toxicity: other routes

No data.

4.7.1.5 Human information

No data.

4.7.1.6 Other relevant information

The toxicity findings of triglyme and tetraglyme were comparable in the oral 28 day study: Both tri- and tetraglyme did not induce any treatment related effect at 62.5 mg/kg bw. Triglyme induced reduced thymus weight in females at 250 mg/kg bw (-21% compared to control). Tetraglyme did not induce any effect at 250 mg/kg bw. At 1000 mg/kg bw, comparable findings were obtained for tri- and tetraglymes. The degree of testes and thymus effects was higher for triglyme. Also the thrombocytes reduction was more pronounced for rats treated with triglyme.

This supports the hypothesis that toxic potency may decrease with chain length in this category.

Table B. 4.7.1.6. Toxicity findings at 1000 mg/kg bw in subacute toxicity studies on tri- and tetraglyme (the values in the parenthesis are relative values to corresponding controls)

Findings	Triglyme	Tetraglyme
Body weight		
males	reduced (-16%)	no effect
females	no effect	no effect
absolute testes weight		
	reduced (-52%)	reduced (-13%)
absolute thymus weight		
males	reduced (-61%)	no effect (-8%)
females	reduced (-56%)	reduced (-19%)
Thrombocytes count		
males	reduced (-30%)	reduced (-15%)
females	reduced (-26%)	reduced (-19%)
Alkaline phosphatase		
males	reduced (-46%)	reduced (-38%)
females	reduced (-36%)	reduced (-29%)
Creatinine		
males	increased (+180%)	increased (+290%)
females	increased (+270%)	increased (+160%)

Also in the 14 day inhalation toxicity study in rats with diglyme adverse effects on testis, thymus and the haematopoetic system were observed which furthermore supports the category approach for tetraglyme with tri-, di-, monoglyme (see Annex 8.2.3 and 8.2.5.) and the metabolite methoxyacetic acid (MAA, see Annex 8.2.5)

4.7.2 Summary and discussion of repeated dose toxicity

In the available repeated dose study for tetraglyme adverse effects, mainly on the male reproductive organs and the haematopoetic system were only observed in the 1000 mg/kg bw day dose group. The observed effects were similar for triglyme and diglyme.

For a justification for the read across approach see section 4.10.3.

For more details on the data for diglyme see Annex 8.2.

4.7.3 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.7.3.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

See 4.7.1.7. and 4.7.2.

4.7.3.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

These repeated dose data are just presented to support the classification for reproductive toxicity and the read across between the source substances in the category of glymes and the related metabolites (see chapter 4.10.3), i.e. the similar toxicological profile of these category members.

The LOAEL of 1000 mg/kg bw day in the 28 day study with tetraglyme (and triglyme) is above the guidance value of the 90 day study LOAELs, i.e. 100 mg/kg bw day, for STOT RE category 2 classification.

4.7.3.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

The data support a similar toxicological profile of the category of glymes.

The classification proposal focuses on reproductive toxicity only.

4.8 Germ cell mutagenicity (Mutagenicity)

Table B. 4.8. Germ cell mutagenicity

Method	Results	Remarks	Reference
bacterial reverse mutation assay (gene mutation) S. typhimurium TA 1535, TA 1537, TA 98, TA 100, TA 1538 and E. coli WP2 (met. act.: with and without) Test concentrations: 0, 4, 20, 100, 500, 2500 and 5000 µg/plate equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Evaluation of results: negative Test results: negative for S. typhimurium TA 1535, TA 1537, TA 98, TA 100, TA 1538 and E. coli WP2(all strains/cell types tested) ; met. act.: with and without cytotoxicity: no vehicle controls valid: yes; positive controls valid: yes	1 (reliable without restriction) Key study Test material : Tetraglyme	Hoechst AG (1984)

4.8.1 Non-human information

4.8.1.1 In vitro data

Four Ames tests are available on tetraglyme and its source (triglyme, diglyme and monoglyme) chemicals. In none of the 4 studies mutagenicity was observed. (For study summaries on the source substances, see Annex 8.2.)

4.8.1.2 In vivo data

Not evaluated.

4.8.2 Human information

4.8.3 Other relevant information

The following information is taken into account for hazard assessment:

- Ames test: negative (4 studies, each on tetraglyme, triglyme, diglyme and monoglyme)
- in vitro UDS assay: negative (3 studies, two on monoglyme and one on diglyme)
- in vitro mammalian cell gene mutation assay: negative (one study on monoglyme)
- in vitro SCE assay: positive (one study on monoglyme)

Further information available:

- in vivo micronucleus test in mice: negative (one study on monoglyme)
- in vivo chromosome aberration test: negative (2 studies, each on diglyme and monoglyme)
- in vivo rodent dominant lethal test: positive (one study on diglyme; limited relevance)

4.8.4 Summary and discussion of mutagenicity

On the basis of all available data and read across information provided in 4.10.3. it is concluded that there is no concern for genotoxicity of tetraglyme as all available, relevant genotoxicity studies are negative.

For a justification for the read across approach see section 4.10.3.

4.8.5 Comparison with criteria

These repeated dose data are just presented to support the classification for reproductive toxicity and the read across between the category of glymes and related metabolites (see chapter 4.10.3), i.e. the similar toxicological profile of these category members.

All available, relevant in vitro genotox studies are negative.

4.8.6 Conclusions on classification and labelling

The data support a similar toxicological profile of the category of glymes.

The classification proposal focuses on reproductive toxicity only

4.9 Carcinogenicity

No data. Not evaluated.

4.10 Toxicity for reproduction

Table B.4.10. Toxicity for reproduction

Method	Results	Remarks	Reference
<p>rat (Wistar) male/female 3 rats/sex/dose group</p> <p>oral: gavage</p> <p>(actual ingested (0; 250 (LD); 500 (MD); 1000 (HD) mg/kg bw))</p> <p>Exposure: 54 days for females; 28 days for males (daily)</p> <p>dose-range finding study to OECD Guideline 421 (Reproduction / Developmental Toxicity Screening Test)</p>	<p>LOAEL (male parent): 1000 (actual dose received) based on: reduced testes and epididymis weight</p> <p>LOAEL (female parent): 500 (actual dose received) based on: prolonged gestation period</p> <p>LOAEL (F1) = 500 mg/kg bw/day (actual dose received) based on: no live pups at 1000 mg/kg bw and reduced number of live pups at 500 mg/kg bw</p>	<p>2 (reliable with restrictions)</p> <p>Key study</p> <p>Test material: Tetraglyme</p>	BSL Bioservice (2011)
<p>rat (Wistar)</p> <p>8 females/dose group</p> <p>oral: gavage</p> <p>250 mg/kg bw (actual ingested)</p> <p>500 mg/kg bw (actual ingested)</p> <p>1000 mg/kg bw (actual ingested)</p> <p>Exposure: in females from the Gestation Day (GD) 5 to GD 19 (daily)</p> <p>dose range finding study to OECD Guideline 414 (Prenatal Developmental Toxicity Study)</p>	<p>LOAEL (maternal toxicity) > 1000 mg/kg bw day: no adverse effects at top dose</p> <p>LOAEL (developmental toxicity) ≤ 250 mg/kg bw/day based on: overall effects external, soft tissue and skeletal abnormalities, ↑ post-implantation loss;</p>	<p>2 (reliable with restrictions)</p> <p>Key study</p> <p>Test material: Tetraglyme</p>	BSL BIOSERVICE (2012)

4.10.1 Effects on fertility

4.10.1.1 Non-human information

In a dose range finding study for OECD Testguideline 421 (repro/dev tox screening study, only 3 animals/sex, not analysed: histopathology, sperm, AGD, nipple retention, T4, TSH) 3 males and 3 females per dose groups were treated **with tetraglyme**, the dose levels being 250, 500 and 1000 mg/kg bw day ([BSL Bioservice 2011](#)).

Parental toxicity in males: Upon clinical observation no significant toxic effect was found. The body weight was comparable for treated and control animals. No clear alteration could be identified upon hematology and clinical biochemistry investigations. All males of 1000 mg/kg bw exhibited reduced organ weight for testes (mean value reduced to 60% of controls) and epididymides (mean value reduced to 70% of controls). One male had testes with a smooth consistency. Furthermore yellow spots on the epididymides were observed for one HD animal.

Parental toxicity in females: Upon clinical observation no significant toxic effect was found. No body weight effect was found for the pre-mating period. The body weight gain was reduced to 30% of the control value for animals of 1000 mg/kg bw during the gestation period. No obvious effect was found for animals of 500 mg/kg bw and 250 mg/kg bw. At doses of 500 and 1000 mg/kg bw day females exhibited an increase in pre-coital interval (control = 2,33 days; 500 mg/kg bw day = 4 days; 1000 mg/kg bw day = 3.67 days) and a prolonged gestation period (control = 22 days; 500 mg/kg bw day = 23 days; the only littering dam in 1000 mg/kg bw day group = 24 days) The body weight gain in the lactation phase was reduced dose dependently for all treated animals (from control to high dose: +8.67g, +2.33g, -1g, -). No clear alteration could be identified for hematology and clinical biochemistry. No clear effect was found for ovary and uterus weights.

F1 toxicity: The copulation index was 100% in all dose groups. The number of implants/dam appears not to be affected (from control to high dose: 12.67, 12, 10.5, 11). However post-implantation loss was increased (from control to high dose: 10.47%, 8.59%, 23.8%, 100%). Consequently at 1000 mg/kg bw no live pups and at 500 mg/kg bw reduced number of live pups were found: 11/dam in 3 dams in control group versus 8/dam in 2 dams in 500 mg/kg bw dose group. The 3rd dam in this dose group was not pregnant though a positive copulation was mentioned. Overall no effect was found for the dose level of 250 mg/kg bw. The body weight development of live born pups was comparable for treated and control animals.

For more details see confidential Annex.

Table B. 4.10.1.1. Summary of effects and conclusion:

Generation	Sex	Effect level	Basis for effect level / Remarks
P	female	>1000	bw gain reduction to 30% of control during gestation, most likely due to embryotoxicity
P	male	1000	Male exhibited reduced testes weight (mean value reduced to 60% of controls) and epididymides weights (mean value reduced to 70% of controls)
P	female	≥ 500 — ≤ 1000 mg/kg bw/day (actual dose received)	females exhibited prolonged pre-coital interval and gestation period
F1	male/female	≥ 500 — ≤ 1000 mg/kg bw/day (actual dose received)	no live pups at 1000 mg/kg bw and reduced number of live pups at 500 mg/kg bw (8/dam vs. 11/dam in control) increased post-implantation loss at 500 mg/kg bw day and 1000 mg/kg bw day (23.8%, 100% vs. 10.47% in control)

Conclusion: Due to the absence of general maternal toxicity up to 1000 mg/kg bw day, specific **fertility** effects in males at 1000 mg/kg bw day and females at 500 and 1000 mg/kg bw day as well as **developmental** effects in terms of affected live pup numbers at 500 and 1000 mg/kg bw day the study indicates reproductive toxicity of tetraglyme in rats.

The main purpose of the studies carried out with the target substance tetraglyme was to verify that the exposure to tetraglyme leads to glyme specific toxicity, thereby to provide scientific justification for using read-across approach. Glymes are known to induce testes toxicity (see chapter 4.7.) and reproduction impairment (no or reduced number of pups, see summaries

below). In the reported study (OECD 421 with deviation of using lower number of animals), testes toxicity as well as reproduction impairment (in terms of number of live pups) could be clearly demonstrated despite of reduced number of test animals.

4.10.1.2 Human information

No human information available.

4.10.2 Developmental toxicity

4.10.2.1 Non-human information

The developmental toxicity of tetraglyme was evaluated based on a dose-range-finding study on tetraglyme and six studies on related source chemicals (tri-, di- and monoglyme).

The study **on tetraglyme** by **BSL BIOSERVICE 2012** (non-GLP, dose-range finding study; rat; oral) is used as bridging study to justify the read-across approach and to verify the presence of significant **developmental** toxicity of tetraglyme: External soft tissue and skeletal abnormalities were identified in the lowest dose group (250 mg/kg bw) in the absence of severe maternal toxicity:

Maternal toxicity: Upon clinical observation no effect indicative of clear toxicity was found. At 1000 mg/kg bw the body weight gain was reduced starting from the gestation day 11, the mean body weight gain up to necropsy amounted to 57% to the corresponding control value, where as adjusted maternal body weight (= body weight gain subtracted by gravid uterus weight) was comparable to the corresponding control value. Together with the dramatically reduced live pups numbers at birth, the reduced maternal body weight gain is not likely to reflect maternal toxicity. The body weight development was not affected for the animals of the dose levels of 500 and 250 mg/kg bw.

Prenatal toxicity: At 1000 mg/kg bw only 3 living pups were delivered by 8 dams and all these pups were visibly abnormal. At 1000 mg/kg bw day and at 500 mg/kg bw the post implantation loss was increased (96.8 at 1000 mg/kg bw day; 14.7 at 500 mg/kg bw; 5.7 in control). In the group of 250 mg/kg bw comparable findings to the control values were obtained.

Fetal toxicity: The evaluation for the dose level of 1000 mg/kg bw was not meaningful due to the reduced living pups and external abnormality. Litter mean weight was slightly but dose dependently decreased at 500 and 1000 mg/kg bw day (from control to high dose: 3.5, 3.24, 2.77, 2.27 gram). Total litter weight was also strongly decreased at 500 and 1000 mg/kg bw day (from control to high dose: 34.56, 34.68, 31.56, 3.45). At dose levels of 500 and 250 mg/kg bw nearly all fetuses exhibited abnormalities. The most notable effects were the paw skeletal malformations such as absent phalanges and absent 4th metacarpal (summarized below in the table). Further, incidences of absent sternebrum, absent hyoid and absent xiphoid were also significantly increased. Increased incidences of incomplete ossification in brain skeletons were remarkable as well. Analysis of fetal viscera revealed a higher incidence in enlarged left and right ventricles or a small thymus in the 500 mg/kg bw dose group. Craniofacial examination revealed an increase in dilated 3rd ventricle as well as dilated lateral ventricles in the 500 mg/kg bw dose group.

For more details see confidential Annex.

Table B.4.10.2.1.2 Summary of effects and conclusion:

Effect type	Effect level	Basis for effect level / Remarks
Maternal toxicity	>1000 mg/kg bw day	No adverse effect at top dose; body weight gain was affected at top dose, but adjusted maternal body weight (= body weight gain subtracted by gravid uterus weight) was comparable to the corresponding control value.
Developmental toxicity	≤ 250 mg/kg bw/day	overall effects, external, soft tissue and skeletal abnormalities at lowest dose examined; at doses of 500 and 1000 mg/kg bw day severe embryotoxicity

Conclusion: At the dose level of 1000 mg/kg bw no healthy pups were born. At dose levels of 500 and 250 mg/kg bw nearly all pups delivered exhibited skeletal malformations. Due to the absence of maternal toxicity at top dose and a developmental LOAEL at the lowest dose as well as the embryotoxic and teratogenic effects observed the study reports that tetraglyme caused developmental toxicity in rats.

Six further studies on source chemicals (triglyme, diglyme, monoglyme, methoxyacetic acid, for study summaries see Annex 8.2.5) comprising investigations in three species, support classification for reproductive toxicity and identify the rabbit to be more sensitive than rat or mouse. The toxicity pattern in rabbit differs from those found in rat and mouse. In rabbit studies the critical effect was found in prenatal data (post-implantation loss), while in rodents the fetal anomalies were more distinctive. The study results are consistent: the developmental toxicity was present in absence of significant maternal toxicity. Two studies on monoglyme are via inhalation. The effects seen in these studies did not differ from those found in oral studies.

4.10.2.2 Human information

No human information available.

4.10.3 Other relevant information: Validity of the category approach used for the human health hazard assessment of tetraglyme

1. Hypothesis for the category approach

The chemical structure of glymes consists of an ethylene glycol ether chain methylated at terminal positions. The target chemical is composed of four ethylene glycol units.

The scientific hypothesis for read across is that the target chemical tetraglyme belongs to the homologues series of glymes (tri-, di- and monoglymes) which form a “chain length category”, where there is an incremental increase in the number of CH₂CH₂O units, with the target substance tetraglyme being the longest chain variant. No data are available for the next longer chain variants pentaglyme and hexaglyme¹. It is assumed that target and other glyme members (mono-, di-, and triglyme) share the same toxic mode of action including similar metabolism products including the reproductive toxicants 2-methoxyacetic acid (2-MAA) and 2-

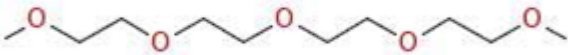
¹ August 2016, accessed: OECD eChemPortal and ECHA substance information

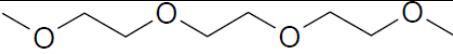
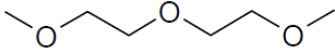
methoxyethanol (2-ME)². No toxicokinetic data are available for tetraglyme, however, for the source substance diglyme toxicokinetic data are available (see appendix 8.2), which demonstrate the formation of the metabolites 2-MAA and 2-ME. The formation of the metabolite 2-MAA metabolites (as well as other possible metabolites) is also suggested for tetraglyme, triglyme and monoglyme when applying the OECD QSARs Toolbox (see table B.4.10.3.4).

It may be assumed that increasing chain lengths could slightly slow down bioavailability or metabolism leading to reduced potency of higher chain variants.

2. Description of target and source substance

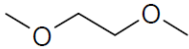
Table B. 4.10.3.2 Description of target and source substance

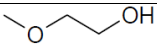
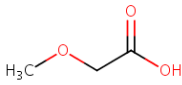
Description of target substance	
Chemical name	Tetraethylene glycol dimethyl ether
Synonyms	Tetraglyme
CAS	143-24-8
Structure	
C&L	Read across from source substances, proposal: CLP/GHS: Repr. 1B; H360

Description of source chemicals	
Chemical name	Triethylene glycol dimethyl ether
Synonyms	Triglyme
CAS	112-49-2
Structure	
*C&L	CLP/GHS: Repr. 1B; H360 Df
Chemical name	Diethylene glycol dimethyl ether
Synonyms	Diglyme
CAS	111-96-6
Structure	
*C&L	CLP/GHS: Repr. 1B; H360 FD

² This hypothesis corresponds to scenario 3 and 4 in the RAAF guidance from ECHA (2015), <http://echa.europa.eu/support/grouping-of-substances-and-read-across>

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Chemical name	Ethylene glycol dimethyl ether
Synonyms	Monoglyme
CAS	110-71-4
Structure	
*C&L	CLP/GHS: Repr. 1B; H360 FD

Description of probably common metabolites (see 4.10.3/chapter 5 for discussion of probability)	
Chemical name	2-Methoxyethanol
Synonyms	2-ME
CAS	109-86-4
Structure	
*C&L	CLP/GHS: Acute Tox. 4; H332, H312, H302, Repr. 1B; H360 FD
Chemical name	Methoxyacetic acid
Synonyms	MAA
CAS	625-45-6
Structure	
*C&L	CLP/GHS: Acute Tox. 4; H302, Skin Corr. 1B, H314; STOT SE 3; H335: C ≥ 5%; Repr. 1B; H360FD

*C&L: refers only for human health hazard

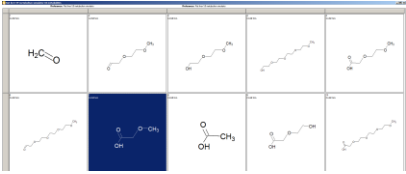
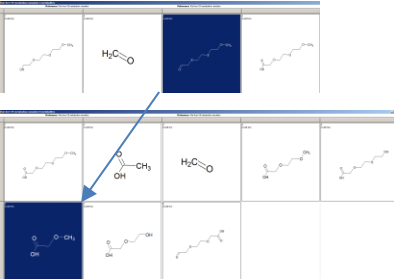
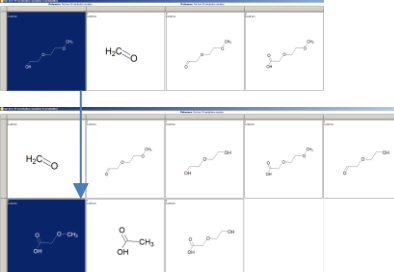
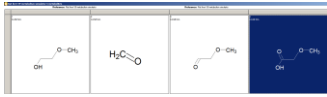
3. Purity / Impurities

See confidential Annex.

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4. Data matrix

Table B. 4.10.3.4. Data matrix for human health hazard assessment, relevant for reproductive toxicity:				
	Target chemical	Source chemicals		
CAS #	143-24-8	112-49-2	111-96-6	110-71-4
Chemical name	Tetraglyme	Triglyme	Diglyme	Monoglyme
Phys-chem				
Molecular weight	222.3 g/mol	178.2 g/mol	134.2 g/mol	90.1 g/mol
Melting point	-30 °C	-45 °C	-68 °C	-58 °C
Boiling point	275 °C	216 °C	162 °C	83 °C
Vapour pressure	0.099 Pa at 20 °C	2.7 Pa at 20 °C	60 Pa at 20 °C	6600 Pa at 20 °C
Water solubility	miscible at every ration	miscible at every ration	miscible at every ration	miscible at every ration
LogPow	-0.84 at 23 °C	-0.52 at 23 °C	-0.36 at 25 °C	-0.21 at 25 °C

Toxicokinetics / Biotransformation				
<p>Basic toxicokinetics and metabolism</p>	<p>OECD QSAR toolboxrat liver S9 metabolism simulator³ suggests qualitatively the formation of MAA (blue) as well as other metabolites structurally similar for the glyme category (oxy, carboxy, hydroxy variants of the basic glyme structure)</p> 	<p>OECD QSAR toolboxrat liver S9 metabolism simulator³ suggests qualitatively the formation of MAA (blue) as well as other metabolites structurally similar for the glyme category (oxy, carboxy, hydroxy variants of the basic glyme structure):</p> 	<p>metabolism studies in-vitro in human and rat and in-vivo in rat and mouse indicate formation of MAA (see section 8.2.1.), i.e. a metabolite shown to induce malformation in mouse</p> <p>OECD QSAR toolboxrat liver S9 metabolism simulator² suggests qualitatively the formation of MAA (blue) as well as other metabolites structurally similar for the glyme category (oxy, carboxy, hydroxy variants of the basic glyme structure):</p> 	<p>OECD QSAR toolboxrat liver S9 metabolism simulator³ suggests qualitatively formation of MAA (blue) as well as other metabolites structurally similar for the glyme category (oxy, carboxy, hydroxy variants of the basic glyme structure):</p> 

³ The current in vitro rat liver metabolic simulator (transformation table) represents an electronically designed set of 509 structurally generalized, hierarchically arranged biotransformation reactions, which are characteristic for the metabolism for in vitro experimental systems such as rodent (mostly rat) liver microsomes and S9 fraction. The principal applicability of this simulator is associated with the reproduction as well as the prediction of the metabolic activation reactions and pathways of xenobiotic chemicals, which may elicit in vitro genotoxicity effects such as bacterial mutagenicity and chromosomal aberrations. Each transformation in simulator consists of source and product structural fragments, and inhibiting "masks". A probability of occurrence is ascribed to each principal transformation, which determines its hierarchy in the transformation list. A training set of xenobiotic chemicals of a wide structural diversity, with experimentally observed metabolic reactions and pathways has been built, using published data on their metabolism in rodent liver microsomes and S9 fraction. The organic compounds in the training set belong to different classes of industrial chemicals, including single and fused-ring arenes, phenols, haloalkanes and haloarenes, aromatic and aliphatic amines, nitroarenes, alkanes and cycloalkanes, alkenes, ethers, carboxylic acids and their derivatives, halogenated hydrocarbons, alcohols, epoxides, N-nitrosoamines, azo chemicals, etc. The data on their metabolism are collected mostly from research publications in the field from selected scientific journals, monographs and websites, and are associated with the commonly observed in vitro liver metabolic reactions of chemicals with different structures. The molecular transformations set consists partly of 25 - 30 abiotic and, also, a few enzyme-controlled reactions believed to occur at a very high rate as compared to the duration of the tests, and the highest priority is assigned to these reactions. This subset of reactions includes also transformations of highly-reactive functional groups and intermediates, such as tautomerizations, arene epoxide rearrangements to phenols, etc. On the whole, the simulator contains also 450 - 470 enzymatic phase I transformations, such as aliphatic C-oxidation, aromatic C-hydroxylation, oxidative N- and O-dealkylation, epoxidation, ester and amide hydrolysis, carbonyl group reduction, nitro and azo group reduction, N-hydroxylation, etc. Additionally, 15 - 20 enzymatic phase II transformations, such as glucuronidation, sulfation, glutathione conjugation, N-acetylation, etc. are included with significantly lower priority than phase I ones.

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Acute toxicity				
Oral	LD50: 3850 mg/kg bw LD50: 5140 mg/kg bw	LD50: 5390 mg/kg bw	LD50: 4760 mg/kg bw	LD50:5370 mg/kg bw
Inhalation	n.a.	n.a.	LC0: 11 mg/L	20 < LC50 < 63 mg/L LC100 at 200 mg/L
Dermal	n.a.	LD50: 6900 mg/kg	n.a.	LD50> 5000 mg/kg bw
Repeated Dose Toxicity				
Subacute	Rat oral LOAEL/NOAEL: 1000/250 mg/kg bw day based on: histopath. thymus and testes; mature sperm ↓; thymus and testis weight ↓; haematopoetic system changes (thrombocyte counts ↓; alkaline phosphatase ↓);	Rat oral 28 days, LOAEL/NOAEL: 1000/250 mg/kg bw day based on: abs. + rel. testis weight ↓, testis size ↓, epididymis weight ↓, isolated foci of necrosis of germinal epithelium, in all animals in seminal vesicles histological evidences of oligospermia and sometimes azoospermia, thymus weight ↓, histopath. thymus; bw gain ↓ (m); water consumption ↓; haematopoetic system changes (leucocyte count ↓ (m), thrombocyte ↓ (m,f)); ALP ↓, bilirubin ↓ + creatinine ↑	Rat respiratory 2 weeks, LOAEC/NOAEC: 370/110 ppm based on on haematopoetic system changes mainly in high dose (thrombocytes ↓, leucocytes ↓, lymphocytes ↓, ALT+ AST+AP+ total protein ↓, bone marrow hyperplasia, spleen + thymus atrophy); prostate weight ↓, seminal vesicle weight ↓, testes weights ↓; testis + epididymis + seminal vesicle + prostate atrophy, spermatogenesis ↓; testis + epididymis histopath.; liver weights ↑ (f) Rat oral 20 days, LOAEL/NOAEL < 684 mg/kg bw d based on abs. and rel. testis weight ↓, testis histopath. ↑, LDH-X activity in testis homogenates ↓, epididymis weight ↓	Mouse oral 5 weeks, LOAEL/NOAEL < 250 mg/kg bw d based on relative testis weight ↓; at 500 mg/kg bw d: atrophy of seminiferous epithelium ↑, combined weight of seminal vesicles and coagulating gland ↓; white blood cell count ↓, red blood cell count & packed cell vol. and/or hemoglobin ↓
Genetic Toxicity in vitro				
Bacterial reverse mutation	Not mutagenic	Not mutagenic	Not mutagenic	Not mutagenic
Gene mutation in	n.a.	n.a.	Not mutagenic	Not mutagenic

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mammalian cells			(UDS test)	(UDS test; HPRT, SCE)
Genetic Toxicity in vivo				
Chromosomal aberration	n.a.	n.a.	Not clastogenic (CA, dominant lethal)	Not clastogenic (MNT; CA)
Reproductive Toxicity				
Fertility	<p>repro/dev. tox screening rat dose-range finding study:</p> <p>male parent LOAEL/NOAEL = 1000/500 mg/kg bw d: testis and epididymis weight ↓</p> <p>female parent LOAEL/NOAEL= 500/250 mg/kg bw day prolonged gestation period;</p> <p>F1 LOAEL/NOAEL = 500/250 mg/kg bw day: ↓ pubs (with 1000 no live pubs)</p>	<p>continuous breeding study with cross-over mating:</p> <p>male parent LOAEL/NOAEL = 1470/830 mg/kg bw d: ↑ liver weight</p> <p>female parent LOAEL/NOAEL = 1470/830 mg/kg bw d: ↓fertility, ↑ liver weight, ↓ pituitary weight</p> <p>F1 LOAEL/NOAEL = 1470/830 mg/kg bw d: ↓live pubs/litter, ↓% pubs born alive, ↓litters/pair</p>	<p>rat dominant rodent lethal test:</p> <p>at 1000 ppm, ↓ pregnancy rates, ↑pre- and post-implantation loss, markedly ↓ male fertility</p>	n.a.
Developmental Toxicity	<p>dev tox rat dose-range finding study:</p> <p>maternal LOAEL > 1000 mg/kg bw d</p> <p>dev.tox. LOAEL ≤ 250 mg/kg bw d: ↑ malformations (paws, absent sternebrum, hyoid, xiphoid), ↑ incidence in incomplete ossification in brain skeletons; at 500 and 1000 mg/kg bw d ↑ post-implantation loss</p>	<p>dev tox rabbit study:</p> <p>maternal LOAEL > 250 mg/kg bw d</p> <p>dev.tox. LOAEL/NOAEL = 125/75 mg/kg bw d: ↑embryo-toxicity: prenatal mortality/litter; with > 175 mg/kg bw d: ↑ malformation (missing toenails, small spleen, hydronephrosis, trend in cardiac malformation)</p> <p>dev tox mouse study:</p> <p>maternal LOAEL > 1000 mg/kg bw d</p> <p>dev.tox. LOAEL/NOAEL = 500/250 mg/kg bw d: ↑ post-implantation loss + malformed live fetuses (neuronal tube, cranio-facial structures, axial skeleton); ↓ fetal</p>	<p>dev tox rabbit study:</p> <p>maternal LOAEL/NOAEL = 175/100 mg/kg bw d: ↑ mortality</p> <p>dev.tox. LOAEL/NOAEL = 50/25 mg/kg bw d: adverse effects on prenatal growth, viability and malformations (axial skeleton, kidney, spleen, cardiovascular system)</p> <p>dev tox mouse study, 4 studies, overall:</p> <p>maternal LOAEL > 500 mg/kg bw d</p> <p>dev.tox. LOAEL = 125-62.5 mg/kg bw d: ↓fetal bw/litter, ↑ nonlive conceptuses/litter; at ≥ 250 mg/kg bw d embryotoxicity +</p>	<p>dev tox rat study</p> <p>maternal LOAEL/NOAEL= 250/120 mg/kg bw d: ↓ body weight (at 1000 mg/kg bw d mortality)</p> <p>dev tox LOAEL/NOAEL < 30 mg/kg bw d: ↑ edema, ↓live birth; at 60 mg/kg bw d: ↑ edema, retarded ossification, ↓ growth, ↑resporptions, ↓ live birth</p> <p>dev tox mouse study, 3 studies, overall:</p> <p>maternal LOAEL/NOAEL < 2000 and > 490 mg/kg bw d: ↑ mortality</p>

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		bw	malformations (exencephaly, skeletal dysmorphogenesis, forelimbs, hindlimbs)	dev tox LOAEL/NOAEL < 250 mg/kg bw d: ↑ malformation (fused ribs & vertebrae), ↑ skeletal variations (extra ribs), ↑ delay of ossification; with > 350 mg/kg bw d: gross abnormalities: exencephaly, caudal defect, umbilical hernia see further inhalation dev tox rabbit and rat study summaries in Annex 8.2.
Harmonized classification				
Human health	<p>proposal: CLP/GHS: Repr. 1B; H360 (without specification for F/f or D/d)</p> <p>Fertility</p> <p>in rats 28 day RDT study and dose range finder for repro/dev tox screening: at 1000 mg/kg bw day ↓ testis and epididymis weight, ↑ gestation length</p> <p>read across to glyme category: similar effects on testis, epididymis, spermatogenesis</p> <p>Development</p> <p>2 dose range finder studies for repro/dev tox rat studies at 250 to 1000 mg/kg bw day: ↓ live pups, ↑ post-implantation loss, ↑ soft tissue and skeletal abnormalities</p> <p>read across to glyme category:</p>	<p>CLP/GHS: Repr. 1B; H360Df</p> <p>rationale for classification in European C&L assessment from 26.01.2001:</p> <p>fertility (Repro Cat. 3, R62)</p> <p>in rats 28 day study testicular toxicity at 1000 mg/kg bw day (↓ testes size, ↓ abs.+rel. testes weight, oligo to azoospermia);</p> <p>in mouse continuous breeding study at 1470 mg/kg bw d, in presence of slight maternal toxicity: ↓ female fertility (% fertile to cohabited); ↓ litters/pair, ↓ live pups/litter</p> <p>reference is given to MAA, which is assumed to represent a metabolite and it is indicated that is induced effects similar to the effects observed in the 28 day study.</p>	<p>CLP/GHS: Repr. 1B; H360FD</p> <p>no summarized arguments for classification are included in the European C&L assessment from 14.12.1998. However from the reported data summary the following can be extracted:</p> <p>Fertility (Repro Cat. 2, R60)</p> <p>inhalation rat RDT 2 weeks, effects in dose range of ~ 100-1000 ppm: ↓ testis & epididymis weight, ↑ atrophy of testis, prostate, seminal vesicles; degeneration of germinal cells in epididymides;</p> <p>inhalation rat dominant-lethal test, effects at 1000 ppm: ↓ male fertility, ↑ pre- and post-implantation loss</p> <p>rat oral RDT 20 days, effects at 684 mg/kg bw day: degeneration of primary and secondary spermatocytes, ↓ testis weight, ↓</p>	<p>CLP/GHS: Repr. 1B; H360FD</p> <p>rationale for classification in European C&L assessment from 26.01.2001:</p> <p>Fertility: Repro cat. 3, R62 - in contradiction (!) to actual entry in CLP Regulation with Repr. Cat. 2/R60, R61 and Repr. 1B/H360FD</p> <p>oral mouse RDT 5 weeks, effects at 250 to 1000 mg/kg bw day: ↓ testis weight, atrophy of seminiferous epithelium;</p> <p>dev tox rat study: ↑ gestation length</p> <p>reference is given to the metabolite MA with the same toxicity profile.</p> <p>Development (Repro Cat. 2,</p>

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	<p>similar effects with embryotoxicity and malformations</p> <p>No specification for F/f or D/d due to uncertainties from 1) limited data documentation, 2) not identical study availability within the glyme source substances and 3) read across.</p>	<p>Development (Repro Cat. 2, R61)</p> <p>3 dev tox studies in mice and 1 dev tox study in rabbit at doses with no or very little maternal toxicity, i.e. 500 and 1000 mg/kg bw day in mice and 175 and 250 mg/kg bw day in rabbits: embryolethality and malformations</p>	<p>testicular lactate dehydrogenase isozyme</p> <p>Reference is given to MAA, which was identified as a metabolite in toxicokinetic rat and mouse studies and for which testicular toxicity data are reported.</p> <p>Development (Repro Cat. 2, R61)</p> <p>1 rat, 1 rabbit, 4 mouse dev. tox. Studies effects at doses without significant maternal toxicity: rat, at 25 to 100 ppm: ↑ incidence of skeletal dev. variations, ↓ fetal weight; rabbit, at 50 -100 mg/kg bw day: ↑ adversely affected implants/litter (resorbed + malformed), ↑ resorptions/litter, ↑ malformations/litter; mouse, at 125 mg/kg bw: ↓ fetal bw/ litter, at 250 mg/kg bw day: ↑ non-live conceptuses/litter, malformed live fetuses/litter</p>	<p>R61)</p> <p>3 dev tox studies in mouse and 1 dev tox study in rat, effects at levels without significant maternal toxicity: mice at 250-490 mg/kg bw day skeletal malformations or variations, at 350 mg/kg bw day gross abnormalities and at 490 mg/kg bw day fetotoxicity; in rats at 30 to 60 mg/kg bw day fetotoxicity</p>
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n.a.: no data available

5. Category approach characterization (according to the ECHA RAAF⁴)

It is assumed that target and other glyme members (mono-, di-, and triglyme) share the same toxic mode of action including similar metabolism products including the reproductive toxicants 2-methoxyacetic acid (2-MAA) and 2-methoxyethanol (2-ME). This corresponds to RAAF scenario 3 and 4:

Scenario 3, Category approach: (Bio)transformation to common compound(s): Variations in the strength of effect(s) observed among source substances. Prediction based on a regular pattern or on a worst-case approach.

Scenario 4, Category approach: Different compounds have the same type of effect(s). Variations in the strength of effect(s) observed among source substances. Prediction based on a regular pattern or on a worst-case approach.

Table B. 4.10.3.5 Read Across Assessment Framework.

Assessment elements (AE) common to all category scenarios	Assessment	Scores (1-5) ⁵
AE C.1 Substance characterization	The target and source substances are clearly described (see table A 1.1.; table B. 4.10.3.2.) and the evidence for similarity of target and source substance purities is sufficient (see point 3 above). Also the category hypothesis is clearly described (see point 1 above).	1
AE C.2 Structural similarity and differences within the category	Homologues series of glymes (tri-, di- and monoglymes) form a “chain length category”, where there is an incremental increase in the number of CH ₂ CH ₂ O units, with the target substance tetraglyme being the longest chain variant.	1
AE C.3 Link of structural similarities and structural differences with the proposed regular pattern	Variations of potency between tetraglyme, triglyme, diglyme and monoglyme were observed in the subacute studies and in the developmental toxicity studies with some tendency towards lower potency of longer chain category members (see data matrix table B.4.10.3/4. and repeated dose toxicity chapter 4.7.1.6). Also experimental variability may contribute to the difference. However it appears that potency is in the medium range for tetra-, tri-, di- and monoglyme (LOAEL and NOAELs between 4 and 400 mg/kg bw d), so that no specific classification limit is needed for any of these category members – and consequently the potency variation is not a concern for the classification purpose.	3
AE C.4 Consistency of effects in the data matrix	The effects within the category are consistent, which is described in the data matrix (see point 4 above) which supports the category hypothesis: The findings in the 28 day repeated dose toxicity studies are comparable for target and for the source substances tri- and diglyme: the target is the male reproductive organ. Further, findings in thymus and altered hematological values are indicative of altered blood system. The findings in reproductive performance (repro/dev. tox screening studies and dev. tox studies) are comparable between the target and the source substances tri-, di- and monoglyme: No live pups and/or reduced number of	1

⁴ <http://echa.europa.eu/support/grouping-of-substances-and-read-across>

⁵ Score 5 is best, score 1 worst; scores are acceptable if ≥ 3

	<p>pubs as well as malformations were the common findings.</p> <p>Rats were exposed to diglyme by inhalation for 14-days (6h/d, 5d/w). The major findings were testicular toxicity and changes in hematopoietic system. The reported study demonstrated that the toxicity profile of diglyme did not differ to that of tri- or tetraglyme and that the exposure routes (oral or by inhalation) did not influence the toxicity of glymes.</p> <p>AMES tests for all glyme category members including the target substance tetraglyme are consistently negative. This supports similar chemical reactivity at the molecular level. Negative genotoxicity is furthermore confirmed for di- and monoglyme in in vitro gene mutation and in vivo chromosomal aberration tests.</p> <p>Acute oral toxicity tests for all glyme category members including the target substance tetraglyme consistently report low acute toxicity (LC50 values above classification needs). This is in agreement with the hypothesis of similar mode of action. Acute inhalation and dermal toxicity tests for tri-, di- and monoglyme confirm the absence of acute toxicity mode of actions of this glyme category.</p> <p>The physchem data differ between the glyme category members according to their chain length.</p> <p>In summary similar effects on reproductive organs and developmental toxicity were observed for the category of glymes (triglyme, diglyme, monoglyme) and the presumed common metabolites 2-ME and MAA and all of these 5 source substances are classified for reproductive toxicity category 1B. Structural similarity of this chain-length category as well as similarity of the experimental toxicological and physchem data matrix (see data matrix table B.4.10.3/4) support the read across of the reproductive toxicity data. All category members and the two metabolites 2-ME and MAA have a harmonized classification as Repr. 1B.</p>	
<p>AE C.5 Reliability and adequacy of the source study(ies)</p>	<p>Detailed study summaries are neither available for the source substances, nor for the target substance.</p> <p>However the reliability of the available studies is adequate, they were evaluated on the basis of the IUCLID entries and considered to meet at least Klimisch score 2 (i.e. acceptable with restrictions).</p> <p>Furthermore the source substances have a harmonized classification and consequently the respective studies are deemed reliable and adequate.</p>	<p>1</p>
<p>AE C.6 Bias that influences the prediction</p>	<p>Data and harmonized classification are only available for the shorter chain variants (tri-, di, monoglyme) and metabolites (2-ME, MAA) in this category of glymes, but not for the longer chain variants.</p> <p>However all repeated dose data including reproduction toxicity data reported in the registration dossiers for the category members were taken into consideration and the experimental data are consistent.</p> <p>Also available data for metabolism products of diglyme (2-ME and MAA) are consistent with the data for the glymes.</p> <p>Search for other structural analogues with the OECD QSAR toolbox (using the organic-functional-groups profiler from US-EPA, strict analogs) did not show similar substances with contradicting toxicity data or profiles.</p>	<p>3</p>

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Scenario 3 assessment elements (AE): (Bio)transformation to common compound(s)	ASSESSMENT	SCORES
<p>AE 3.1 Formation of common (identical) compound(s)</p> <p>AND</p> <p>AE 3.2 The biological target(s) for the common compound(s)</p> <p>AND</p> <p>AE 3.3 Exposure of the biological target(s) to the common compound(s)</p>	<p>Due to the structural similarity of the glyme chain length category members similar toxicokinetics and similar metabolism is assumed. In fact similar effects were observed in the available studies for the category members (see data matrix above in point 4) and this also supports this assumption.</p> <p>Furthermore in metabolism studies with diglyme including human and rat in vitro studies as well as rat and mouse in vivo studies 2-ME and subsequent to this MAA were identified as metabolites (see Annex 8.2.1.). In the developmental toxicity study on the target chemical tetraglyme (dose range findings study for OECD 414) the most notable findings were paw skeletal malformations, which is also known to be typical for MAA (see study summaries in Annex 8.2.5. above). Formation of MAA is also suggested by the OECD QSAR toolbox rat liver S9 metabolism simulator for all of the 4 glymes. Furthermore this simulator also suggests structural similarity of the metabolites from the 4 glymes, as all suggested metabolites represent oxy-, carboxyl- and hydroxyl- variants of the common glyme structure (see table B. 4.10.3.4.). Also these other metabolites may be responsible for the reproductive toxicity effects.</p> <p>Taken together these pieces of evidence provide some support for the hypothesis of similar metabolism of the glyme category.</p>	3
AE 3.4 The impact of parent compounds	See scenario 4 assessment elements	
AE 3.5 Formation and impact of non-common compounds	Due to the high structural similarity (Homologues series of glymes) and consistent toxicological and physicochemical matrix similar metabolism is expected. However no experimental metabolism data are available to support this claim.	3

Scenario 4 assessment elements (AE): Different compounds have the same type of effect(s)	ASSESSMENT	SCORES
AE 4.1 Compounds the test organism is exposed to	see above: AE C.1 Substance characterization	1
AE 4.2 Common underlying mechanism, qualitative aspects	see above: AE C.2 Structural similarity and differences within the category AND AE C.4 Consistency of effects in the data matrix	1
AE 4.3 Common underlying mechanism, quantitative aspects	see above: AE C.3 Link of structural similarities and structural differences with the proposed regular pattern	3
AE 4.4 Exposure to other compounds than those linked to the prediction	see above: AE 3.5 Formation and impact of non-common compounds	3
AE 4.5 Occurrence of other effects than covered by the hypothesis and justification	For the purpose of this CLH Dossier other endpoints than reproductive toxicity are only included in order to describe similarity of mode of action. Acute toxicity and local corrosive effects from the metabolites 2-ME and MAA appear additional to the reproductive toxicity effects. These additional effects of the low weight metabolites are likely due to the shorter chain length and higher polarity and higher concentration and bolus application compared to the systemic exposure kinetics from bio-transformed longer chain glymes.	1

Overall the category approach appears sufficiently robust for classification of tetraglyme for reproductive toxicity category 1B.

4.10.4 Summary and discussion of reproductive toxicity

The assessment of reproductive toxicity is based on data for tetraglyme itself as well as on data for structural “chain length category members” (triglyme, diglyme, monoglyme) as well as the toxic metabolites 2-methoxyethanol (2-ME) and methoxyacetic acid (MAA).

For tetraglyme a dose-range finding study for a rat reproduction developmental toxicity screening test was carried out with an overall LOAEL of 500 mg/kg bw d and adverse reproductive toxicity effects in the absence of general parental toxicity. **Adverse effects for fertility** were observed in terms of reduced testis weight (minus 40%, 1000 mg/kg bw d) and epididymis weight (minus 30%, 1000 mg/kg bw d) and prolonged gestation (500 and 1000 mg/kg bw d). **Adverse effects for development** were observed in terms of reduced number of live pups (500 mg/kg bw d) or absence of live pups (1000 mg/kg bw d). The observed **adverse effects on fertility** were **confirmed** in an oral gavage 28 day study in terms of testis weight (minus 15%), germinal epithelium histopathology (in 2 of 5 animals) and significantly reduced mature sperm. Within this study furthermore histopathology of thymus, thymus weight and haematopoietic system changes were observed. The overall LOAEL was 1000 mg/kg bw day (top dose). The **developmental effects** were **confirmed** in a further dose-range finding study for a rat developmental toxicity study. Post-implantation loss (at 1000 mg/kg bw nearly total, at 500 mg/kg bw significant) and soft tissue and skeletal abnormalities (nearly all foetuses) were observed in the absence of maternal toxicity with an overall LOAEL below 250 mg/kg bw d. These observed adverse effects serve to demonstrate concern for reproductive toxicity (fertility as well as development) and show toxicological similarity to the glyme chain-length-category members triglyme, diglyme and monoglyme as well as the metabolites 2-ME and MAA.

The read-across hypothesis is outlined and discussed in chapter 4.10.3. above. In short the **glyme category** members show **similar developmental toxicity** in terms of embryotoxicity and malformations in rabbit, rat and mouse developmental toxicity studies in the absence of severe maternal toxicity. Furthermore also **similar adverse effects on fertility** were observed in the 28 day rat studies for triglyme and diglyme in terms of adverse effects on testis, epididymis and spermatogenesis. In these studies also adverse effects on thymus and the haematopoietic system were observed. Acute LC50 values above classification needs and no AMES genotoxicity was observed for tetraglyme as well as for tri-, di- and monoglyme which supports similar chemical reactivity of the glyme category. Due to structural similarity within the glyme category and due to similar effects observed in the toxicity studies also similar toxicokinetics and metabolism is assumed. For diglyme metabolism to 2-ME and MAA was shown within various metabolism studies (including human in vitro test) and due to similarity of developmental effects observed for MAA and tetraglyme, MAA is hypothesised to represent a common metabolite. All of the source substances tri-, di- and monoglyme as well as the metabolites 2-ME and MAA already have a harmonized classification for Repro. 1B.

4.10.5 Comparison with criteria

The criteria for classification with **lactation effects, H362 are not met**, since no specific toxicological or toxicokinetic data are available to support such a classification and also none of the source substances (members of the glyme category and the presumed common metabolites 2-ME and MAA) is classified for lactation effects.

The criteria for classification as **Cat 1A, H360 is not met**, since no relevant human data are available.

The criteria for classification as **Cat 2, H361 are also not met**, since there is more than “*some evidence from ... experimental animals, possibly supplemented with other information ...*”

In contrast the criteria for classification as **Cat 1B, H360 are considered to be met**, i.e.

‘...clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects’.

Within one sub-acute oral rat study and within dose range finding studies for a rat reproduction developmental toxicity screening study and for a rat developmental toxicity study adverse effects were observed for tetraglyme with regard to **fertility** (adverse effects on weight and histopathology of testis, histopathology of epididymis, spermatogenesis, gestation length) and **development** (reduced or no live pups or post-implantation loss as well as malformations). All these effects were observed in the absence of severe general or parental toxicity and at or below maximal standard testing doses of 1000 mg/kg bw day⁶.

Similar effects on reproductive organs and developmental toxicity were observed for the category of glymes (triglyme, diglyme, monoglyme) and the presumed common metabolites 2-ME and MAA and all of these 5 source substances are classified for reproductive toxicity category 1B. Structural similarity of this chain-length category as well as similarity of the experimental toxicological and physchem data matrix support the read across of the reproductive toxicity data.

Variations of potency between tetraglyme, triglyme, diglyme and monoglyme were observed in the subacute studies and in the developmental toxicity studies with some tendency towards lower potency of longer chain category members (see data matrix table B.4.10.3.4. and repeated dose toxicity chapter 4.7.1.6 and Annex 8.2.3 and 8.2.5). Also experimental variability may contribute to the difference. However it appears that potency is in the medium range for tetra-, tri-, di- and monoglyme (LOAEL and NOAELs between 4 and 400 mg/kg bw d), so that no specific classification limit is needed for any of these category members – and consequently the potency variation is not a concern for the classification purpose.

It can be concluded that members of the glyme category cause similar effects on fertility and development. There are indications for reduced potency with increasing chain length, however,

⁶ It is noted that ECHA Guidance R7a, page 478 indicates: “If a range-finding study indicates adverse effects on fertility but the effects do not meet the criteria for Reproductive toxicity Category 1B, it is recommended that the main study should be designed to confirm the findings from the range-finding study. However, if the results from the range-finding study meet the criteria for Reproductive toxicity Category 1B reproductive toxicants, the adaptation of Column 2 may apply and further studies (including the main study) may not be needed.”

there is not sufficient evidence to support classification in a lower category or specific concentration limits for mixture classification for tetraglyme.

In order to account for remaining uncertainties from the read across approach it is **not proposed to further specify the classification for fertility and/or developmental effects**: Clear **developmental** effects of tetraglyme (reduced/no live pups, post-implantation loss, malformations) were observed at moderate doses of 250 mg/kg bw d that did not appear secondary to maternal effects. The clear **fertility** effects with tetraglyme (i.e. testis weight minus 15% in 28d study, minus 40% in repro/dev tox screening study; epididymis weight minus 30% in repro/dev tox screening study; germinal epithelium histopath. in 2/5 animals in 28d study, significantly reduced mature sperm in 28d study; prolonged gestation period in repro/dev tox screening study) – were only observed at the limit dose of 1000 mg/kg bw day. Furthermore diglyme and monglyme as well as the potential metabolites 2-ME and MAA are classified with H360FD, but triglyme, the structurally nearest neighbour to tetraglyme in the category of glymes, is classified with H360Df. However, the reason why triglyme was categorized differently compared to the other glymes and the potential metabolites is not clear: According to the classification dossiers (from 2001 and 1998) in all cases, classification for fertility effects is based on effects on testis, epididymis and spermatogenesis in repeated dose toxicity studies. Only for diglyme in addition a rat dominant lethal test is available indicating reduced male fertility. However, indicating f instead of F because of the lack of data is not considered adequate, especially in the case where classification is supported by read across. In addition the classification dossier for monoglyme available to the dossier submitter (Repr. Cat. 3, R62) is inconsistent with the Annex VI to the CLP regulation (Repr. Cat 2, R60). Acknowledging the uncertainties related to testing, assessment, data documentation and read across, it is supported not to indicate f/F or d/D.

4.10.6 Conclusions on classification and labelling

Tetraglyme shall be classified for Repr. 1B, H360

4.11 Other effects

No data available. Not evaluated.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated.

6 OTHER INFORMATION

No data.

7 REFERENCES

Bossert NL et al. (1992). Reproductive toxicity of triethylene glycol and its diacetate and dimethyl ether derivatives in a continuous breeding protocol in Swiss CD1 mice. *Fundamental applied toxicology*, 18: 602-608.

BSL BIOSERVICE (2012). Dose Range Finding Study for Prenatal Development Toxicity Study in Wistar Rats with Tetraethyleneglycoldimethylether. Testing laboratory: BSL BIOSERVICE. Report no.: 113562. Owner company: Clariant.

- BSL Bioservice (2011). Dose Range Finding Study for Combined Repeated Dose Oral Toxicity with Reproduction/Developmental Toxicity Screening Test in Wistar Rats with Tetraethyleneglycoldimethylether. Testing laboratory: BSL Bioservice. Report no.: 113561. Owner company: Clariant.
- Daniel; F. B. et al. (1991). Bis(2-Methoxyethyl) Ether: Metabolism and Embryonic Disposition of a Developmental Toxicant in the Pregnant CD-1 Mouse. *Fund. Appl. Toxicol.*, 16; 567-575. Testing laboratory: no data. Report no.: n. a. Owner company: n. a. Study number: n. a. Report date: 1990-12-18.
- George, J. D. et al. (1987). Teratologic Evaluation of Triethylene Glycol Dimethyl Ether (CAS No. 112-49-2) Administered to New Zealand White Rabbits on Gestational Days 6 Through 19. NTP study NTIS No. PB 87-181657/GAR. Testing laboratory: Triangle Research Institute, NC, USA. Report no.: NTIS No. PB 87-181657/GAR. Owner company: n. a. Study number: n. a. Report date: 1987-01-09.
- Geogr, J. D. et al (1987). The Developmental Toxicity of Triethylene Glycol Dimethyl Ether in Mice. *Fund Appl. Tox.* 9, 173-181. Testing laboratory: Triangle Research Institute, NC, USA. Report no.: n. a. Owner company: n. a. Study number: n. a. Report date: 1985-07-06.
- Hardin B.D. and Eisenmann C. (1987). Relative potency of four ethylene glycol ethers for induction of paw malformations in the CD-1 mouse. *Teratology* 35: 321-328
- Hoechst (1982). Akute orale Toxizität von LP 1713 (Tetraethyleneglycoldimethylether) an weiblichen Ratten. Testing laboratory: Hoechst Aktiengesellschaft Pharma Forschung Toxikologie. Report no.: 425/82. Owner company: Clariant. Report date: 1982-07-29.
- Hoechst AG (1979a). Akute orale Toxizität von Diethylenglykol-dimethylether (Diglyme, Produkt der ICI) an weiblichen Ratten. Testing laboratory: Hoechst AG, Pharma Forschung Toxikologie. Report no.: 376/79. Owner company: Clariant. Study number: 79.0376. Report date: 1979-06-20.
- Hoechst AG (1981a). Akute orale Toxizität von Monoethylenglykoldimethylether Monoglyme an weiblichen Ratten. Testing laboratory: Hoechst AG, Pharma Forschung Toxikologie. Report no.: 192/81. Owner company: Clariant. Study number: 81.0192. Report date: 1981-04-22.
- Hoechst AG (1981c). A Mutagenicity Screening of 356/81 A in Bacteria (Ames Test). Testing laboratory: Höchst Aktiengesellschaft, Pharma Research, Frankfurt, Germany. Report no.: 526/81. Owner company: Clariant. Study number: 526/81. Report date: 1981-09-07.
- Hoechst AG (1982a). Akute orale Toxizität von Triethylenglykoldimethylether-Hoechst an weiblichen Ratten. Testing laboratory: Hoechst AG, Pharma Forschung Toxikologie. Report no.: 82.0178. Owner company: Clariant. Study number: 82.0178. Report date: 1982-04-15.
- Hoechst AG (1984). Tetraethylenglykoldimethylether weitsiedend Study of the mutagenic potential in strains of Salmonella typhimurium (Ames Test) and Escherichia coli. Testing laboratory: Pharma Research Toxicology HOECHST AKTIENGESELLSCHAFT. Report no.: 84.0625. Owner company: Clariant.
- Hoechst AG (1988a). Monoethylenglykoldimethylether HOECHST, Prüfung auf embryotoxische Wirkung an Wistar-Ratten bei inhalativer Verabreichung. n. a. Testing laboratory: Hoechst AG, Pharma Forschung Toxikologie und Pathologie, Frankfurt, Germany. Report no.: 88.0110. Owner company: Clariant. Study number: 88.0110. Report date: 1988-02-15.
- Hoechst AG (1988b). Monoethylenglykoldimethylether HOECHST, Prüfung auf embryotoxische Wirkung an Russen-Kaninchen bei inhalativer Verabreichung. n. a. Testing laboratory: Hoechst AG, Pharma Forschung Toxikologie und Pathologie, Frankfurt, Germany. Report no.: 88.0003. Owner company: Clariant. Study number: 88.0003. Report date: 1988-01-05.
- Hoechst AG (1992a). Tetraethylenglykoldimethylether, rein Subakute orale Toxizität (28 Applikationen in 29 Tagen) an männlichen und weiblichen Wistar-Ratten. Testing laboratory: Pharma Entwicklung Zentrale Toxikologie Hoechst Aktiengesellschaft. Report no.: 91.0582. Owner company: Clariant. Report date: 1992-06-03.
- Hoechst AG (1992b). Triethylenglykoldimethylether, rein Subakute orale Toxizität (28 Applikationen in 29 Tagen) an männlichen und weiblichen Wistar-Ratten. n. a. Testing laboratory: Hoechst AG, Pharma Entwicklung, Zentrale Toxikologie, Frankfurt, Germany. Report no.: 92.0371. Owner company: Clariant. Study number: 92.0371. Report date: 1992-05-26.

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- Höchst AG (1992). Triethylenglykoldimethylether rein Study of the mutagenic potential in strains of Salmonella typhimurium (Ames Test). Testing laboratory: Höchst Aktiengesellschaft, Pharma Development Toxicology, Frankfurt, Germany. Report no.: 91.1171. Owner company: Clariant. Study number: 91.1171. Report date: 1992-01-24.
- Jagannath, D. R. (1979). Mutagenicity Evaluation of Diethylenglycol-Dimethylether in the Ames Salmonella/Microsome Plate Test. Testing laboratory: Litton Bionetics, Inc.; Kensington, USA. Report no.: 743/79. Owner company: Clariant. Study number: 743/79. Report date: 1979-10-17.
- Larese Filon, A. et al. (1999). Skin absorption in vitro of glycol ethers. *Int. Arch. Occup. Environ. Health* 72. Testing laboratory: no data. Report no.: n. a. Owner company: n. a. Study number: n. a. Report date: 1999-05-09.
- Leonhardt D.E., Coleman L.W., Bradshaw W.S. (1991). Perinatal toxicity of ethylene glycol dimethyl ether in the rat. *Reproductive Toxicology* 5: 157-162
- McGregor et al. (1983). *Toxicol. Appl. Pharmacol* 70: 303-316
- Morrissey RE. (1988). Association of sperm, vaginal cytology and reproductive organ weight data with results of continuous breeding preproduction studies in Swiss CD1 mice. *Fundamental applied toxicology* 11(2): 359-371
- Nagano K., Nakayama E., Oobayashi H., Nishizawa T., Okuda H., Kazunori Y. (1984). Experimental studies on toxicity of ethylene glycol alkyl ethers in Japan. *Environmental Health Perspectives* 57: 75-84
- Plasterer M.R., Bradshaw W.S., Booath G.M., Carter M.W. (1985). Developmental toxicity of none selected compounds following prenatal exposure in the mouse: naphthalene, p-nitrophenol, sodium selenite, dimethyl phthalate, ethylenethiourea and four glycol ether derivatives. *Journal of Toxicology and Environmental Health* 15: 25-38.
- Price, C. J. et al (1985). Teratologic Evaluation of Diethylene Glycol Dimethyl Ether (CAS No. 111-96-6) Administered to CD-1 Mice on Gestational Days 6 through 15. NTP study NTIS No. PB 86-135233; Triangle Research Institute, NC, USA. Testing laboratory: Triangle Research Institute, NC, USA. Report no.: NTIS No. PB 86-135233. Owner company: n. a. Study number: n. a. Report date: 1985-08-30.
- Price, C. J. et al (1987). Teratologic Evaluation of Diethylene Glycol Dimethyl Ether (CAS No. 111-96-6) Administered to New Zealand White Rabbits on Gestational Days 6 through 19. NTP study NTIS No. PB 87-209532; Triangle Research Institute, NC, USA. Testing laboratory: Chemistry and Life Sciences Research Triangle Institute, NC27709-2194, USA. Report no.: NTIS No. PB 87-209532. Owner company: n. a. Study number: n. a. Report date: 1987-09-06.
- Price, J. C. et al (1987). The Developmental Toxicity of Diethylene Glycol Dimethyl Ether in Mice. *Fund. Appl. Toxicol.*, 8; 115-126. Testing laboratory: Chemistry and Life Sciences, Center for Life Sciences and Toxicology, Research Triangle Institute, NC, USA. Report no.: n. a. Owner company: n. a. Study number: n. a. Report date: 1985-07-06.
- Rasjad C, Yamashita K, Datu AR, Yasuda M (1991), Pattern of limb malformations in mice induced by methoxyacetic acid, *Hiroshima J Med Sci. Sep;40(3):93-9*.
- Richards, D. E. et al. (1993). Comparative Metabolism of Bis(2-Methoxyethyl) ether in Isolated Rat Hepatocytes and in the Intact Rat: Effects of Ethanol on in vitro Metabolism. *Arch. Toxicol.*, 67; 531-537. Testing laboratory: no data. Report no.: n. a. Owner company: n. a. Study number: n. a. Report date: 1993-06-14.
- Schuler R.L. Hardin B.D., Niemeier R.W., Booth G., Hazelden K., Piccirillo V., Smith K. (1984). *Environmental Health Perspectives* 57: 141-146
- Schwetz, B. A. et al (1992). The Developmental Toxicity of Diethylene and Triethylene Glycol Dimethyl Ethers in Rabbits. *Fund. Appl. Toxicol.*; 238-245. Testing laboratory: Chemistry and Life Sciences, Center for Life Sciences and Toxicology, Research Triangle Institute, NC, USA. Report no.: n. a. Owner company: n. a. Study number: n. a. Report date: 1992-01-24.
- Smyth, H. F. jr., Seaton, J., Fischer, L. (1941). THE SINGLE DOSE TOXICITY OF SONIE GLYCOLS AND DERIVATIVES. *JOURNAL OF INDUSTRIAL HYGIENE A) TD TOXICOLOGY* vol. 23, no. 6. Testing laboratory: Mellon Institute, Pittsburgh, Pa. Report date: 1941-04-30.
- Tirmenstein, M. A. (1993). Comparative Metabolism of Bis(2-Methoxyethyl) ether by Rat and Human Hepatic Microsomes: Formation of 2-Methoxyethanol. *Toxicology In Vitro* 7, 645-652. Testing laboratory: no data. Report no.: n. a. Owner company: n. a. Study number: n. a. Report date: 1993-01-20.

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Valentine, R. et al. (1998). Subchronic Inhalation Toxicity of Diglyme. Food Chem. Toxicol., 37; 75-86. Testing laboratory: DuPont Haskell Laboratory for Toxicology and Industrial Medicine, DE, USA. Report no.: n. a. Owner company: n. a. Study number: n. a. Report date: 1998-06-07.

8 ANNEXES

8.1 Annex 1: Study summaries of source substances

(as presented in the registration dossier of tetraglyme and amended with more detailed information)

8.1.1 Toxicokinetics and Metabolism

No toxicokinetic and metabolism data are available for Tetraglyme. Instead metabolism data are provided for Diglyme and Monoglyme. The details of these studies may not be essential for the conclusion on classification of tetraglyme, but the studies are nevertheless summarized here in Annex 8.2. to explain the quality of the related data. For an explanation of the relevance of these toxicokinetic studies carried out with Diglyme for read across to Tetraglyme see section 4.10.3.

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Table B.8.2.1. Toxicokinetics

Method	Results	Remarks	Reference
<p>in vitro study</p> <p>Exposure regime: Single application, 30 min incubation</p> <p>Doses/conc.: 1 mM Diethylene glycol dimethyl ether (Diglyme)</p> <p>Human and rat microsomes were incubated with Diethylene glycol dimethyl ether. The formation of 2-Methoxyethanol was determined.</p>	<p>Metabolites identified: yes; 2-Methoxyethanol</p> <p>2-Methoxyethanol identified as metabolite in rat and human microsomes; P450 IIE1 (aniline hydroxylase) appears to be involved as key enzyme</p>	<p>2 (reliable with restrictions)</p> <p>weight of evidence read-across from supporting substance</p> <p>Test material: Diglyme</p>	<p>Tirmens tein, M.A. (1993)</p>
<p>mouse (CD-1) female, pregnant</p> <p>oral: gavage</p> <p>Exposure regime: Single application gd11, gavage</p> <p>Doses/conc.: 500 mg/kg bw equivalent or similar to OECD Guideline 417 (Toxicokinetics)</p>	<p>Metabolites identified: yes, 2-(2-methoxyethoxy) ethanol; (2-methoxyethoxy) acetic acid; 2-methoxyethanol; methoxyacetic acid in urine and embryonic tissue</p> <p>no bioaccumulation potential based on study results (> 97% of the administered dose is excreted as metabolite via urine)</p>	<p>2 (reliable with restrictions)</p> <p>weight of evidence read-across from supporting substance</p> <p>Test material: Diglyme</p>	<p>Daniel; F.B. et al. (1991)</p>
<p>rat (Sprague-Dawley) male</p> <p>oral: gavage</p> <p>Exposure regime:</p> <p>a. in vivo study: single application</p> <p>b. in vitro study: rats used in the induction study (primary hepatocytes) received Ethanol (15% v/v) in their drinking water on the 5 days prior to hepatocyte isolation</p> <p>Doses/conc.:</p> <p>a. in vivo study: 5.1 mmol [¹⁴C]-Diglyme/kg bw (148 μCi/kg bw)</p> <p>b. in vitro study: 1, 10, 30 or μM [¹⁴C]-Diglyme</p> <p>The metabolism of Diglyme was investigated in both settings (in vitro and in vivo) a. Rats were treated with a single dose of the radiolabelled test item and urine was examined for Diethylene glycol dimethyl ether and its metabolites.</p> <p>b. Freshly prepared rat hepatocytes were incubated with radiolabelled Diethylene glycol dimethyl ether and metabolites were detected.</p>	<p>Metabolites identified: yes; (2-methoxyethoxy) acetic acid; 2-(2-methoxyethoxy) ethanol; methoxyacetic acid; 2-methoxyethanol</p> <p>no bioaccumulation potential based on study results</p>	<p>2 (reliable with restrictions)</p> <p>weight of evidence read-across from supporting substance</p> <p>Test material: Diglyme</p>	<p>Richards , D.E. et al. (1993)</p>

Table B.8.2.1 (continuation Toxicokinetics)

Method	Results	Remarks	Reference
Dermal absorption study in vitro human (n.a.) male Coverage (dermal absorption study): in vitro study Exposure regime: 30, 60, 90, 120, 150, 180 and 240 min. Doses/conc.: 0.2 mL In vitro skin permeation using human skin was measured with the Franz method. equivalent or similar to OECD Guideline 428 (Skin Absorption: In Vitro Method)	Absorption: Lag time: 36 ± 3 min for diglyme, 39 ± 3 min for monoglyme Flux at steady state permeation: 0.952 ± 0.340 mg/cm ² /h for diglyme, 3.434 ± 1.897 mg/cm ² /h for monoglyme Permeation values of mixture (glycol ether 30% + acetone 70%) Lag time: 49 ± 28 min for diglyme; 35 ± 27 min for monoglyme Flux at steady state permeation: 0.674 ± 0.305 mg/cm ² /h for diglyme, 0.837 ± 0.474 mg/cm ² /h for monoglyme, Total recovery: not determined	2 (reliable with restrictions) weight of evidence read-across from supporting substance Test material: Diglyme and Monoglyme	Larese Filon, A. et al. (1999)

Non-human information

Tirmenstein, M.A. (1993): Human and rat hepatic microsomes were incubated with diglyme. The rat microsomes catalysed the NADPH-dependent cleavage of the central ether linkage of diglyme yielding 2-methoxyethanol and 2-(2-methoxyethoxy) ethanol. Microsomes isolated from phenobarbital- or ethanol-pretreated rats exhibited an increased capacity to cleave diglyme to 2-methoxyethanol. This ethanol-induced increase in 2-methoxyethanol formation was not observed if incubations contained the cytochrome P450 IIE1 inhibitor Isoniazid. Pretreatment of rats with diglyme significantly increased microsomal P-450 levels, P-450 associated enzyme activities and the conversion of diglyme to 2-methoxyethanol. Human hepatic microsomes also catalysed the NADPH-dependent cleavage of diglyme to 2-methoxyethanol. The formation of 2-methoxyethanol from diglyme correlated with the aniline hydroxylase activity (P450 IIE1) levels measured in human hepatic microsomes.

Daniel; F.B. et al. (1991): An embryotoxic oral dose of diglyme, 3.73 mmol/kg bw (500 mg/kg bw), administered on gestation day 11 to pregnant CD-1 mice was metabolised predominantly by O-demethylation to 2-(2-methoxyethoxy) ethanol with subsequent oxidation to (2-methoxyethoxy) acetic acid. Urinary excretion of this metabolite over 48 hours amounted to 63 +/-2% of the dose. A smaller percentage of the administered dose was metabolised at the (central) ether linkage to produce 2-methoxyethanol, which was further metabolised by alcohol dehydrogenase to methoxyacetic acid. The total excretion within 48h amounted > 97% Urinary excretion of methoxyacetic acid, a potent developmental toxicant, amounted to 28 +/-1% of the administered dose by 48 hours and was the second most prominent urinary metabolite. Unchanged diglyme and

methoxyacetic acid were detected in the embryonic tissues from these animals and embryos harvested after the initial 6 -hour period showed detectable amounts of only methoxyacetic acid. The average amount of methoxyacetic acid per embryo was calculated to be 1.5 +/-1.0 µmol (5.9 mmol/kg bw) at the 6 -hour termination time.

Richards, D.E. et al. (1993): The metabolism of diglyme was studied in isolated rat hepatocytes and in intact rat. Male Sprague-Dawley rats were used in both studies. Primary hepatocytes were cultured as monolayers and incubated with [14C]-diglyme at 1, 10, 30 and 50 µM for up to 48 hours. For the in vivo study, rats were given single oral doses of [14C]-diglyme at 5.1 mmol/kg bw and urine was collected for up to 96 hours. The principal metabolite from primary rat hepatocytes and in the urine was (2-methoxyethoxy) acetic acid (approx. 67% of the administered dose after 48 hours). Other prominent metabolites common to both systems included 2-(2-methoxyethoxy) ethanol, methoxyacetic acid, 2-methoxyethanol and diglycolic acid. Diglyme was demonstrated to be not cytotoxic to rat hepatocytes.

Human information

See 4.1.1. summary of **Tirmenstein 1993** including human microsomes.

Larese Filon, A. et al. (1999): An in vitro skin absorption study was performed applying Diethylene glycol dimethyl ether to dermatomed human skin. For diglyme, the lag time was reported to be 36 ± 3 min and the flux at steady state permeation was 0.952 ± 0.340 mg/cm²/h. For monoglyme, the lag time was reported to be 39 ± 3 min and the flux at steady state permeation was 3.434 ± 1.897 mg/cm²/h. A mixture of 30% monoglyme or diglyme in acetone showed slightly reduced flux at steady state, probably due to reduced concentration in applied dose, i.e. 0.674 ± 0.305 mg/cm²/h for diglyme, 0.837 ± 0.474 mg/cm²/h for monoglyme. Based on the obtained results, it can be concluded that diglyme and monoglyme are dermally readily bioavailable.

8.1.2 Acute toxicity of source substances

Table 8.2.2. Acute toxicity of source substances

Method	Results	Remarks	Reference
rat (Wistar) female oral: gavage equivalent or similar to OECD Guideline 401 (Acute Oral Toxicity)	LD50: 5390 mg/kg bw (female)	2 (reliable with restrictions) read-across from supporting substance: Triglyme	Hoechst AG (1982a)
rat (Wistar) female oral: gavage equivalent or similar to OECD Guideline 401 (Acute Oral Toxicity)	LD50: 4760 mg/kg bw (female)	2 (reliable with restrictions) read-across from supporting substance: Diglyme	Hoechst AG (1979a)
rat (Wistar) female oral: gavage equivalent or similar to OECD	LD50: 5370 mg/kg bw (female)	2 (reliable with restrictions) read-across from supporting substance:	Hoechst AG (1981a)

Guideline 401 (Acute Oral Toxicity)		Monoglyme	
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Summary:

Oral:

- female rats, LD50 (triglyme): 5390 mg/kg bw (supporting data)
- female rats, LD50 (diglyme): 4760 mg/kg bw (supporting data)
- female rats, LD50 (monoglyme): 5370 mg/kg bw (supporting data)

Further information available:

Inhalation:

- male/female rats, LC0 (diglyme; 7h exposure): 11 mg/L -> 19.3 mg/L (4h calculated exposure)
- male/female rats, LC0 (monoglyme; 1h exposure): 240 mg/L -> 60 mg/L (4h calculated exposure)
- rats, LC50 (monoglyme; 6h exposure): > 20 mg/L

Dermal:

- male rats, LD50 (triglyme): > 6900 mg/kg bw

It can be concluded that source substances relevant for read across to tetraglyme have low acute toxicity.

8.1.3 Repeated dose toxicity studies of source substances

Table 8.2.3. Repeated dose toxicity studies of source substances

Method	Results	Remarks	Reference
rat (Hoe: WISKf (SPF71)) male/female subacute (oral: gavage) 0, 62.5, 250, 1000 mg/kg bw/d (actual ingested) Exposure: 29 days (once per day) OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents)	LOAEL: 1000 mg/kg bw/day (actual dose received) (male/female) based on: abs. + rel. testis weight ↓, testis size ↓, epididymis weight ↓, isolated foci of necrosis of germinal epithelium, in all animals in seminal vesicles histological evidences of oligospermia and sometimes azoospermia, thymus weight ↓, histopath. thymus; bw gain ↓ (m); water consumption ↓; haematopoetic	2 (reliable with restrictions) supporting study read-across from supporting substance: Triglyme	Hoechst AG (1992b)

	<p>system changes (leucocyte count ↓ (m), thrombocyte ↓ (m,f)); ALP ↓, bilirubin ↓ + creatinine ↑</p> <p>NOEL: 62.5 mg/kg bw/day</p> <p>(actual dose received)</p> <p>(female) (decreased thymus weight at 250 mg/kg bw/d)</p>		
<p>rat (CrI:CD BR) male/female</p> <p>subacute (inhalation: vapour) (nose only)</p> <p>110, 370, 1100 ppm (analytical conc.)</p> <p>Vehicle: clean air</p> <p>Exposure: 6 h/d (5 d/week for 2 weeks)</p> <p>equivalent or similar to OECD Guideline 412 (Repeated Dose Inhalation Toxicity: 28/14-Day)</p>	<p>LOAEC male: 370 ppm (male) based on haematopoetic system changes mainly in high dose (thrombocytes ↓, leucocytes ↓, lymphocytes ↓, ALT+AST+AP+ total protein ↓, bone marrow hyperplasia, spleen + thymus atrophy); prostate weight ↓, seminal vesicle weight ↓, testes weights ↓; testis + epididymis + seminal vesicle + prostate atrophy, spermatogenesis ↓; testis + epididymis histopath.; liver weights ↑ (f)</p> <p>LOAEC female: 1100 ppm based on changes in haematopoeticsystem</p>	<p>2 (reliable with restrictions)</p> <p>supporting study read-across from supporting substance: Diglyme</p>	<p>Valentine, R. et al. (1998)</p>
<p>rat (Sprague-Dawley) male</p> <p>subacute (oral, gavage in water)</p> <p>0 (control), 684 mg/kg bw d (actually ingested)</p> <p>No of rats: 90/group</p> <p>Exposure: 20 d</p> <p>Post-exposure observation: up to 8 w</p> <p>5 rats killed at 2d intervals during exposure and weekly in post-exposure period</p> <p>Evaluation: body weight; organ weight and histology of testes, epididymides, thymus glands, brains; LDH-X enzyme activity</p>	<p>LOAEL ≤ 684 mg/kg bw d</p> <p>body weight after 18d exp. ↓; absolute and relative testis weight after 10d exp. ↓; absolute weight of testes, epididymides, thymus glands after 20d exp. ↓, no full recovery till 8w post-exp.</p> <p>degenerative changes in testis histopath. from mild after 8d exp. to marked after 18d exp.; no full recovery after 8w post-exp.</p> <p>decreased LDH-X activity in testis homogenates</p>	<p>2 (reliable with restrictions)</p> <p>supporting study read-across from supporting substance: Diglyme</p>	<p>Publication 1989, summarized in registration dossier for diglyme (therein listed in section Toxicity to reproduction under heading: other studies 002)</p>
<p>Mouse (JCL-ICR) male</p> <p>subacute (oral, gavage in water)</p> <p>0 (control), 250, 500, 1000 mg/kg</p>	<p>LOAEL ≤ 250 mg/kg bw d</p> <p>based on: relative testis weight ↓</p> <p>≥ 500 mg/kg bw d: Histopath.</p>	<p>2 (reliable with restrictions)</p> <p>supporting study read-across from supporting</p>	<p>Publication 1984, cited in registration dossier for diglyme (therein</p>

<p>bw d (actually ingested)</p> <p>No of mice: 5 males/dose</p> <p>Exposure: 5d/week for 5 weeks</p> <p>Post-exposure observation: 1d</p> <p>Evaluation: body weight; testis weight, combined weight of seminal vesicles and coagulating glands, histology, haematology</p>	<p>dose related ↑ of atrophy of seminiferous epithelium; white blood cell count ↓; combined weight of seminal vesicles and coagulating gland slightly ↓; red cell count, packed cell volume and/or hemoglobin content ↓</p>	<p>substance: Monoglyme</p>	<p>listed in section Toxicity to reproduction under heading "other studies 005")</p>
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Repeated dose toxicity oral

Hoechst AG 1992b reports an oral gavage 28 day rat study with **triglyme**: All animals survived and no clinical signs were noted at any dose level. No neurological or ophthalmological effects or changes in mucosa were noted. Except for the males of the 1000 mg/kg bw/d group which showed a decreased body weight gain (-16% compared to control), the body weight gain of all animals was not affected. There were no effects upon the mean daily food and water consumption observed in all test groups.

There were no changes in haematology and clinical chemistry noted at 62.5 and 250 mg/kg bw dose groups. At 1000 mg/kg bw the leucocyte count was decreased in male rats; the thrombocyte count was decreased in all animals of the high dose group (-30% for males, -26% for females). At 1000 mg/kg bw/d the bilirubin and creatinin concentration was significantly increased (+180% for males and +270% for females). The pH in urine of all animals of the high dose group was decreased.

Relative organ weights were within the control range with the exception of a decreased testis (-52%) of the males in the high dose group. The thymus weight was also significantly decreased in all animals of the 1000 mg/kg bw/d group (-61% for males, -56% for females). Females of the 250 mg/kg bw group had decreased thymus weight (-21%).

The size of testes was reduced in males of the 1000 mg/kg bw 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/d dose group which caused oligo- and aspermia. In both males and females involution of thymus was observed in the high dose group.

Repeated dose toxicity inhalation

Valentine, R. et al. (1998): Groups of 20 male and 10 female rats were exposed by nose-only inhalation 6 hours/day, 5 days/week for 2 weeks to either 0 (control), 110, 370 or 1100 ppm **diglyme**. 2-Methoxyethanol was applied as positive control. Rats were sacrificed immediately following exposure, after a 14-day recovery period, or after 42 and 84 days of recovery (males only). Parameters investigated included in-life observations and body weights, clinical pathology, and histopathology with organ weights.

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. Although these effects were more severe at the higher concentrations tested, partial

or complete recovery was seen by 84 days post-exposure. Changes in the haematopoietic system occurred in both sexes and involved the bone marrow, spleen, thymus, leucocytes and erythrocytes. The testicular effects of diglyme were somewhat less pronounced than those seen with 2-methoxyethanol. The NOEC for repeated inhalation exposure to diglyme in female rats is 370 ppm. For males, the NOAEC was derived at 110 ppm

Further studies from the registration dossier of diglyme and monoglyme are summarized in the table 8.2.3. above in order to further support the evidence for testicular toxicity of the glyme category.

8.1.4 Germ cell mutagenicity (mutagenicity)

Table 8.2.4. Germ cell mutagenicity

<p>bacterial reverse mutation assay (gene mutation) Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538 (met. act.: with and without) Test concentrations: Study I: 4, 20, 100, 500, 2500, 10000 µg/plate Study II: 4, 20, 100, 500, 2500, 5000 µg/plate equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)</p>	<p>Evaluation of results: negative Test results: negative for S. typhimurium, other: TA98, TA100, TA1535, TA1537, TA1538(all strains/cell types tested) met. act.: with and without cytotoxicity: no ; negative controls valid: yes; positive controls valid: yes</p>	<p>2 (reliable with restrictions) Supporting study read-across from supporting substance: Triglyme</p>	<p>Höchst AG (1992)</p>
<p>bacterial reverse mutation assay (gene mutation) Salmonella thyphimurium TA98, TA100, TA1535, TA1537, TA1538 (met. act.: with and without) Test concentrations: 0.005, 0.01, 0.1, 1.0, 5.0, 10.0, 25.0 and 50.0 µL/plate equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)</p>	<p>Evaluation of results: negative Test results: negative for Salmonella th. TA98, TA100, TA1535, TA1537 and 1538 (all strains/cell types tested) ; met. act.: with and without ; cytotoxicity: no ; vehicle controls valid: yes; positive controls valid: yes</p>	<p>2 (reliable with restrictions) Supporting study read-across from supporting substance: Diglyme</p>	<p>Jagannath, D.R. (1979)</p>
<p>bacterial reverse mutation assay (gene mutation) Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538 (met. act.: with and without) E. coli WP2 uvr A (met. act.: with</p>	<p>Evaluation of results: negative (with and without metabolic activation) Test results: negative for TA98, TA100, TA1535, TA1537, TA1538 (all strains/cell types</p>	<p>2 (reliable with restrictions) Supporting study read-across from supporting substance:</p>	<p>Hoechst AG (1981c)</p>

<p>and without)</p> <p>Test concentrations: 0, 0.004, 0.02, 0.1, 0.5, 2.5 and 10 µL</p> <p>OECD Guideline 471 (Bacterial Reverse Mutation Assay)</p>	<p>tested) ;</p> <p>met. act.: with and without ; vehicle controls valid: yes; negative controls valid: yes; positive controls valid: yes</p> <p>negative for E. coli WP2 uvr A(all strains/cell types tested) ;</p> <p>met. act.: with and without ; vehicle controls valid: yes; negative controls valid: yes; positive controls valid: yes</p>	<p>Monoglyme</p>	
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Ames tests are available on the source chemicals triglyme, diglyme and monoglyme. In none of the 4 studies mutagenicity was observed.

8.1.5 Toxicity for reproduction

Table 8.2.5.1 Toxicity for reproduction

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<p>rabbit (New Zealand White) oral: gavage 75 mg/kg bw/d (main study) (actual ingested) 125 mg/kg bw/d (main study) (actual ingested) 175 mg/kg bw/d (main study) (actual ingested) 250 mg/kg bw/d (main study) (actual ingested) Exposure: gd 6 through 19 (14 days) (daily) OECD Guideline 414 (Prenatal Developmental Toxicity Study)</p>	<p>LOAEL (maternal toxicity): > 250 mg/kg bw day (actual dose received) (corrected maternal weight gain was not significantly affected by treatment, but gravid uterine weight exhibited a dose-related decreasing trend) LOAEL (developmental toxicity): 125 mg/kg bw day (actual dose received) (increase in embryo toxicity: prenatal mortality/litter at 125 and 175 mg/kg bw/d; at 175 mg/kg bw d malformations (missing toenails, small spleen, hydronephrosis, trend in cardiac malformation)</p>	<p>2 (reliable with restrictions) Supporting study read-across from supporting substance: Triglyme, purity 98.5%</p>	<p>George, J. D. et al. (1987) Schwetz, B.A. et al (1992)</p>
<p>mouse (CD-1) oral: gavage 0 mg/kg bw/d (actual ingested) 250 mg/kg bw/d (actual ingested) 500 mg/kg bw/d (actual ingested) 1000 mg/kg bw/d (actual ingested) Exposure: gd 6 through 15 (10 days) (daily) equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)</p>	<p>LOAEL (maternal toxicity): > 1000 mg/kg bw/day (actual dose received): increased relative liver weight at 500 and 1000 mg/kg bw day not considered adverse LOAEL (developmental toxicity) = 500 mg/kg bw/day (actual dose received): decreased fetal body weight, adversely affected conceptuses/litter[↑] (i.e. post-implantation loss + malformation: neuronal tube, cranio-facial structures, axial skeleton)</p>	<p>2 (reliable with restrictions) Supporting study read-across from supporting substance: Triglyme, purity > 98%</p>	<p>Geogr, J.D. et al (1987)</p>
<p>mouse (Swiss CD-1), 20 pairs per treatment group, 40 pairs for control group oral, drinking water 0%, 0.25%, 0.5%, 1% ~ 440, 830, 1470 mg/kg bw d continuous breeding study with cross over mating trial</p>	<p>male parent LOAEL/NOAEL = 1470/830 mg/kg bw d: [↑] liver weight female parent LOAEL/NOAEL = 1470/830 mg/kg bw d: [↓]fertility (fertile/cohabited at top dose: 9/19 vs. control 16/20) , [↑] liver weight, [↓] pituitary weight F1 LOAEL/NOAEL = 1470/830 mg/kg bw d: [↓]live pups/litter (6 vs. control 12), [↓]%pups born alive (0.8 vs. control 0.99) , [↓]litters/pair (4.2 vs 4.8)</p>	<p>4 (RMS copy from classification dossier from 2001) supporting study read-across from supporting substance: triglyme</p>	<p>Bossert NL et al. (1992) Morrissey RE. (1988).</p>
<p>rabbit (New Zealand White) oral: gavage 25 mg/kg bw/d (actual ingested) 50 mg/kg bw/d (actual ingested)</p>	<p>LOAEL (maternal toxicity): 175 mg/kg bw/day ([↑] mortality) LOAEL (developmental toxicity): 50 mg/kg bw/day (adverse effects on prenatal growth,</p>	<p>2 (reliable with restrictions) Supporting study read-across from supporting</p>	<p>Price, C.J. et al (1987) Schwetz, B.A. et al (1992)</p>

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<p>100 mg/kg bw/d (actual ingested) 175 mg/kg bw/d (actual ingested) Exposure: gd 6 through 19 (14 days) (daily) OECD Guideline 414 (Prenatal Developmental Toxicity Study)</p>	<p>viability and malformations: axial skeleton, kidney, spleen, cardiovascular system)</p>	<p>substance: Diglyme, purity > 99%</p>	
<p>mouse (CD-1) oral: gavage 62.5 mg/kg bw/d (analytical conc.) 125 mg/kg bw/d (analytical conc.) 250 mg/kg bw/d (analytical conc.) 500 mg/kg bw/d (analytical conc.) Exposure: gd 6 through 15 (10 days) (daily) equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)</p>	<p>LOAEL (maternal toxicity) > 500 mg/kg bw/day (actual dose received): no effect at highest tested dose level LOAEL (developmental toxicity): 62.5 mg/kg bw/day (actual dose received) based on: embryotoxicity: ↓ fetal bw/litter, ↑ nonlive conceptuses/litter at 125 mg/kg bw/d (at ≥ 250 mg/kg bw d embryotoxicity + malformations: exencephaly, skeletal dysmorphogenesis, forelimbs, hindlimbs; gravid uterine weight and number of live fetuses per live litter was significantly decreased at all doses)</p>	<p>2 (reliable with restrictions) Supporting study read-across from supporting substance: Diglyme, purity > 99%</p>	<p>Price, C.J. et al (1985) Price, J.C. et al (1987)</p>
<p>rat (CD), male, 10 animals/group rodent dominant lethal test inhalation 250, 1000 ppm Exposure: 7h/day, 5 days</p>	<p>at 1000 ppm ↓ pregnancy rates, ↑ pre- & postimplantation loss, markedly ↓ male fertility; due to cytotoxic effect on the germinal cells and the resulting male infertility the dominant lethal test is not adequate for the evaluation of genotoxicity</p>	<p>4 (RMS copy from classification dossier from 2001) supporting study read-across from supporting substance: diglyme</p>	<p>McGregor et al., (1983)</p>
<p>rat (Hoe: WISKf (APF71)) inhalation: vapour (whole body) 10 ppm (0.037 mg/L) (analytical conc.) 32 ppm (0.12 mg/L) (analytical conc.) 100 ppm (0.374 mg/L) (analytical conc.) Exposure: 6 hours per day (daily) OECD Guideline 414 (Prenatal Developmental Toxicity Study)</p>	<p>LOAEC (maternal toxicity): > 0.374 mg/L air (no effects) LOAEC (developmental toxicity): 0.12 mg/L air, based on retarded ossification, ↑ incidence of skeletal malformations and soft tissue effects</p>	<p>2 (reliable with restrictions) Supporting study read-across from supporting substance: monoglyme, purity > 99%</p>	<p>Hoechst AG (1988a)</p>
<p>rabbit (Hoe: HIMK (SPF Wiga)) inhalation: vapour (whole body)</p>	<p>LOAEC (maternal toxicity): 0.187 mg/L air (slightly decreased food consumption)</p>	<p>2 (reliable with restrictions)</p>	<p>Hoechst AG (1988b)</p>

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<p>5 ppm (0.019 mg/L) (analytical conc.)</p> <p>16 ppm (0.06 mg/L) (analytical conc.)</p> <p>50 ppm (0.187 mg/L) (analytical conc.)</p> <p>Exposure: 6 hours per day (daily)</p> <p>OECD Guideline 414 (Prenatal Developmental Toxicity Study)</p>	<p>LOAEC (developmental toxicity): 0.187 mg/L air: ↓ vitality within the first 24 hours and skeletal abnormalities</p>	<p>Supporting study</p> <p>read-across from supporting substance: monoglyme, purity > 99%</p>	
<p>rat (Sprague Dawley)</p> <p>gavage</p> <p>0, 30, 60, 120, 250, 500, 1000 mg/kg bw d</p> <p>Exposure: daily GD 8-18</p>	<p>maternal LOAEL/NOAEL= 250/120 mg/kg bw d: ↓ body weight (at 1000 mg/kg bw d mortality)</p> <p>dev tox LOAEL/NOAEL < 30 mg/kg bw d: ↑ edema, ↓ live birth; at 60 mg/kg bw d: ↑ edema, retarded ossification, ↓ growth, ↑ resorptions, ↓ live birth</p>	<p>4 (RMS copy from classification dossier from 2001)</p> <p>supporting study</p> <p>read-across from supporting substance: monoglyme</p>	<p>Leonhardt et al., 1991</p>
<p>mouse</p> <p>gavage</p> <p>0, 250, 350, 490 mg/kg bw d</p> <p>Exposure: daily GD 7-10</p>	<p>maternal LOAEL/NOAEL > 490 mg/kg bw d</p> <p>dev tox LOAEL/NOAEL < 250 mg/kg bw d: ↑ malformation (fused ribs & vertebrae), ↑ skeletal variations (extra ribs), ↑ delay of ossification; with > 350 mg/kg bw d: gross abnormalities: exencephaly, caudal defect, umbilical hernia</p>	<p>4 (RMS copy from classification dossier from 2001)</p> <p>read-across from supporting substance: monoglyme</p>	<p>Nagano et al., 1984</p>
<p>mouse</p> <p>gavage</p> <p>0, 361 mg/kg bw day</p> <p>Exposure: GD 11</p>	<p>maternal LOAEL/NOAEL > 361 mg/kg bw d</p> <p>dev tox LOAEL/NOAEL < 361 mg/kg bw d: malformations (forepaws & hindpaws)</p>	<p>4 (RMS copy from classification dossier from 2001)</p> <p>read-across from supporting substance: monoglyme, purity > 99%</p>	<p>Hardin et al. 1987</p>
<p>mouse</p> <p>gavage</p> <p>2000 mg/kg bw d</p> <p>Exposure GD 7-14</p>	<p>maternal LOAEL/NOAEL < 2000 mg/kg bw d: ↓ maternal survival (to 74%)</p> <p>dev tox LOAEL/NOAEL < 2000 mg/kg bw d: no surviving pups</p>	<p>4 (RMS copy from classification dossier from 2001)</p> <p>read-across from supporting substance: monoglyme, purity > 99%</p>	<p>Plasterer et al., 1985</p> <p>Schuler et al., 1984</p>
<p>mouse (Jcl:ICR)</p> <p>oral</p> <p>10 mmol/kg bw</p> <p>Exposure: single dose at gd 10.5,</p>	<p>NOAEL < 10 mmol/kg bw Based on limb/metacarpal, phalanges/digit defects</p>	<p>2 (reliable with restrictions)</p> <p>Supporting study</p> <p>read across from</p>	<p>Rasjad C, Yamashita K, Datu AR, Yasuda M (1991),</p>

<p>11 or 11.5 Analysis of fetuses at gd 15.5 for external and skeletal malformations</p>		<p>supporting substance: methoxyacetic acid (MAA)</p>	
<p>rat (Alpk/AP) oral 118, 295, 590 mg/kg bw d single application</p>	<p>significant decrease in testes weight along with spermatocyte damage (at all doses)</p>	<p>4 (RMS copy from classification dossier from 2001 read-across from supporting substance: methoxyacetic acid (MAA)</p>	<p>Foster, P.M., et al 1987. Toxicology 43(1): 17-30</p>
<p>mouse (C57Bl/CnexCH/Cne F1) oral 50, 100, 300, 600, 900 mg/kg bw d single application</p>	<p>changes in mouse germ cells and in testicular morphology</p>	<p>4 (RMS copy from classification dossier from 2001 read-across from supporting substance: methoxyacetic acid (MAA)</p>	<p>Spano M. et al. 1991. J. Toxicol. Environ. Health 34(1): 157-176</p>
<p>mouse (CD-1) oral in drinking water 140, 240, 390 mg/kg bw d continuous breeding fertility assessment, 2 generations exposed</p>	<p>adverse reproductive and fertility effects seen at all doses including testicular atrophy, reduced fertility and reduced number of live pups/litter (none at the high dose). F1 animal sin the low dose receiving MAA as their parents also demonstrated adverse reproductive effects and non (when mated) had live youngs.</p>	<p>4 (RMS copy from classification dossier from 2001 read-across from supporting substance: methoxyacetic acid (MAA)</p>	<p>George J.D. et al. 1986. PB86-164274, Methoxy Acetic ACid, Reproduction and Fertility Assesmetn in CD-1 Mice when administered in drinking water, NTP/86-040 (J-10183)</p>
<p>5 mouse studies, 3 rat studies, 1 rabbit study all oral route (except one rat i.p. and one rat inhalation) all single application studies (except rabbit with dosing daily from GD 7 to 19)</p>	<p>limb defects in fetuses, limb bud defects in embryos, malformation of tail, digit malformations, hydronephrosis, hydrocephalus, kidney pelvis dilation, heart malformation, (in rabbit also decreased litter size)</p>	<p>4 (RMS copy from classification dossier from 1998) read-across from supporting substance: methoxyacetic acid (MAA)</p>	<p>Classification dossier for MAA from 14.12.1998</p>
<p>rat, mouse, rabbit studies</p>	<p>Exposure of male rats to 300 ppm vapour has been shown to result in infertility due to testicular atrophy which is, at least partially, reversible. A no effect level for fetotoxicity and teratogenicity of 10 ppm has been demonstrated in inhalation studies in the rat, mouse and rabbit.</p>	<p>4 (RMS copy from classification dossier from 2001 read-across from supporting substance: 2-methoxyethanol (2-ME)</p>	<p>Classification dossier for 2-ME from 16.03.1989</p>

For informing on **fertility effects** the repeated dose studies for the source chemicals (listed in chapter 8.3.2.) are available and in this chapter further to this two more studies are listed:

Bossert NL et al. (1992) and Morrissey RE. (1988) report a mouse (Swiss CD-1) continuous breeding study with cross-over matings for triglyme. 20 pairs were included in the treatment group, 40 pairs for the control group. Exposure was oral, via drinking water at concentrations of 0%, 0.25%, 0.5%, 1%, corresponding to 0, 440, 830, 1470 mg/kg bw d.

Table 8.2.5.2. Fertility effects (for more details see confidential annex)

adverse effects	no adverse effects
decreases of fertility and reproductive parameters, dose-related and statistically significant at 1%: -litters/pair: 4.2 ± 0.2 (controls 4.8 ± 0.1) -live pups/litter: 6.0 ± 0.8 (controls : 12.2 ± 0.3) -proportion of pups born alive: 0.8 ± 0.4 (controls: 0.99 ± 0.00)	
trend to dose-related decrease (significant at the highest tested dose) in number of live pups/litter	
in cross-mating study, fertility of females exposed to 1% was decreased: number fertile/ number cohabited: 9/19 (= 47%) (controls: 16/20 (=80%))	in cross mating study no change in male fertility at any dose tested
	no macro or histological changes in sexual organs and no changes were noted in the quality of the sperm
at 1%, effects noted in adults were: -increase of the relative an absolute liver weight -decrease of pituitary weight in females	

McGregor et al. (1983) report one rodent dominant lethal test for diglyme. 10 male animals per group were included in the study. Exposure was via inhalation at 250 and 1000 ppm, for 7 hours/day and 5 days per week.

Table 8.2.5.3 Fertility effects

adverse effects	no adverse effects
at 1000 ppm reduced pregnancy rates	
at 1000 ppm increased pre- and post-implantation loss	
at 1000 ppm markedly reduced male fertility	
(due to the cytotoxic effect on the germinal cells and the resulting male infertility the dominant-lethal-test is not adequate for the evaluation of genotoxicity)	

For developmental toxicity six studies on source chemicals (triglyme, diglyme, monglyme, methoxyacetic acid, 2-methoxyethanol) comprising investigations in three species, support

classification for reproductive toxicity and identify the rabbit to be more sensitive than rat or mouse. The toxicity pattern in rabbit differs from those found in rat and mouse. In rabbit studies the critical effect was found in prenatal data (post-implantation loss), while in rodents the fetal anomalies were more distinctive. The study results are consistent: the developmental toxicity was present in absence of significant maternal toxicity. Two studies on monoglyme are via inhalation. The effects seen in these studies did not differ from those found in oral studies.

George, J. D. et al. (1987) and Schwetz, B.A. et al (1992) tested the developmental toxicity of the source chemical **triglyme in rabbits:**

Maternal toxicity: Minor signs of toxicity included lacrimation, alopecia, weight loss, and absence of feces. During the study two animals in the 75 mg/kg bw/d group died from causes that could not be directly attributed to experimenter error. Specifically, one dam died on gd 18 while aborting her litter. The other dam was found dead on gd 18 without exhibiting distinctive clinical signs and upon necropsy exhibited an intact esophagus but hemorrhagic lungs. Thus, the mortality for rabbits exposed to Triglyme was 0%, 8.3%, 0%, 0%, and 0% for the control through high dose groups, respectively. Maternal body weight did not differ significantly among treatment groups on gd 0, gd 6, or on gd 12. On gd 19, maternal body weight exhibited a significant decreasing trend with the 175 mg/kg bw/d group representing the NOEL. Maternal weight gain during the treatment period exhibited a decreasing trend in the 175 mg/kg bw/d group and above significantly below controls. Corrected maternal weight gain was not significantly affected by treatment, but gravid uterine weight exhibited a dose-related decreasing trend. Absolute maternal liver weight increased in a dose-related manner with 75 mg/kg bw/d representing a NOEL.

Developmental toxicity:

Table 8.2.5.4. Developmental toxicity (for more details see confidential annex)

Adverse effects	No adverse effects
Of the pregnant does surviving until the scheduled sacrifice, the number of the does with entirely resorbed litters and no live fetuses was 1, 1, 2, 2, and 7 for the 0, 75, 125, 175, and 250 mg/kg bw/d groups, respectively.	There was no significant difference among the treated groups in the number of corpora lutea per doe, or the number of implantation sites per litter.
The percent preimplantation loss, however, exhibited a significant increasing trend that appeared to be primarily caused by the effect size in the high dose group. The percent resorptions per litter (LOAEL 125 mg/kg bw/d group), the percent non-live implants per litter (LOAEL = 250 mg/kg bw/d group), and percents adversely affected implants per litter (LOAEL: 175 mg/kg bw/d) each increased in a dose-dependent manner.	
The % of litters with non-live implants exhibited a significant treatment effect that was caused by the effect size in the 175 and 250 mg/kg bw/d groups. The % of litters with adversely affected implants exhibited an increasing trend with both the 175 and 250 mg/kg bw/d dose groups significantly above controls. For litters with live fetuses, the number of live fetuses/litter exhibited a significant decreasing trend that was caused by the effect size in the 125, 175, and 250 mg/kg bw/d dose groups, with 75 mg/kg bw/d representing the NOAEL.	The % dead fetuses/litter and % litters with resorptions or with dead fetuses were not significantly affected by Triglyme treatment

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	Triglyme had not significant effect on the sex ratio of live litters, average fetal body weight/litter, or average male or female fetal body weight/litter.
Triglyme treatment caused a significant increasing trend in the % fetuses having one or more malformations/litter, with the 175 mg/kg bw d (51.3 ± 7.1) and 250 mg/kg bw/d (75.2 ± 5.1) dose groups significantly increased over controls (7.3 ± 2.6)	
The % litters with one or more malformed fetuses exhibited a significant dose effect with the 175 and 250 mg/kg bw/d groups significantly above controls, but the increase in this parameter was not strictly dose-related at lower doses.	
For both external and visceral malformations, 125 mg/kg bw/d was the NOAEL. Malformations observed most frequently included missing toenails in fetuses 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.	

Table 8.2.5.5. Summary of effects and conclusion:

Effect type	effect level	Basis for effect level / Remarks
maternal toxicity	>250 mg/kg bw day	Maternal weight gain during the treatment period exhibited a decreasing trend with the 175 mg/kg bw/d group and above significantly below controls. However corrected maternal weight gain was not significantly affected by treatment, but gravid uterine weight exhibited a dose-related decreasing trend.
developmental toxicity	125 mg/kg bw day (actual dose received)	increase in embryo toxicity (in terms of post-implantation loss) at 125 and 175 mg/kg bw day, increased malformations at 175 mg/kg bw day and 250 mg/kg bw day

Conclusion: Due to the difference of maternal LOAEL and developmental LOAEL as well as the embryotoxic and teratogenic effects observed the study reports that triglyme caused developmental toxicity in rabbits.

George, J. D. et al. (1987) tested the developmental toxicity of the source chemical **triglyme in mice**

Maternal toxicity: No maternal deaths, morbidity, or distinctive clinical signs were observed during the study. Minor clinical signs (piloerection, rough coat, lethargy, weight loss, and vaginal bleeding) were not consistently dose related and thus were apparently not directly associated with Triglyme treatment. Maternal body weight at sacrifice (gd 17), weight gain during gestation, weight gain during treatment, and corrected maternal weight gain were unaffected by treatment. Relative maternal liver weight, but not absolute liver weight, was increased significantly at 500 and 1000 mg/kg bw/d.

Developmental toxicity:

Table 8.2.5.6. Developmental toxicity (for details see confidential annex)

Adverse effects	No adverse effects
	Gravid uterine weight decreased in a dose-related manner, but was not significantly depressed compared to controls at any dose level.
	The number of live fetuses/litter was unaffected by treatment.
	When indices of embryo/fetal toxicity were expressed as % of implants/litter, there was no effect on the % of resorptions/litter, the % of late fetal deaths/litter, or the % of post-implantation loss/litter.
The % of adversely affected conceptuses/litter (postimplantation loss + malformed live fetuses) exhibited a dose-related trend toward an increase, with the 250 mg/kg bw/d dose group being a NOEL. (11.09% at 1000 mg/kg bw and 0.27% in controls)	
The average fetal body weight/litter was significantly	

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decreased at 500 and 1000 mg/kg bw/d.	
	The fetal sex ratio was not affected by Triglyme treatment.
The % of malformed live fetuses/litter and % of litters with one or more malformed fetuses were both significantly increased at 1000 mg/kg bw/d, as a result of a significant increase in both external and skeletal, but not visceral malformations at the 1000 mg/kg bw/d dose level. (59% in 1000 mg/kg bw d vs. 3.85% in controls)	
Malformations observed at external examination included primarily neural tube closure defects and cleft palate. Skeletal malformations, observed in stained preparations solely affected the axial region (fused ribs, fused sternbrae). The two visceral malformations observed (missing left kidney and ureter) occurred in a single fetus in the 1000 mg/kg bw/d dose group.	

Table 8.2.5.7. Summary of effects and conclusion

Effect type	Effect level	Basis for effect level / Remarks
maternal toxicity	1000 mg/kg bw/day (actual dose received)	No adverse effect ad highest dose level; increased relative liver weight at 500 and 1000 mg/kg bw day not considered adverse
developmental toxicity	500 mg/kg bw/day (actual dose received)	decreased fetal body weight, adversely affected conceptuses/litter in terms of post-implantation loss + malformed live fetuses

Conclusion: Due to the difference of maternal LOAEL and developmental LOAEL as well as the embryotoxic and teratogenic effects observed the study reports that triglyme caused developmental toxicity in mice.

Price et al. 1987 tested the developmental toxicity of the source substance **diglyme** in rabbits by gavage

Maternal toxicity: A clearcut maternal toxicity was evident only at the highest dose level (175 mg/kg bw/d). From 75 to 100% of the bred females in the various groups were pregnant. There was an increase in treatment-related maternal mortality at the highest dose level (15 vs. 4% in controls). Clinical signs were limited to a decreased fecal output at the high dose level. There was a loss of weight during the days of chemical treatment at dose levels of 50 mg/kg bw/d and above, with the difference from control statistically significant at each of these dose levels.

A decrease in weight of the gravid uterus was observed that was statistically significant at the two highest dose levels (100 and 175 mg/kg bw/d). The body weight gain, corrected for gravid uterine weight, did not differ from that of the control group in any of the dose groups.

Developmental toxicity:

Table 8.2.5.8. Developmental toxicity

Adverse effects	No adverse effects
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There was a significant increase in prenatal mortality at the two highest dose levels (100 and 175 mg/kg bw/d). This loss was due largely to the total resorption of 2 of 21 litters at 100 mg/kg bw day and 5 of 17 litters at 175 mg/kg bw day. Resorptions/ litter: 3, 2, 6, 21, 44% from control to high dose.	At necropsy, all groups were similar in the number of implantations and the percentage pre-implantation loss.
	There was no evidence of a sex difference in either of these parameters - live fetuses/litter of fetal body weight.
There was a significant decreasing trend in fetal body weight but no group mean was significantly different from that of the controls.	
In contrast, female pups tended to have more malformations than males, with percentage of malformed fetuses per litter being significantly increased at both 100 and 175 mg/kg bw/d among female pups and only at the highest dose level among males. malformed fetus/litter: 4, 3, 12, 37, 51% from control to high dose	
The malformations observed were diverse, being identified primarily in the visceral and skeletal structures. The two most frequently observed malformations were fused ribs in 17% (13/77) and hydronephrosis in 23% (18/77) of high-dose fetuses. In addition, 19% of the high-dose fetuses exhibited clubbing of the fore- and hindlimbs, a condition that was classified as an anatomical variation in the present study, but for which the incidence increased with dose. The percentage of adversely affected (includes resorptions, late fetal death and malformed fetuses) implants/litter was significantly increased above control at dose levels of 50 mg/kg bw/d and above.	

Table 8.2.5.9. Summary of effects and conclusion

Effect type	Effect level	Basis for effect level / Remarks
maternal toxicity	175 mg/kg bw/day	increased mortality at top dose level
developmental toxicity	50 mg/kg bw/day	adverse effects on prenatal growth, viability and malformations

Conclusion: Due to the difference of maternal LOAEL and developmental LOAEL as well as the embryotoxic and teratogenic effects observed the study reports that diglyme caused developmental toxicity in rabbits.

Price et al. 1985 tested the developmental toxicity of the source substance **diglyme** in mice by gavage.

Maternal toxicity: No maternal deaths, morbidity, or dose-related clinical signs were observed during the study. Maternal body weight at sacrifice (gd 17), weight gain during gestation, and weight gain during treatment were each decreased in a dose-related manner, and each measure was significantly reduced at 250 and 500 mg/kg bw/d relative to controls. No effect of Diglyme was observed on corrected maternal weight gain (gravid uterine weight was significantly decreased at all doses). Maternal liver weight, but not relative liver weight, was decreased significantly at the high dose.

Developmental toxicity:

Table 8.2.5.10. Developmental toxicity

Adverse effects	No adverse effects
The gravid uterine weight per dam, as well as the number of live fetuses per live litter, was significantly decreased at all doses of Diglyme.	
When indices of postimplantation mortality were expressed as a percentage of implants per litter or as a percentage of litters with intrauterine deaths, only the 250 and/or 500 mg/kg bw/d groups were significantly elevated above the vehicle group. nonlive conceptuses/litter: 5, 8, 7, 12, 50%	
Average fetal body weight/litter was significantly decreased at 125, 250, and 500 mg/kg bw/d, but not at 62.5 mg/kg bw/d.	
	The fetal sex ratio was not affected by Diglyme treatment.
The percentage of malformed live fetuses/litter and the percentage of litters with one or more malformed live fetuses were both significantly increased at 250 and 500 mg/kg bw/d, and a wide variety of malformation types (encephaly, limb and digit malformations, cleft palate) was observed. malformed life fetuses/litter: 0.4, 0, 2, 24, 96% from control to top dose	
When malformation incidence was analyzed by general category, fetuses with either external (gross) malformations or skeletal malformations occurred in a significantly higher proportion of litters in the 250 and 500 mg/kg bw/d groups. Malformations observed upon external examination included primarily neural tube closure defects, limb and digit malformations, craniofacial malformations, and abdominal wall defects. A number of malformations classified as "external" also involved the underlying appendicular skeleton, specifically malformations of the limbs, digits and tail. Skeletal malformations affecting the axial region (i.e. fused, branched or missing ribs; fused, misaligned, or missing arches and vertebral centra) could only be observed in stained preparations.	
Visceral malformations occurred in a higher number of litters only at the high dose (500 mg/kg bw/d), and	

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involved primarily the heart, major vessels, and urinary tract.	
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Table 8.2.5.11 Summary of effects and conclusion

Effect type	Effect level	Basis for effect level / Remarks
maternal toxicity	>500 mg/kg bw/day (actual dose received)	no effect at highest tested dose level
developmental toxicity	62.5 mg/kg bw/day (actual dose received)	Significant embryotoxicity with ≥ 62.5 mg/kg bw/d and malformations at ≥ 250 mg/kg bw day;

Conclusion: Due to the difference of maternal LOAEL and developmental LOAEL as well as the embryotoxic and teratogenic effects observed the study reports that diglyme caused developmental toxicity in mice.

Leonhardt et al. 1991 reports a developmental study in the rat (SD) with the source substance **monoglyme**. Exposure was carried out via gavage at doses of 0, 30, 60, 120, 250, 1000 mg/kg bw d, daily between GD 8-18. Pregnant females were weighted daily from GD0 and either sacrificed at GD 19 or permitted to give birth.

Maternal toxicity: at 1000 mg/kg bw d, 4/6 (66%) females died, also nasal and rectal bleeding were reported. Weight loss was observed severe at 1000 mg/kg bw d and 500 mg/kg bw d, less severe but significant at 250 mg/kg bw d.

At 120 mg/kg bw d a decrease in the weight gain pattern was reported, but this may be related to the 100% resorption rate observed from 120 mg/kg bw d.

At 60 and 30 mg/kg bw d the weight gain pattern was almost comparable to controls except a modest reduction in weight gain at 60 mg/kg bw d at GD 19 (probably related to late fetal death).

Developmental Toxicity:

Table 8.2.5.12 developmental toxicity

adverse effects	no adverse effects
at 250 mg/kg bw d: 100% resorption, with death at GD10 at 120 mg/kg bw d: 100% resorption, with death between GD 12-14 at 60 mg/kg bw d: 16% foetolethality (vs. 3% in control) between GD 16-18	
at 60 mg/kg bw d: increase in gestation length: >21 days for 12/15 produced litters at 30 mg/kg bw d: increase in gestation length: >21 days for 5/14 produced litters	
at 60 mg/kg bw d: decrease in % live births: 51% vs. 98% in controls; decrease in survival rate at day 1: 2% survival rate vs. 93% in controls – probably due to maternal behavior, a lack of maternal care was reported at this dose level	
at 30 mg/kg bw d: slight reduction of % live births: 92% vs. 98% in control slight reduction of survival rate: 89% vs. 93% in control	
at 60 mg/kg bw d: abnormalities in terms of: edema: 28% affected fetuses/litters (40% affected litters), retardation of bone ossification (53% affected litters) and reduced growth (7% smaller than controls)	
at 30 mg/kg bw d: edema: 5% affected fetuses/litters	skeletal and soft tissue analysis did not reveal any specific teratogenic defects in offspring exposed to 30 and 60 mg/kg bw d

Nagano et al. 1984 reports a mouse developmental toxicity study with **monglyme**. Exposure was carried out via gavage at 0, 250, 350, 490 mg/kg bw d, daily from GD 7-10.

No significant maternal toxicity was evidenced.

Developmental toxicity

Table 8.2.5.13 developmental toxicity

adverse effects	no adverse effects
490 mg/kg bw d: increase in dead foetuses	
350 and 490 mg/kg bw d: increase in gross abnormalities: exencephaly, caudal defect, umbilical hernia	
all groups: increased incidence of malformation (fused ribs and vertebrae) and skeletal variations (extra ribs and delay of ossification)	

Hardin et al. 1987 reports a mouse developmental toxicity study with **monoglyme**. Exposure was carried out via gavage at 0 and 361 mg/kg bw d at GD 11.

No maternal effects were observed.

Developmental toxicity

Table 8.2.5.14 developmental toxicity

adverse effects	no adverse effects
increase in forepaws and hindpaws malformation	no differences in live or dead fetuses
	no external gross malformations

Plasterer et al. 1985 and Schuler et al. 1984 report a mouse developmental toxicity study. Exposure was carried out via gavage with 2000 mg/kg bw d from GD 7 to 14. Maternal survival decreased to 74% and there were no surviving pups. No other data were available for maternal toxicity.

Hoechst AG 1988a tested the developmental toxicity of the source substance **monoglyme** in rats by whole body inhalation.

Maternal toxicity: All animals survived. No clinical signs were noted at any dose level. Body weight gain and food consumption was within the control range. The organ weights were within the control range. No changes occurred at any dose level with the exception of one dam in which all embryos were found dead in the 100 ppm dose group.

Developmental toxicity:

Table 8.2.5.10 Developmental toxicity

Adverse effects	No adverse effects
Examination of litters	
There was a slight decrease of foetal weight observed in	Body weight of control foetuses and foetuses of the 10

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the 32 ppm (=low dose) group and the body weight of the foetuses of the 100 ppm (=high dose) group was considerably decreased.	ppm dose group was regular.
The foetuses of the 100 ppm (=high dose) group showed a retarded development.	
Resorptions as well as dead foetuses were found in the 100 ppm (=high dose) group.	
Resorptions were present in all groups (control and treatment group), but the number of resorptions at the 100 ppm (=high dose) level was increased compared to the others.	
The number of viable foetuses was considerably decreased in the 100 ppm (=high dose) dose group.	
	Placenta weight was unaffected.
Skeletal examination	
	One foetus of the control group showed anophthalmia and 5 foetuses had malformations of the extremities. 9 foetuses of the control group showed malformations of the sternum and ribs. In the 10 ppm dose group no anomalies were observed; the ossification was not affected.
4 foetuses of the 32 ppm dose level showed anomalies of the extremities (abnormal orientation, malformations and haematoma) and 2 of them had an open eyelid. 2 foetuses of the 32 ppm dose level had a malformation of the scapula.	
In the 100 ppm dose group 11 foetuses had malformations of the extremities and scapula (crooked, shortened). One foetus of the high dose group had a shortened tail and 4 foetuses showed subcutaneous oedema	
The ossification of the foetuses of the 32 ppm and 100 ppm dose group was considerably retarded. In these dose groups fragmented thoracic and lumbar vertebrae were observed.	
The number of foetuses showing malformations of ribs was significantly increased at exposure to 32 ppm and 100 ppm of the test substance.	
Soft tissue examination	
Blood in the pericardium and enlarged ureter were observed in foetuses of the 32 ppm and 100 ppm dose group.	

Table 8.2.5.11 Summary of effects and conclusion

Effect type	Effect level	Basis for effect level / Remarks
maternal toxicity	>0.374 mg/L air	no effects

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developmental toxicity	0.12 mg/L air	based on retarded ossification and increased incidence of skeletal malformations and soft tissue effects at 0.12 mg/L
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Conclusion: Due to the absence of maternal effects up to top concentration and developmental effects in terms of retarded ossification and increased malformations with a LOAEL of 0.12 mg/L air the study indicates that **monoglyme** caused developmental toxicity in rats by inhalation exposure.

Hoechst AG 1988b tested the developmental toxicity of the source substance **monoglyme** in rats by whole body inhalation.

Maternal toxicity: All animals survived. With the exception of one abortion in the 16 ppm dose group no serious clinical signs were noted at any dose level. The urine of one rabbit at the 16 ppm level was stained red on day 9 of gestation. Body weight gain of all animals in the 0, 5 and 16 ppm dose group was not affected. During the first week of treatment the body weight of the animals of the 50 ppm dose group was decreased. Within the second week of treatment this effect disappeared. There were no effects upon the mean daily food consumption observed at the 5 ppm dose level. The food consumption of the animals of the 50 ppm and 16 ppm dose level was slightly decreased during the exposure period. The effect diminished after the last treatment. The organ weights were within the control range. No changes occurred at any dose level with the exception of one abortion in the 16 ppm dose group. Grey areas on the kidney surface were found in one control animal, 4 animals of the 5 ppm dose group, 2 animals of 16 ppm dose group and 1 animal of the high dose group.

Developmental toxicity

Table 8.2.5.12 Developmental toxicity

Adverse effects	No adverse effects
Examination of litters	
The vitality of the litters within the first 24 hours after Caesarean section at 50 ppm exposure was considerably decreased.	There was no effect on foetal development and body weight observed at any dose level. Sex ratio was regular.
Skeletal examination (foetuses):	
	One foetus of the control group had an abnormal orientation of the fore-paws and an umbilical hernia.
In the 5 ppm dose group 3 foetuses with skull malformations were found.	
One foetus of the 16 ppm dose level showed a retarded skeletal development and multiple malformations of skull, spine and extremity (left fore-paw almost completely missing).	
In the 50 ppm dose group 10 foetuses had an abnormal orientation of one or both fore-paws. Two foetuses showed skull malformations.	
Irregularity of the skull ossification was observed in 2 /1 /3 /8 foetus from control to high dose group.	
There was an increased incidence of rib anomalies	The skeletal development of viable foetuses was not

(statistically significant).	affected by treatment compared to the control group.
Soft tissue examination (foetuses):	
	Cases of lung anomalies and blood within the chest were present in all groups
	Enlarged stomachs were observed in the 16 ppm and 50 ppm dose groups as well as in the control group.
2 foetuses of the high dose group had red-bordered spots on the skin (mandible, neck and below the eyes).	

Table 8.2.5.13 Summary of effects and conclusion

Effect type	Effect level	Basis for effect level / Remarks
maternal toxicity	0.187 mg/L air	slightly decreased food consumption
developmental toxicity	0.187mg/L air	decreased vitality within the first 24 hours and skeletal abnormalities at 0.187mg/L

Conclusion: Due to the maternal effects of minor severity at 0.06 mg/L and severe developmental effects in terms of skeletal abnormalities of the fetuses at the same concentration level the study indicates that **monoglyme** caused developmental toxicity in rabbits by inhalation exposure.

Rasjad C et al 1991. The study investigated the pattern of limb malformations induced in mice by **methoxyacetic acid (MAA)**. Pregnant Jcl:ICR mice were given orally at gestational day (gd) 10.5, 11.0, or 11.5 (vaginal plug = gd 0) a single dose of MAA 10 mmol/kg of body weight. Fetuses were examined at gd 15.5 for external and skeletal malformations. Limb defects were maximum in frequency and severity after administration at gd 11.5 and comprised cutaneous or osseous syndactyly and ectrodactyly of digits, metacarpals or phalanges.

The **classification dossier for MAA (14.12.1998)** lists further **5 mouse studies, 3 rat studies, 1 rabbit study**, all by oral route (except one rat i.p. and one rat inhalation), all with single application (except rabbit with dosing daily from GD 7 to 19). Several malformations were observed as limb defects in fetuses, limb bud defects in embryos, malformation of tail, digit malformations, hydronephrosis, hydrocephalus, kidney pelvis dilation, heart malformation. In rabbit also decreased litter size was observed.

The **classification dossier for 2-methoxyethanol (31.03.1992)** summarizes the following data:

Fertility: Exposure of rats, mice and rabbits by inhalation has led to degenerative changes in the testicular epithelium and disruption of spermatogenesis. In one study at 1000ppm, damage to the seminiferous tubules resulted in cessation of spermatogenesis. No-effect levels of 100 and 300 have been established for rats and mice respectively and 30 ppm is a minimum effect level in the rabbit.

Similar changes have been observed following oral administration in rats at 100 mg/kg/day and in mice at 250 mg/kg/day. The no-effect level was 50 mg/kg/day.

The reversibility of these effects has been demonstrated in rats. Exposure of male rats to 300ppm of 2-methoxyethanol vapour for 13 weeks prior to mating resulted in 80% infertility immediately after treatment due to testicular atrophy. This was shown to be at least partially reversible, with

50% being fertile after a further 13 weeks. Mating and female fertility were both apparently unaffected by the treatment.

No effects on fertility were observed in males or females at doses of up to 100 ppm. No dominant lethal effects were noted at the 300ppm dose level 13 weeks post exposure. Other studies confirm that oral or intraperitoneal administration can cause testicular effects in rats and probably also in guinea pigs and hamsters. There is sufficient evidence to indicate that these effects are due to the metabolite metlioxyacetic acid.

Development: Exposure of rats and rabbits during pregnancy to vapour levels in excess of 200ppm resulted in total embryomortality. Some evidence of maternal and foetal toxicity was apparent in rats, mice and rabbits exposed to 50ppm during pregnancy. At this exposure level, major foetal abnormalities were observed in rabbits, with only slight signs of maternal toxicity. A no-effect level of 10ppm for both teratogenicity and foetotoxicity has been demonstrated in inhalation studies in the rat, mouse and rabbit.

Oral administration of 2-methoxyethanol to pregnant mice resulted in the death of virtually all foetuses at 500 mg/kg/day and of 50% at 250 mg/kg/day. There was evidence of teratogenicity and delayed development at doses as low as 31 mg/kg/day in rats and foetotoxicity has been demonstrated at 40 mg/kg/day by the subcutaneous but not the percutaneous route.

Some studies suggest that the alkoxyacetic acid metabolite may be the proximate teratogen.