Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

2-methoxyethyl acrylate

EC Number: 221-499-3
CAS Number: 3121-61-7

CLH-O-0000001412-86-202/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

9 March 2018

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

2-methoxyethyl acrylate

EC Number: **221-499-3**

CAS Number: **3121-61-7**

Index Number: **-**

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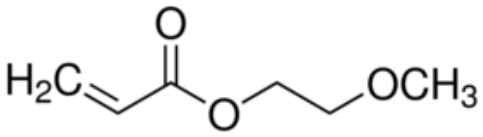
Version number: 3 **Date: February 2017**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	2-methoxyethyl acrylate 2-methoxyethyl prop-2-enoate Ethylene glycol methyl ether acrylate
Other names (usual name, trade name, abbreviation)	2-MEA
ISO common name (if available and appropriate)	Not relevant
EC number (if available and appropriate)	221-499-3
EC name (if available and appropriate)	2-methoxyethyl acrylate
CAS number (if available)	3121-61-7
Other identity code (if available)	Not relevant
Molecular formula	$C_6H_{10}O_3$
Structural formula	
SMILES notation (if available)	COCCOC(=O)C=C
Molecular weight or molecular weight range	130.14 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not relevant
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not relevant
Degree of purity (%) (if relevant for the entry in Annex VI)	98% (w/w)

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)***
2-methoxyethyl acrylate EC no.: 221-499-3	98% (w/w)	None	See table below

*As published in ECHA website on February 2016

Classification		Number of notifiers
Hazard class and category code	Hazard statement code	
Flam. Liq. 3	H226	62
Acute Tox. 4	H302	43
Acute Tox. 3	H311	43
Acute Tox. 3	H331	41
Acute Tox. 4	H332	12
Skin Corr. 1C	H314	38
Skin Irrit. 2	H315	22
Skin Sens. 1	H317	40
Eye Dam. 1	H318	40
Eye Irrit. 2	H319	21
Repr. 1 B	H360	40
STOT RE 2	H373	29
STOT SE 3	H335	10
Aquatic Chronic 3	H412	38
Aquatic chronic 2	H411	11

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Confidential. No impurity is considered relevant for the classification of 2-MEA.

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
Mequinol EC no.: 205-769-8	Stabiliser	50 – 100 ppm	Acute tox 4*, H302 Skin sens 1, H317 Eye Irrit. 2 , H319	-	-

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No existing Annex VI entry										
Dossier submitters proposal	tbd	2-methoxyethyl acrylate	221-499-3	3121-61-7	Flam Liq. 3 Acute Tox. 4 Acute Tox. 3 Skin Corr. 1C Eye Dam. 1 Skin Sens. 1 Muta. 2 Repr. 1B	H226 H302 H331 H314 H318 H317 H341 H360FD	Dgr GHS 02 GHS 05 GHS 06 GHS 08	H226 H302 H331 H314 H317 H341 H360FD	EUH071		
Resulting Annex VI entry if agreed by RAC and COM	Tbd	2-methoxyethyl acrylate	221-499-3	3121-61-7							

Tbd: to be determined

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class needs not to be applied based on chemical structure of the substance	No
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids		Yes
Flammable solids	Hazard class not applicable	No
Self-reactive substances	Hazard class not applicable	No
Pyrophoric liquids	Hazard class needs not to be applied based on chemical structure of the substance	No
Pyrophoric solids	Hazard class not applicable	No
Self-heating substances	Hazard class needs not to be applied based on chemical structure of the substance	No
Substances which in contact with water emit flammable gases	Hazard class needs not to be applied based on chemical structure of the substance	No
Oxidising liquids	Hazard class needs not to be applied based on chemical structure of the substance	No
Oxidising solids	Hazard class not applicable	No
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Data conclusive but not sufficient for classification	No
Acute toxicity via oral route		Yes
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route		Yes
Skin corrosion/irritation		Yes
Serious eye damage/eye irritation		Yes
Respiratory sensitisation	Data lacking	Yes
Skin sensitisation		Yes
Germ cell mutagenicity		Yes
Carcinogenicity	Hazard class not assessed in this dossier	No
Reproductive toxicity		Yes
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	Conclusive but not sufficient for classification	Yes
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Hazard class not assessed in this dossier	No
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

2-methoxyethyl acrylate (2-MEA) has not previously been assessed for harmonised classification by RAC or TC C&L.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

A substance with CMR classification is normally subject to harmonised classification (Art. 36 CLP regulation). 2-methoxyethyl acrylate is currently not classified according to Annex VI of CLP. However, based on a screening developmental reproductive toxicity study, it is warranted to classify 2-methoxyethyl acrylate as Repr. 1B. Although data were insufficient for classification, respiratory sensitisation is also discussed in the dossier. Moreover, following submission of a new *in vivo* study after decision no. TPE-D-2114300801-66-01/F to investigate Germ cell mutagenicity, this endpoint has been assessed in the dossier and it is concluded that 2-MEA warrant to be classified Muta. 2.

Furthermore, differences in self classifications for acute toxicity by oral or inhalation route, skin sensitisation, skin irritation/corrosion, seirous eye damage/eye irritation and STOT RE justify the need for action at Community level since:

- Based on the local lymph node assay performed with 2-methoxyethyl acrylate, classification as Skin Sens. 1 is warranted.
- Based on available animal data, 2-methoxyethyl acrylate shall be classified for skin corrosion, serious eye damage.
- Based on the available data, classification for acute toxicity by oral and inhalation route are warranted

Physico-chemical hazards have been assessed and is thus reported in the dossier.

5 IDENTIFIED USES

The substance is manufactured and used at industrial sites only. The sectors of end-uses are: manufacture of bulk, fine chemicals, rubber, plastics products, printing and reproduction of recorded media.

6 DATA SOURCES

The data sources used for this report include the aggregated dataset of the REACH registration dossier as available on 08 January 2016. A litterature search on pubmed and science direct was conducted for relevant studies up to February 2016. Subject words were used for the literature search including “2-methoxyethyl acrylate”, “ethylene glycol monomethyl ether acrylate”.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Colourless transparent liquid	Chemicals Evaluation and Research Institute, Japan, 2005 (Registration dossier, IUCLID 5)	Visual inspection Purity: 99.81%
Melting/freezing point	- 45°C	Chemicals Evaluation and Research Institute,	Measured OECD Guideline 102 (DSC)

Property	Value	Reference	Comment (e.g. measured or estimated)
		Japan, 2005 (Registration dossier, IUCLID 5)	Purity: 99.81%
Boiling point	164°C	Chemicals Evaluation and Research Institute, Japan, 2005 (Registration dossier, IUCLID 5)	Measured OECD Guideline 103 (Siwoloboff method) Purity: 99.81%
Relative density	1.012 g/cm^3 at 20°C	CRC Handbook of Chemistry and Physics, 86th edition, 2005 (Registration dossier, IUCLID 5)	Handbook data
Vapour pressure	399 Pa at 30°C 931 Pa at 40°C 1660 Pa at 50°C 281 Pa at 25°C	Chemicals Evaluation and Research Institute, Japan, 2005 (Registration dossier, IUCLID 5)	Measured OECD Guideline 104 (static method) Purity: 99.81% Extrapolated value OECD Guideline 104 (static method) Purity: 99.81%
Surface tension	Based on the chemical structure, surface activity is not expected.	Registration dossier, IUCLID 5	
Water solubility	144 g/L at 20°C (pH=5.3)	Chemicals Evaluation and Research Institute, Japan, 2005 (Registration dossier, IUCLID 5)	Measured OECD Guideline 105 (flask method) Purity: 99.9%
Partition coefficient n-octanol/water	Log Pow=0.9 at 25°C	Chemicals Evaluation and Research Institute, Japan, 2005 (Registration dossier, IUCLID 5)	Measured OECD Guideline 117 (HPLC method) Purity: 99.81%
Flash point	59°C at 101.3 kPa	Tremain, S.P., 2012 (Registration dossier, IUCLID 5)	Measured EU Method A.9 (closed cup method) Purity: 99.9%
Flammability	Flammable liquid	Registration dossier, IUCLID 5	Based on flash point.
Explosive properties	There are no chemical groups associated with explosive properties present in the molecule.	Registration dossier, IUCLID 5	Statement
Self-ignition temperature	246°C at 101.1 kPa	Tremain, S.P., 2012	Measured

Property	Value	Reference	Comment (e.g. measured or estimated)
		(Registration dossier, IUCLID 5)	EU Method A.15 Purity: 99.9%
Oxidising properties	On the basis of the chemical structure the substance is incapable of reacting exothermically with combustible materials.	Registration dossier, IUCLID 5	Statement
Stability in organic solvents and identity of relevant degradation products	The stability of the substance is not considered to be critical.	Registration dossier, IUCLID 5	Statement
Dissociation constant	The substance has no dissociable groups.	Registration dossier, IUCLID 5	Statement
Viscosity	Study is ongoing.		The test will be conducted after a decision on the requirement to carry out the proposed test has been taken according to the procedure laid down in Regulation (EC) 1907/2006.
Corrosive to metals	Corrosion rate: Aluminium Test Piece: max. 0.06 mm/year Steel Test Piece: max. 0.03 mm/year	Shimbori, K., 2012 (Registration dossier, IUCLID 5)	Measured UN Test C.1 (UN RTDG, Manual of Tests and Criteria, Part III, Section 37, paragraph 37.4). Purity: ≥99.9%

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 8: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
Statement	There are no chemical groups associated with explosive properties present in the molecule. Not explosive.		Registration dossier

8.1.1 Short summary and overall relevance of the information provided on explosive properties

The assessment of explosives properties of 2-MEA is based on a statement on the chemical structure of the substance. Data provided are considered as relevant.

8.1.2 Comparison with the CLP criteria

According to CLP criteria, a substance shall not be classified as explosive when there are no chemical groups present in the molecule associated with explosive properties as given in Table A6.1 in Appendix 6 of the UN RTDG, Manual of Tests and Criteria.

8.1.3 Conclusion on classification and labelling for explosive properties

Based on chemical structure, it is considered that the substance has no explosive properties according to the CLP criteria.

8.2 Flammable gases (including chemically unstable gases)

Not relevant.

8.3 Oxidising gases

Not relevant.

8.4 Gases under pressure

Not relevant.

8.5 Flammable liquids

Table 9: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
EU Method A.9 – Flash point (closed cup method)	59°C at 101.3 kPa	Measured Purity: 99.9%	Tremain, S.P., 2012 (Registration dossier)

8.5.1 Short summary and overall relevance of the provided information on flammable liquids

The assessment of flammability of 2-MEA is based on the flash point of the substance, determined according to the EU method A.9 – Flash Point (closed-cup method). Data provided are considered as relevant.

8.5.2 Comparison with the CLP criteria

According to CLP criteria, "Flammable liquids" means a liquid having a flash point of not more than 60°C, they are classified in three categories based on their boiling point and their flash point. The substance has a flash point of 59°C which corresponds to a Category 3 flammable liquid.

8.5.3 Conclusion on classification and labelling for flammable liquids

Based on the flash point, it is concluded that the substance is classified as Category 3 Flammable liquid (H226: Flammable liquid and vapour) according to the CLP criteria.

8.6 Flammable solids

Not relevant.

8.7 Self-reactive substances

Table 10: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference
Statement	Not self-reactive substance There are no chemical groups		Registration dossier

Method	Results	Remarks	Reference
	present in the molecule associated with explosive or self- reactive properties		

8.7.1 Short summary and overall relevance of the provided information on self-reactive substances

The assessment of self-reactive properties of 2-MEA is based on a statement on the chemical structure of the substance. Data provided are considered as relevant.

8.7.2 Comparison with the CLP criteria

According to CLP criteria, the classification procedure for self-reactive substance does not need to be applied when there are no chemical groups present in the molecule associated with explosive or self- reactive properties as given in Table A6.1 and A6.2 in Appendix 6 of the UN RTDG, Manual of Tests and Criteria.

8.7.3 Conclusion on classification and labelling for self-reactive substances

Based on chemical structure, it is considered that the substance has no self- reactive properties according to the CLP criteria.

8.8 Pyrophoric liquids

Table 11: Summary table of studies on pyrophoric liquids

Method	Results	Remarks	Reference
Statement	Not pyrophoric substance. Regarding the experience in handling and use, pyrophoric properties are not to be expected.		Registration dossier

8.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

The assessment of pyrophoric properties of 2-MEA is based on a statement on experience in handling and use of the substance. Data provided are considered as relevant.

8.8.2 Comparison with the CLP criteria

According to CLP criteria, the classification procedure for pyrophoric liquids does not need to be applied when experience in manufacture or handling shows that the substance or mixture does not ignite spontaneously on coming into contact with air at normal temperatures.

8.8.3 Conclusion on classification and labelling for pyrophoric liquids

Based on the experience in use, it is concluded that the substance has no pyrophoric properties according to the CLP criteria.

8.9 Pyrophoric solids

Not relevant.

8.10 Self-heating substances

Table 12: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
Statement	Not self-heating substance. As the substance is a liquid, no self-heating properties is expected.	-	Registration dossier

8.10.1 Short summary and overall relevance of the provided information on self-heating substances

The assessment of self-heating properties of 2-MEA is based on a statement on the physical state of the substance. Data provided are considered as relevant.

8.10.2 Comparison with the CLP criteria

According to CLP criteria, self-heating substances are classified in two categories following the results of the test described in Part III, Sub-section 33.3.1.6 of the UN RTDG, Manual of Tests and Criteria.

The Guidance on the Application of the CLP Criteria states that in general, the phenomenon of self-heating applies only to solids. The surface of liquids is not large enough for reaction with air and the test method is not applicable to liquids. Therefore liquids are not classified as self-heating.

Self-heating properties of liquid should be considered only if the substance is absorbed on a large surface.

8.10.3 Conclusion on classification and labelling for self-heating substances

As the substance is a liquid, it is concluded that the substance is not classified as self-heating.

8.11 Substances which in contact with water emit flammable gases

Table 13: Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference
Statement	Not a substance which in contact with water emits flammable gases. Regarding the chemical structure and the experience in handling and use, the substance is not expected to emit flammable gases in contact with water.		Registration dossier

8.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

The assessment of flammability on contact with water of 2-MEA is based on a statement on experience in handling and use and on the chemical structure of the substance. Data provided are considered as relevant.

8.11.2 Comparison with the CLP criteria

According to CLP criteria, the classification procedure for substances which in contact with water emit flammable gases does not need to be applied when the chemical structure of the substance or mixture does

not contain metal or metalloids or experience in handling and use shows that the substance does not react with water or if the substance or mixture is known to be soluble in water to form a stable mixture.

8.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

Based on chemical structure and on the experience in handling and use, it is concluded that the substance is not classified as substance which in contact with water emit flammable gases.

8.12 Oxidising liquids

Table 14: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
Statement	Not oxidising On the basis of the chemical structure the substance is incapable of reacting exothermically with combustible materials.		Registration dossier

8.12.1 Short summary and overall relevance of the provided information on oxidising liquids

The assessment of oxidising properties of 2-MEA is based on a statement on the chemical structure of the substance. Data provided are considered as relevant.

8.12.2 Comparison with the CLP criteria

According to CLP criteria, for organic substance or mixture containing oxygen in their chemical structure, the classification for oxidizing liquids does not need to be applied if oxygen is chemically bonded only to carbon or hydrogen.

The chemical structure of 2-MEA contains oxygen which is chemically bonded only to carbon.

8.12.3 Conclusion on classification and labelling for oxidising liquids

Based on chemical structure, it is considered that the substance has no oxidising properties according to the CLP criteria.

8.13 Oxidising solids

Not relevant

8.14 Organic peroxides

Not relevant.

8.15 Corrosive to metals

Table 15: Summary table of studies on the hazard class corrosive to metals

Method	Results	Remarks	Reference

Method	Results	Remarks	Reference
UN Test C.1 (UN RTDG, Manual of Tests and Criteria, Part III, Section 37, paragraph 37.4).	Not corrosive to metal Corrosion rate: Aluminium Test Piece: max. 0.06 mm/year Steel Test Piece: max. 0.03 mm/year	Measured Purity: ≥99.9%	Shimbori, K., 2012

8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

The assessment of the hazard class corrosive to metals of 2-MEA is based on the corrosion rate on aluminium test piece and steel test piece immersed in the liquid substance at 55 °C for 7 days, following the test described in Part III, Section 37, paragraph 37.4 of the UN RTDG, Manual of Tests and Criteria. Data provided are considered as relevant.

8.15.2 Comparison with the CLP criteria

According to CLP criteria, substances of hazard class corrosive to metals are classified in a single hazard category on the basis of the outcome of the test described in Part III, Section 37, paragraph 37.4 of the UN RTDG, Manual of Tests and Criteria, if corrosion rate on either steel or aluminium surfaces exceeding 6.25 mm per year at a test temperature of 55 °C when tested on both materials.

Corrosion rate on aluminium test piece and steel test piece are max. 0.06 mm/year and max. 0.03 mm/year respectively, meaning the substance is not corrosive to metal according to CLP criteria.

8.15.3 Conclusion on classification and labelling for corrosive to metals

Based on the corrosion rate, it is concluded that the substance is not corrosive to metal.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

In a GLP study following EU method A.9 (closed-cup method), the flash point was determined to be 59°C ± 2°C after measurement and correction for the atmospheric pressure.

The DS proposed to classify 2-methoxyethyl acrylate as flammable liquid category 3: flammable liquid and vapour, but did not propose to classify for any other physical hazard classes.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

The only hazard class assessed in the CLH dossier was flammability; other physical hazards were not assessed. There are no chemical groups associated with explosive or self-reacting properties present in the molecule. On the basis of the chemical structure,

the substance is incapable of reacting exothermically with combustible materials. Based on the experience with handling and use, pyrophoric properties are not to be expected.

The substance has a flash point of 59°C, which was determined under GLP conditions and following EU method A.9. This value is within a range of flash point ≥ 23°C and ≤ 60°C, therefore 2-methoxyethyl acrylate meets classification criteria for flammable liquids category 3. Taking that into account, RAC supports the classification 2-methoxyethyl acrylate as **flammable liquid category 3 (Flam. Liq. 3; H226 "Flammable liquid and vapour")**, as proposed by the DS.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

There are no experimental studies available in which the toxicokinetic properties of 2-methoxyethyl acrylate (2-MEA) were investigated.

2-MEA is highly soluble in water (144 g/L), has a high vapour pressure of 281 Pa at 25 °C and a molecular weight of 130.14g/mol.

The low partition coefficient (log K_{ow}) of 0.9 suggests a low potential to accumulate in biological systems. Based on the physico-chemical properties and the systemic toxicity observed in toxicity studies performed by oral and inhalation routes of administration, 2-MEA is expected to be bioavailable.

There are no experimental data available concerning the metabolism of 2-MEA.

Ester hydrolysis to acrylic acid and an alcohol has been shown to be the principal metabolic pathway of acrylates (Silver and Murphy, 1981, Millers *et al*., 1981, Ghanayem *et al*., 1987). This is the case also for methacrylate such as methylmetacrylate (Borak *et al*., 2009).

QSAR estimation using the OECD Toolbox v.3.4 Rat liver S9 metabolism simulator results in eight metabolites reported in the table 16 below.

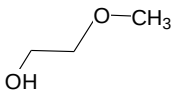
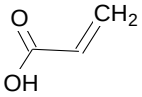
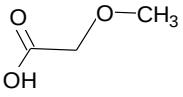
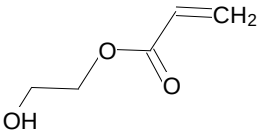
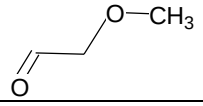
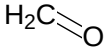
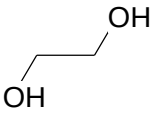
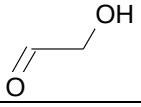
Based on the expected enzymatic cleavage of the ester bond, it is anticipated that acrylic acid and 2-methoxyethanol will be the main primary metabolites of 2-MEA.

Based on the known metabolite pathway of 2-methoxyethanol, methoxyacetic acid, methoxyacetaldehyde ethylene glycol and formaldehyde are expected to be degradation products of 2- methoxyethanol (See figure 1 below from WHO, 2009 on 2-methoxyethanol).

According to the EU RAR of 2002 on acrylic acid, acrylic acid is rapidly metabolised by oxidative pathways to carbon dioxide which is formed via acrylyl-CoA by the non-vitamin-B12-dependent pathway of mammalian propionate. About 80% of an ingested dose of acrylic acid is exhaled as carbon dioxide within 24 hours. In urine poorly characterised substances of a higher polarity than those of acrylic acid are detected. Unmetabolised acrylic acid was not found in urine. However, a small proportion of 3-hydroxypropionic acid as major urinary metabolite of absorbed acrylic acid was identified.

Based on the OECD QSAR toolbox, three other acrylates (2 unknown compounds and 2-hydroxyethyl acrylate) may also be formed.

Table 16: Summary table of predicted metabolites of 2-MEA

Simulated metabolites	Structure	Harmonised classification (CMR and sensitising properties)	Self-classification (CMR and sensitising properties)
2- methoxyethanol CAS no 109-86-4		Repr. 1B H360 FD	Repr. 1B, H360 FD
Acrylic acid CAS no 79-10-7		No classification for CMR or sensitising properties	No self-classification for CMR or sensitising properties
Methoxyacetic acid CAs no 625-45-6		Repr. 1B , H360 FD	Repr. 1B , H360 FD
2-hydroxyethyl acrylate CAS no 818-61-1		No classification for CMR or sensitising properties	No self-classification for CMR or sensitising properties
Methoxyacetaldehyde CAS no 10312-83-1		No Harmonised classification	Skin sens 1
Formaldehyde CAS no 50-00-0		Carc. 1B Muta 2 Resp. Sens 1 Skin sens 1A	Carc. 1B Muta 2 Resp. Sens 1 Skin sens 1A
Ethylene glycol CAS no 107-21-1		No classification for CMR or sensitising properties	No self-classification for CMR or sensitising properties
Glycollaldehyde CAS no 141-46-8		No Harmonised classification	Skin sens 1

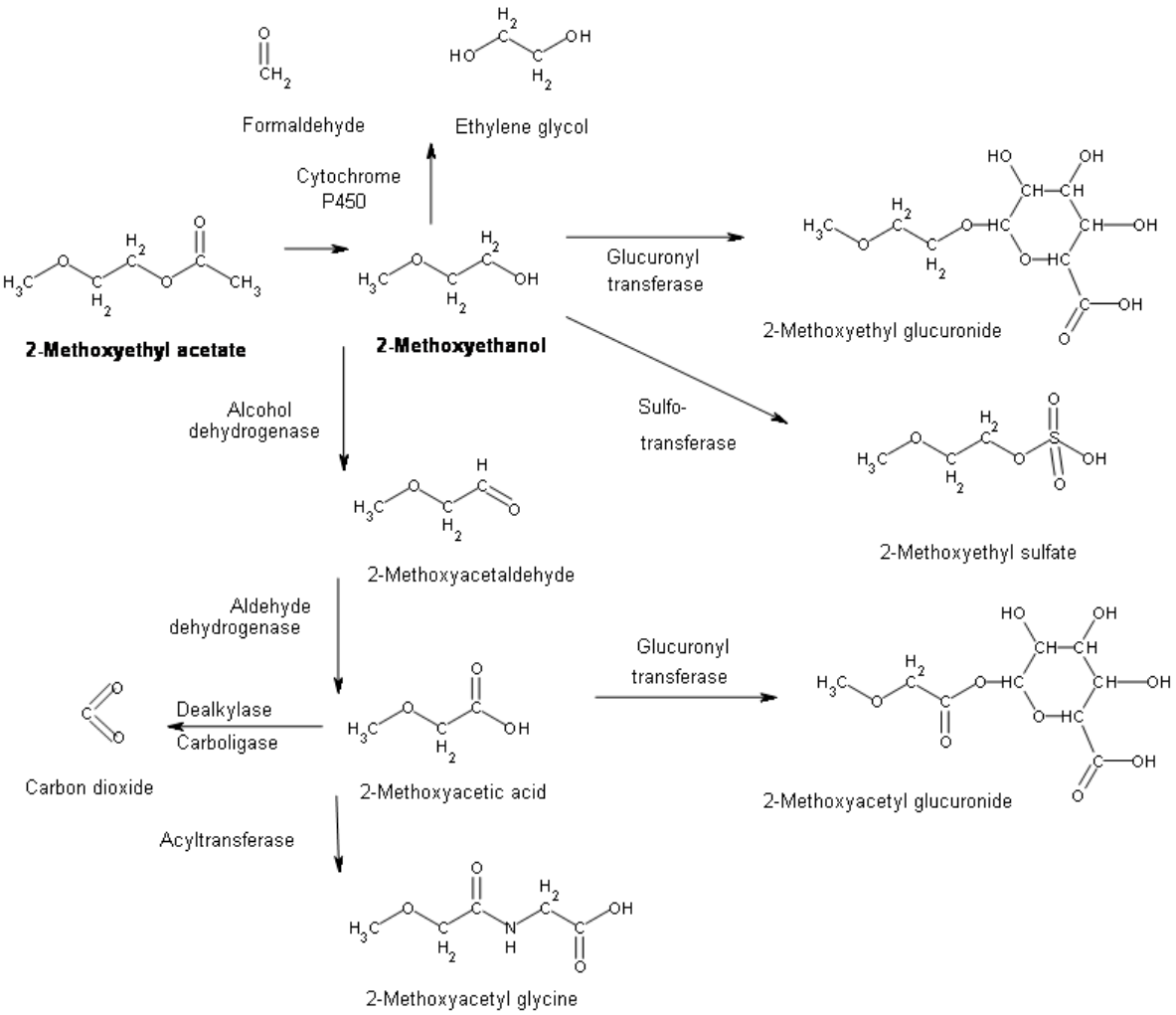


Figure 1: Metabolic pathways of 2-methoxyethanol (WHO, 2-Methoxyethanol, 2009)

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Table 17: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Equivalent to OECD 401 (Acute Oral toxicity) 2 (reliable with restriction) Oral: gavage Limitations: only dead animals were necropsied; no	SD Rats 5/sex/dose	2-MEA	Acute single exposure 252, 353.5, 505, 555.5, 606 mg/kg bw	404 mg/kg bw (95% CL =343.4-464.6)	Rhône-Poulenc Inc., 1980

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
histopathology, prior to GLP					
Equivalent to OECD 401 (Acute oral toxicity) 2 (reliable with restriction) oral: gavage Limitations: No details on analytical purity of the test substance; limited details on test animals and environmental conditions; prior to GLP	Wistar male rats	2-MEA	Single exposure 505, 1010, 2020 mg/kg bw	818 mg/kg bw/d (95%CL = 596-1131)	Union Carbide Corporation study, 1968

CL: confidence limits

Detailed study summaries are available in Annex I of the CLH report.

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

In an acute oral toxicity study, rats were administered 2-MEA via gavage (Rhône-Poulenc Inc., 1980). Five rats per sex and dose received the following dose levels: 252.5, 353.3, 505.0, 555.5, 606.0 mg/kg bw. The mortality was 0, 2, 2, 4 and 5 for males and 0, 2, 3, 4 and 5 for females, respectively, listed by increasing dose levels. Autopsy of dead animals revealed pulmonary haemorrhages. No clinical signs were noted. Based on the results, the oral LD_{50} in rats was 404 mg/kg bw.

The acute toxicity of the test substance was also assessed in a study similar to OECD 401, in which 5 male rats per group received the test substance via oral gavage at dose levels of 252.5, 1010 and 2020 mg/kg bw (Union Carbide Corporation, 1968). Mortalities were observed in 4/5 animals and 5/5 animals treated with 1010 and 2020 mg/kg bw, respectively. No mortalities were observed in animals administered the lowest dose (252.5 mg/kg bw). However, at this dose level, sluggish behaviour was observed in the animals during the 14-day observation period. In all surviving animals of the 252.5 and 1010 mg/kg bw/day, no effects on body weights were noted. At necropsy, congestion was observed in the lungs and the abdominal viscera of treated animals. Based on the probit method, the oral LD_{50} value in rats was calculated to be 818 mg/kg bw.

10.1.2 Comparison with the CLP criteria

The LD_{50} values are within the range (300-2000 mg/kg bw) established for classification as Acute tox. 4 – H302 under regulation (EC) 1272/2008 criteria.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the available acute oral toxicity studies, 2-MEA needs to be classified **Acute tox. 4 "Harmful if swallowed"** – **H302**

10.2 Acute toxicity - dermal route

Not evaluated.

10.3 Acute toxicity - inhalation route

Table 18: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value** LC_{50}	**Reference**
Similar to OECD 403 2 (reliable with restriction) Limitations: prior to GLP and OECD guideline, no details on analytical purity of the test substance; limited details on inhalation exposure as well as on test animals and environmental conditions	Male Wistar rats 6/group	2-MEA, no data on MMAD	Whole body exposure 4h exposure Vapour 1.4; 2.7; 5.4 mg/L	2.7 mg/L (95% CL = 1.9-3.8)	Union Carbide Corporation study, 1968

Detailed study summary is available in Annex I.

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

The acute inhalation toxicity of 2-methoxyethyl acrylate was investigated in male rats using a whole body exposure system (Union Carbide Corporation, 1968). In a preliminary test, 6 animals per group were exposed to the test substance at target concentrations of 10.4, 9.6 and 8.4 mg/L for periods of 15 min, 30 min and 1 h, respectively. Since mortalities already occurred at 9.6 mg/L, concentrations used in the main study were lowered to 5.3, 2.7 and 1.3 mg/L and animals (6 per concentration) were exposed to the test substance for 4 h. At 2.7 and 5.3 mg/L, mortalities were observed between Days 1 and 3 in 3/6 and 6/6 animals, respectively. No mortality occurred in animals treated with 1.3 mg/L up to the end of the 14-day observation period. Clinical signs observed in the animals involved swollen abdomen, laboured breathing and gasping. Furthermore, irritation of the eyes, nose and extremities was noted during exposure to the test substance. Necropsy of rats dying during the study revealed slight haemorrhage of lungs and blood in intestines. In two of the three surviving rats at 2.7 mg/L areas of focal consolidation scattered throughout the lungs were observed at necropsy. All others showed nothing remarkable. Body weights in all surviving animals were not affected by treatment. Based on the results, the LC_{50} value in rats was 2.7 mg/L.

10.3.2 Comparison with the CLP criteria

The LC_{50} value for 2-MEA as vapour are in range (2-10 mg/L) for classification as Acute tox. 3 –H331 under regulation (EC) no. 1272/2008 criteria.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on the available acute inhalation toxicity study, 2-MEA is classified **Acute tox. 3 "Toxic if inhaled" – H331**

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Oral Route

Two studies equivalent to OECD TG 401 (non GLP) are available.

In the first study five SD rats/sex/dose were exposed to 2-methoxyethyl acrylate via gavage. The mortality was 0, 2, 2, 4 and 5 for males, and 0, 2, 3, 4 and 5 for females, which died after being exposed to 252.0, 353.5, 505.0, 555.5 or 606.0 mg/kg bw, respectively. Autopsy of the dead animals revealed pulmonary haemorrhages. No clinical signs were noted. The LD_{50} was calculated at 404 mg/kg bw (95% CL = 343.4-464.6).

In the second study, 5 Wistar male rats were exposed via gavage to 2-methoxyethyl acrylate. The number of rats which were found dead after being treated with 505, 1010 or 2020 mg/kg bw was 0, 4 and 5, respectively. The resulting LD_{50} was 818 mg/kg bw.

The DS proposed to classify 2-methoxyethyl acrylate as Acute Tox. category 4; H302 "Harmful if swallowed". The DS did not establish an Acute Toxicity Estimate (ATE) value.

Dermal Route

No data provided.

Inhalation Route

One study similar to OECD TG 403 is available. Six males Wistar rats were exposed to 2-methoxyethyl acrylate vapour by inhalation, whole body. The number of animals which were found dead between day 1 and 3 after being exposed for 4h to 1.4, 2.7 and 5.4 mg/L was 0, 3 and 6, respectively. At necropsy, congestion was observed in the lungs and the abdominal viscera of treated animals. Based on this data, LC_{50} was calculated to be 2.7 mg/L.

The DS proposed to classify 2-methoxyethyl acrylate as Acute Tox. category 3; H331 "Toxic if inhaled". The DS did not establish an ATE value.

Comments received during public consultation

One MSCA supported the DS proposal to classify 2-methoxyethyl acrylate for acute oral and inhalation toxicity.

Assessment and comparison with the classification criteria

Oral Route

Both rat oral LD_{50} values were within the range of 300-2000 mg/kg bw established as a

criterion for classification as Acute Tox. 4; H302.

An ATE value of 404 mg/kg bw was established by RAC based on the lowest LD_{50} value for the preferred test species for acute toxicity by the oral route (rat).

Dermal Route

Not evaluated.

Inhalation Route

The LC_{50} value for inhalation of 2-methoxyethyl acrylate as vapour was 2.7 mg/L, therefore the LC_{50} is within the range 2-10 mg/L criterion for classification of toxic vapours as Acute Tox. 3; H331.

AAn ATE value of 2.7 mg/L was established by RAC based on the only available LC_{50} value for preferred test species for acute toxicity by the inhalation route (rat).

RAC is of the opinion that 2-methoxyethyl acrylate warrants classification as **Acute Tox. 4; H302 "Harmful if swallowed"** and **Acute Tox. 3; H331 "Toxic if inhaled"**, as proposed by the DS.

The proposed **ATE values are 404 mg/kg bw for the oral route and 2.7 mg/L for the inhalation route (as vapour)**.

10.4 Skin corrosion/irritation

Table 19: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
Equivalent to OECD 404, non GLP 4(not assignable) Deviations: 24h instead of 4h, open condition, no postexposure period, only 0.01 mL instead of 0.5 mL, only short summary available	5 Rabbits	2-MEA	0.01 mL 24-h exposure	Immediately after exposure to the test substance, very slight to slight irritation was observed in 1/5 and 4/5 animals, respectively	Union Carbide Corporation study, 1968
Equivalent to OECD 404 Non GLP 2(reliable with restriction) Deviations: 24h instead of 4h, occlusive test condition on both abraded and intact skin, only two reading points, the study was terminated at 72h	6 NZ rabbits	2-MEA	0.5mL 24-h exposure	Mean Skin irritation scores on intact skin: at 24h: Erythema: 3 Edema: 3 at 72h: Erythema: 3.17 Edema: 2.5 No differences between intact and abraded application sites	Rhône-Poulenc Inc., 1980a
Equivalent to OECD 404 Non GLP	6 NZ rabbits	2-MEA	1mL 4-h exposure	No corrosive effects at 4h readings. Skin Corrosion in 5/6 animals at 48h exposure	Rhône-Poulenc Inc., 1980b

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
2(reliable with restriction) Deviations: 1mL instead of 0.5 mL, only 4 and 48h readings. The study was terminated at 48h, individual scores not given					

Detailed study summaries are available in Annex I of the CLH report.

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

The Union Carbide Corporation study (1968) is not considered suitable for classification purposes. Indeed, only 0.01mL of test material was applied under open condition.

In the study of Rhone-Poulenc Inc (1980a), a 48-hour observation time was not included and involve a 24-hour test material exposure followed by observations at 24 hour and 72 hours. The test material was patched both on abraded and on intact skin of six rabbits. Twenty four hours instead of 4h were used under occlusive dressing condition. Pronounced responses at the 72 hours time point was observed. Reversibility of the effects was not studied.

In the skin irritation study of Rhone-Poulenc Inc (1980b), corrosive effects have been observed at 48h post-exposure but not after 4-h exposure.

10.4.2 Comparison with the CLP criteria

Visible necrosis was seen at 48h after 4-hour exposure in rabbits (Rhone-Poulenc Inc., 1980b). As the responses were observed after exposure longer than 1 hour, skin Corr. 1A and 1B are not appropriate. According to the CLP criteria 2-MEA has to be classified Skin Corr. 1C, H314.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

2-MEA is a corrosive substance to skin and classification **Skin Corrosion category 1C, H314 "Causes severe skin burns and eye damage"** is warranted.

Due to 2-MEA high vapour pressure, 2-MEA may be inhaled and since 2-MEA is classified for skin corrosivity, the supplementary hazard statement **EUH071 " Corrosive to respiratory tract"** is considered warranted.

RAC evaluation of skin corrosion/irritation
Summary of the Dossier Submitter's proposal Only two of the three studies (cf. Background Document, BD; Table 19) equivalent to OECD TG 404 (non GLP) can be taken into account for classification purpose. The Union Carbide Corporation study (1968), was not considered suitable, because, only 0.01 mL of test material (instead of 0.5 mL) was applied under non-occlusive condition amongst

other deviations.

In these two studies, the test material was patched both on abraded and on intact skin of six New Zealand White (NZW) rabbits for 24h instead of 4h and under occlusive dressing conditions. In the first study, a 48h observation time was not included, and involved a 24h test material exposure followed by observations at 24h and 72h. Pronounced responses at the 72 hours' time point was observed. Reversibility of the effects were not studied. Rabbits presented a mean score of 3 for erythema and oedema at 24h and 3.17 for erythema and 2.5 for oedema after 72h. No difference between intact and abraded skin was observed.

In the second study, no sign of corrosion was observed at 4h readings, but at 48h, 5/6 NZW rabbits presented skin corrosion (individual scores not given).

The DS proposed to classify 2-methoxyethyl acrylate as Skin Corr. 1C; H314, and to add the supplementary hazard statement EUH071 "Corrosive to respiratory tract".

Comments received during public consultation

One MSCA supported the DS proposal to classify as skin corrosion category 1C.

Assessment and comparison with the classification criteria

Visible necrosis was seen at 48h after 4-hour exposure in rabbits in the second study. As the necrotic responses were observed only after an exposure of longer than 1 hour, the classification criteria for Corr. 1A and 1B were not met and according to the CLP criteria 2-methoxyethyl acrylate should be classified Skin Corr. 1C; H314.

Noting that relevant criteria were met, RAC supports the classification 2-methoxyethyl acrylate as **Skin Corr. 1C; H314 "Causes severe skin burns and eye damage"** as proposed by the DS.

EUH071 (corrosive to respiratory tract):
The following points were considered in the assignment of EUH071 :

1. Acute inhalation test data (cf. CLH report, table 18): there are no data on irritation/corrosion on the airway epithelium after exposure to vapours or aerosols of 2-methoxyethyl acrylate. The results from the available inhalation study meet the criteria for classification (see above).

   Rats that died (3/6) at the mid concentration (2.7 mg/L; 4h exposure) had slight haemorrhage in the lungs and blood in the intestines, while 2 out of 3 survivors had areas of focal consolidation scattered throughout the lungs. Clinical signs of laboured breathing was observed after 4h exposure to the low dose of the vapour (1.4 mg/L) which also caused eye irritation after a few minutes and subsequently skin irritation. Gasping was observed after 2h exposure to the high dose (5.4 mg/L), at which all animals died.

2. Corrosivity to the skin: visible necrosis was seen at 48h after 4-hour exposure in rabbits. The necrotic responses were observed after exposure of longer than 1 hour. According to the CLP criteria 2-methoxyethyl acrylate should be classified as Skin Corr. 1C; H314 - Causes severe skin burns and eye damage

3. 2-methoxyethyl acrylate may be inhaled: it has a high vapour pressure (281 Pa at 25°C).

According to the CLP Regulation, Annex II, section 1.2.6, EUH071 is applied "*in addition to classification for inhalation toxicity, if data are available that indicate that the mechanism of toxicity is corrosivity".* Substances have to be supplementary labelled with EUH071, if there is a possibility of exposure via inhalation, taking into consideration the saturated vapour concentration and the possibility of exposure to particles or droplets of inhalable size as appropriate (chapter 3.8.2.5 of Guidance).

According to "Acute toxicity study" (OECD TG 403) with 2-methoxyethyl acrylate, exposure to concentrations where mortalities occurred (i.e.9.8 mg/L and above) causes congestion in the lungs and areas of focal consolidation scattered throughout the lungs.

In conclusion: there is no experimental evidence that 2-methoxyethyl acrylate injures the epithelium of the respiratory tract but taking into account general the corrosive properties of 2-methoxyethyl acrylate seen in the skin and eye damage/irritation studies, in combination with a high vapour pressure (281 Pa at 25°C), inhalation of vapour could lead to irritation/corrosion of the mucous membranes of respiratory tract and pulmonary injury. **RAC therefore supports to use the supplemental hazard statement EUH071**.

10.5 Serious eye damage/eye irritation

Table 20: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, Klimish score, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **- Observations and time point of onset** **- Mean scores/animal** **- Reversibility**	**Reference**
Similar to OECD 405 4(not assignable) Deviations: Original report not available and documentation insufficient for assessment.	Albino rabbits,	2-MEA	6 animals/dose 0.001, 0.005, 0.02, 0.1, and 0.5 mL 24h exposure	24h reading: severe corneal injury was observed in 3 eyes treated with 0.02 mL of the undiluted test substance. Minor to moderate injury was observed in the eyes after treatment with 0.005 mL of the undiluted test substance (no further details)	Union Carbide Corporation, 1968
Similar to OECD 405 2(reliable with restriction) Deviations: Study termination at day 7	NZ rabbits	2-MEA	0.1mL 6 animals Single exposure without washing or 30s exposure	Mean 24-72h score/6 animals: Conjunctivae redness: 2.67 Conjunctivae oedema: 3.88 Iris: 0.2 Cornea: 1.7 Only iris effects were fully reversible at day 7.	Rhône-Poulenc Inc., 1980c

Detailed study summaries are available in Annex I of the CLH report.

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

The eye irritation potential of 2-methoxyethyl acrylate was investigated in New Zealand Albino rabbits according to EPA guideline 40 CFR 163.81.4 (Rhône-Poulenc Inc., 1980c). The undiluted test substance (0.1 mL) caused serious and irreversible damage to the eyes on conjuctivea and cornea. The reversibility of the effects was not shown at the end of the observation period (day-7).

The union Carbide Corporation study (1968) is not considered suitable for classification. Nevertheless, the study gives supporting evidence that the undiluted test substance (0.02 mL) caused severe corneal injury to the eyes after an exposure period of 24 h in all tested albino rabbits. Even minor to moderate injury was observed in the eyes of the animals after treatment with 0.005 mL of the undiluted test substance after 24 h.

10.5.2 Comparison with the CLP criteria

Severe eye effects were observed in conjunctivae and cornea in rabbits in the Rhône-Poulenc Inc., 1980c study. The mean scores of the 6 rabbits meet the criteria for eye irritation category 2. The reversibility of the effects were not studied until 21 day post exposure period. Nevertheless, eye scores of 3 to 4 were still observed in 5/6 rabbits after the 7 days post-exposure period in conjunctivae.
Therefore, 2-MEA is considered to cause irreversible damage to the eyes and support classification as Eye dam. 1 – H318 “Causes serious eye damage” according to the CLP criteria.

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

2-MEA is a severe eye irritant. As 2-MEA needs to be classified as Skin corr. 1C, the risk of severe damage to eyes is considered implicit. Therefore, the substance is classified for **Eye damage, category 1, H318 “Causes serious eye damage”** but will not be labelled for serious eye damage.

Since 2-MEA was assessed as corrosive to skin and eyes, a potential for respiratory tract irritation is considered to be very likely. According to Regulation (EC) 1272/2008, classification for corrosivity is considered to implicitly cover the potential to cause respiratory tract irritation and so the additional classification is considered to be superfluous.

RAC evaluation of serious eye damage/irritation
Summary of the Dossier Submitter’s proposal The eye irritation potential of 2-methoxyethyl acrylate was investigated in New Zealand Albino rabbits according to EPA guideline 40 CFR 163.81.4. The undiluted test substance (0.1 mL) caused serious and irreversible damage to conjunctivae and cornea of the eyes. Mean 24-72h scores for the 6 rabbits were >2 for conjunctivae redness, >2 for conjunctivae oedema and >1 for corneal opacity. The reversibility of the effects were not shown at the end of the observation period (day 7). In this case, this data set is consistent with the criteria for Eye Irrit. Cat. 2. Considering the effects can be regarded as severe since some scores were higher than 3. The union Carbide Corporation study (1968) is not considered suitable for classification in

its own right. Nevertheless, the study gives supporting evidence that the undiluted test substance (0.02 mL) caused severe corneal injury to the eyes after an exposure period of 24h in all tested albino rabbits, while minor to moderate injury was observed in the eyes of the animals after treatment with 0.005 mL of the undiluted test substance.

The DS proposed to classify 2-methoxyethyl acrylate as Eye Damage 1 H318; "Causes serious eye damage".

Comments received during public consultation

One MSCA supported the DS proposal to classify 2-methoxyethyl acrylate as Eye. Dam. 1; H318.

Assessment and comparison with the classification criteria

Severe eye effects were observed in conjunctivae and cornea in rabbits in a study similar to OECD TG 405. In this study all animals showed effects on the cornea and conjunctivae. The mean scores of the 6 rabbits (average: 24+48+72h) were:
- cornea: 1.0; 1.3; 2.0; 2.0; 2.0; 2.0
- conjunctivae: redness – 2.0; 2.3; 2.3; 2.0; 2.0; 3.0; swelling – 3.3; 4.0; 4.0; 4.0; 4.0; 4.0
- iris: 0.0; 0.0; 0.0; 0.0; 0.3; 1.0

The reversibility of the effects in animals were not studied until 21 day post exposure period, but eye scores of 3 to 4 were still observed in 5/6 rabbits after the 7 days post-exposure period in conjunctivae. The effects are not expected to reverse.
Therefore, RAC supports the classification 2-methoxyethyl acrylate as **Eye. Dam. 1; H318 "Causes serious eye damage"** as proposed by the DS.

10.6 Respiratory sensitisation

No specific animal or human data available on 2-MEA.

10.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

Table 21: Summary table of other studies relevant for respiratory sensitisation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
SAR (structural alert)	2-MEA	OECD QSAR Toolbox v.3.4 Profiler: Respiratory sensitisation	2-MEA hit the alert : acrylates Proposed mechanism: A Michael addition mechanism has been suggested to be responsible for the ability of chemicals containing this structural alert to react with proteins in the lung.)	Enoch, S.J., et al., Development of Mechanism-Based Structural Alerts for Respiratory Sensitization Hazard Identification. Chemical Research

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
				in Toxicology, 2012. 25(11): p. 2490-2498
Danish QSAR database (Requested on February 2016)	2-MEA	(Q)SAR predicted profile for respiratory sensitisation in humans Software used are : CASE Ultra, Leadscope, SciQSAR	Leadscope predict positive results and the prediction was inside the applicability domain of the model, CASE Ultra and SciQSAR give positive prediction but the prediction was outside the applicability domain. Overall the battery of test predict positive results but outside applicability domain.	-
SAR (structural alert)	2-MEA	DEREK v5.0.2	No alert flagged. This is expected as DEREK v5.0.2 does not contain respiratory sensitisation structural alerts referring to Acrylates.	-

According to the OECD QSAR database, acrylates have been suggested to be capable of reacting with proteins in the lung *via* a direct Michael addition mechanism. Leadscope also predict positive results for this substance. DEREK nexus do not predict respiratory sensitisation for 2-MEA as no structural alerts for acrylates were developed in the model.

With regard to the predicted metabolites only formaldehyde has an harmonised classification for respiratory sensitisation. Furthermore, respiratory sensitisation has not been reported with the two expected main metabolites 2-methoxyethanol or acrylic acid.

No human or animal data are available specifically on 2-MEA on respiratory sensitisation in the literature. Furthermore, since data to get a clear understanding of the sensitising properties of members within the group of acrylate are currently not available, no classification is proposed for 2-MEA.

10.6.2 Comparison with the CLP criteria

No data are available in both human and animals.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

No classification for respiratory sensitisation is warranted based on insufficient data.

RAC evaluation of respiratory sensitisation
Summary of the Dossier Submitter's proposal No specific animal or human data was available on 2-methoxyethyl acrylate. According to the OECD QSAR Toolbox, version 3.4., acrylates have been suggested to be

capable of reacting with proteins in the lung via a direct Michael addition mechanism. The Leadscope Toxicity Database also predicts positive results for this substance. DEREK nexus does not predict respiratory sensitisation for 2-methoxyethyl acrylate as no structural alerts for acrylates were developed in the model.

With regard to the predicted metabolites (WHO, 2009; see CLH report, Table 16) only formaldehyde (a known metabolite of 2-methoxyethanol) has a harmonised classification for respiratory sensitisation. Respiratory sensitisation has not been reported for the expected primary metabolites of 2-methoxyethyl acrylate, 2-methoxyethanol and acrylic acid.

No human or animal data are available specifically on 2-methoxyethyl acrylate on respiratory sensitisation.

DS did not proposed a classification 2-methoxyethyl acrylate as respiratory sensitizer.

Comments received during public consultation

One MSCA supported an opinion that data to classify 2-methoxyethyl acrylate for respiratory sensitisation are insufficient.

Assessment and comparison with the classification criteria

No data are available in both human and animals.

RAC is of the opinion that the available data are insufficient to classify 2-methoxyethyl acrylate as a respiratory sensitizer in agreement with the DS.

10.7 Skin sensitisation

Table 22: Summary table of animal studies on skin sensitisation

Method, guideline, Klimish score, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
Local lymph node assay OECD 429, GLP 1(reliable without restriction)	CBA/Ca Mice 4 females/group Vehicle: acetone/olive oil 4:1	2-MEA	0, 25, 50, 100 %	Sensitising Stimulation index results: 25%: 9.20 50%: 12.84 100%: 11.55	Study report, 2012a

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

The local lymph node assay (LLNA) performed with 2-MEA was positive (SI > 3 at 25% and above). The dossier and literature do not contain human data on 2-MEA. Nevertheless, acrylates is a class of chemical known to be contact allergens.

10.7.2 Comparison with the CLP criteria

The results of local lymph node assay demonstrate the sensitising properties of 2-MEA. A classification Skin Sens 1, H317 "may cause an allergic reaction" is considered warranted since positive data are available.

The criteria for subcategorisation of skin sensitizers based on LLNA study is an estimated concentration to produce a stimulation index of 3 (EC_3) ≤ 2% for sub-category 1A and EC_3 value > 2% for sub-category 1B.

An EC_3 value could not be derived adequately as all stimulation index values exceed 3 and were not linear. Thus, a derivation of an EC_3 value may be associated with great uncertainty.

Therefore a classification Skin Sens. 1, H317 without sub-categorisation is proposed.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Based on a LLNA assay, 2-MEA has to be classified as **Skin Sensitiser, Category 1, H317 "May cause an allergic skin reaction"** according to the CLP criteria.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The dossier does not contain any human data on 2-methoxyethyl acrylate. One local lymph node assay (LLNA) test, conducted according to OECD TG 429 and GLP, is available to assess sensitizing potential of 2-methoxyethyl acrylate. The LLNA performed on CBA/Ca Mice (three groups; 4 females/group) was positive (Stimulation index SI > 3 at 25% and above).

Nevertheless, the acrylates is a class of chemical known to contain contact allergens.

The results of the LLNA study demonstrate the sensitising properties of 2-methoxyethyl acrylate.

The DS proposed a classification Skin Sens. 1; H317 "May cause an allergic skin reaction" resulting from the availability of positive data.

Comments received during public consultation

One MSCA supported that data from animal tests fulfils the criteria to classify as Skin Sens. 1 without subcategorization, and thus agreed to classify as proposed by DS.

Assessment and comparison with the classification criteria

The LLNA performed on CBA/Ca Mice with 2-methoxyethyl acrylate was positive, with a stimulation index higher than 3 (SI >9.2% and above) at the concentrations of 25%, 50% and 100%. The EC_3 value is not available and there is no experimental data with

which to calculate it.

Since all tested concentration were above 2%, there are no data to exclude the possibility that at lower concentrations (≤ 2%) 2-methoxyethyl acrylate does not stimulate cell proliferation with a stimulation index above 3, therefore it is not possible to exclude that the substance meets classification criteria for category 1A. It is not known if the result is positive at a concentration of ≤2%, therefore it is not possible to assign a subcategory.

In the opinion of RAC, the substance warrants classification as **Skin Sens. 1; H317 "May cause an allergic skin reaction"** with no subcategorization.

10.8 Germ cell mutagenicity

Table 23: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, Klimish score, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Bacterial reverse mutation Similar to OECD 471 2 (reliable with restriction) Limitations: - 4 strains instead of 5 recommanded -non GLP - limited data on test system and conditions - dose rationale not specified - no analytical purity, - positive controls not specified	2-MEA	*S. typhimurium* TA 100, TA 1535, TA 97, TA98 With and without rat or hamster S9mix Pre-incubation method and plate test with vapour from the test liquid	Negative with and without metabolic activation	Confidential report available in REACH registration IUCLID file, 1991
Bacterial reverse mutation OECD 471, GLP 1 (Reliable without restriction)	2-MEA	*S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 and *E. coli* WP2 uvrA Test concentrations: 5-5000 µg/plate With and without rat S9 mix	Negative with and without metabolic activation	Confidential report available in REACH registration IUCLID file, 2012
In vitro mammalian cell gene mutation OECD 476, GLP 1 (Reliable without restriction)	2-MEA	L5178Y lymphoma cells: mouse (with and without rat met. Act.) Test concentrations: 4h treatment (-S9 mix): 0.63, 1.25, 2.5, 5, 10, 20, 30, 40 µg/mL	Positive with and without metabolic activation. The increase is mainly due to small colony formation ±S9	Confidential report available in REACH registration IUCLID file, 2013

Method, guideline, Klimish score, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		4h treatment (+S9 mix): 20.25, 40.5, 81, 162, 324, 432, 540 and 648 µg/mL		
Mammalian chromosomal aberration test OECD 473, GLP 1 (Reliable without restriction)	2-MEA	Cultured peripheral human lymphocytes With and without rat S9mix Test concentrations: - 4h treatment (-S9 mix): 10, 20, 40 µg/mL - 4h treatment (+S9 mix): 320, 480, 640 µg/mL	Positive with metabolic activation. Negative without S9 (short exposure period only performed)	Confidential report available in REACH registration IUCLID file, 2013

Table 24: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
In vivo alkaline comet assay OECD 489, GPL 2(reliable with restriction) Limitations: - Negative controls were slightly below historical control data but the relevance of this observation is questionable as Only very low number of animals were included in the historical control data, - No historical control data for non-glandular stomach.	2-MEA	2 single treatment within 24-h Sacrifice 4-h after final treatment Doses: 120, 240, 480 mg/kg bw Positive control: N-methyl-N-nitrosurea 7 animals/group except 5 in the positive control group Tissues: liver, non-glandular and glandular stomach Vehicle: PBS	Negative in liver. Equivocal in glandular stomach and positive in non-glandular stomach. Histopathological findings: Degenerative changes in the epithelium of the non-glandular stomach and glandular stomach was noted, with dose-related increased severity of effects in the non-glandular stomach. These are signs of cytotoxicity at the site of contact. Positive control: positive.	Confidential study report, 2016

Detailed study summaries are available in Annex I of the CLH report.

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

- *In vitro*

Two gene mutation assays in bacteria (Ames test) were conducted with 2-MEA. No increase in the mean revertant number of colonies was observed at any of the concentrations tested in both experiments with or without rat or hamster S9.

2-MEA was also tested for its potential to cause gene mutations in the mouse lymphoma assay according to OECD 476. The potential mutagenicity of the test substance on the thymidine kinase, TK +/- locus of the L5178Y mouse lymphoma cell line was investigated after 4 h exposure. The concentration range of the test substance was 0.63 to 40 µg/mL in the absence of metabolic activation and 20.25 to 648 µg/mL in the presence of metabolic activation. The test substance induced toxicologically significant dose-related increases in the mutant frequency at the TK +/- locus in L5178Y cells both with and without metabolic activation. The increases in mutant frequency observed were mainly due to small colony formation, indicating clastogenic activity resulting in structural chromosome damage. 2-MEA is, therefore, considered to be mutagenic under the conditions of the test. 2-MEA was more cytotoxic without S9 than in presence of S9 as shown by the higher tested concentrations with S9. The clastogenic potential observed in the chromosome aberration test was taken as confirmatory evidence for the mutagenicity of the test substance under *in-vitro* test conditions.

The potential of 2-MEA to induce chromosomal aberrations was tested in cultured peripheral human lymphocytes according to OECD 473. The lymphocytes were exposed to 2-MEA for 4h with or without metabolic activation followed by 20h culture in treatment-free media prior to cell harvest. The concentration range of the test substancewas 10 to 40µg/mL in the absence of S9 mix and 320 to 640 µg/mL in the presence of S9 mix. The test substance did not induce any statistically significant increases in the frequency of cells with aberrations in the absence of S9 mix (4-h exposure). In the presence of metabolic activation, the test substance induced a statistically significant increase in the frequency of cells with aberrations, at a dose level of 640 µg/mL. The test substance was therefore considered to be clastogenic to human lymphocytes under the conditions of the test. The substance appeared around 10 times more cytotoxic without S9 than in presence of S9. Nevertheless, a positive result without S9 cannot be excluded since long-term treatment (e.g. 24-h) was not performed.

Overall, 2-MEA is considered genotoxic *in vitro* with and without metabolic activation.

- *In vivo*

An *in vivo* mammalian alkaline comet assay was performed with 2-MEA on male rats, according to OECD guideline 489 and under GLP conditions. Male rats (7/dose) were administered 120, 240 and 480 mg/kg bw of the test substance for 2 consecutive days (at 0 and 24 h). The animals were sacrificed 4 hours after the second dose administration and samples of the liver, glandular stomach and non-glandular stomach tissues were taken from each animal. 1/7 animals in the high-dose group died within 24 h; no reason for the mortality was given in the report. The remaining 6/7 rats had a hunched posture for approximately 1 h after each dosing. The positive control substance produced a marked increase in the % tail intensity value in all the investigated tissues. The negative control was slightly below the historical control values observed in glandular stomach. However, only a very low number of animals were included in the historical control data (11 animals). No significant change in the percentage tail intensity in the liver tissue was observed between the treatment groups and control group. A dose-related significant increase in the mean of median percentage tail intensity in the glandular stomach tissue was noted in all dose groups, and in the mean percentage tail intensity in the mid- and high dose group, compared with the control group respectively. However, the increase fell within the range of the historical negative control data. But the limited dataset of historical control data (only 11 animals) question the adequacy of using these values. A significant increase in the percentage tail intensity was also observed in the non-glandular stomach tissue of the mid- and high-dose

groups, compared to the control group (mean percentage tail intensity and mean of median percentage tail intensity).
In this study, the results of the histopathological examination of the non-glandular stomach showed that 2-MEA had a dose-related cytotoxic effect at the site of contact: inflammation and degeneration of the glandular- and non-glandular stomach tissues in the mid- and high dose animals (See table 21 of Annex I of the CLH report). The inflammation and degeneration effects are considered to be a result of the corrosive properties of the test substance and were more severe in non-glandular stomach than in glandular stomach. However, statistically significant increase in the mean percentage tail intensity in the non-glandular stomach was already observed at the lowest dose showing only minimal concomitant histopathological findings in the non glandular-stomach. Moreover, in the non-glandular stomach, the increase in cytotoxicity was clearly dose-related at the mid and high dose level but was not correlated with an increased genotoxic response. This result suggests that the genotoxic response cannot only be explained by a cytotoxic response. Therefore, the results in non-glandular stomach are considered true intrinsic genotoxic response. Based on the results of the comet assay, the test substance 2-MEA is considered positive *in vivo* under the conditions of this test at the site of contact in non-glandular stomach.

10.8.2 Comparison with the CLP criteria

Germ cell mutagens category 1 in the CLP regulation is dedicated to "Substances known to induce heritable mutations or to be regarded as if they induce mutations in the germ cells of humans. The classification in Category 1A is based on positive evidence from human epidemiological studies.

No human data are available with 2-MEA, therefore Muta. 1A is not appropriate.

The classification in Category 1B is based on:
– "positive results from *in vivo* heritable germ cell mutagenicity tests in mammals; or
– positive results from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells *in vivo*, or by demonstrating the ability of the substance or its metabolites to interact with the genetic material of germ cells; or
– positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people".

According to the CLP criteria the classification in Category 2 is based on:
"– Positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from:

– Somatic cell mutagenicity tests *in vivo*, in mammals; or
– Other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays."

In the ECHA guidance on the application of CLP criteria (v.4.1, June 2015), it is also stated that "It is also warranted that where there is evidence of only somatic cell genotoxicity, substances are classified in cat. 2. This holds true especially for those genotoxicants which are incapable of causing heritable mutations because they cannot reach the germ cells (e.g. genotoxicants only acting locally, 'site of contact' genotoxicants). **This means that if positive results *in vitro* are supported by at least one positive local *in vivo*, somatic cell test, such an effect should be considered as enough evidence to lead to classification in Category 2.**"

The equivocal and positive results obtained in glandular and non-glandular stomach, respectively, give evidence that 2-MEA may react at the site of contact at all doses tested and induce local genotoxicity. As human do not have a forestomach, the extrapolation to humans may be questionable. However, humans have comparable squamous epithelial tissues in the oral cavity and the upper two-thirds of the oesophagus (CLP guidance 2015, page 375). Therefore, the substance is considered to have genotoxic potential that can be evidenced in humans at the route of entry.

There is neither *in vivo* heritable germ cell mutagenicity test nor tests in human germ cells available with 2-MEA. Some evidence are available on the ability of 2-MEA or most probably its metabolites (e.g. 2-ethoxyexanol is classify Repr. 1B, H360FD) to interact with the genetic material of germ cells as effects on the spermatogenesis were observed in the combined repeated dose toxicity study with reproduction /developmental toxicity screening test (OECD 422) (Study report, 2012b). Detoxification with regard to the genotoxic potential of the substance may occur in liver as shown by the negative result in this organ from the comet assay *in vivo* and the decreased cytotoxicity in presence of metabolic activation *in vitro*. But, with regard to the genotoxicity potential of the test substance, this is not supported by the *in vitro* assays as positive results were observed with and without metabolic activation in the MLA. In addition, the test substance was positive with S9 and negative without S9 in the Mammalian chromosomal aberration test. However, a positive result without S9 cannot be excluded since long-term treatment according to OECD guideline was not performed.

Overall, 2-MEA fulfils the criteria for category 2. Positive local *in vivo* genotoxic response was supported by the positive *in vitro* gene mutation assay and *in vitro* chromosomal aberration assay.

Due to the absence of mutagenicity test on germ cells, a category 1B cannot be judged adequate at this time. Therefore, further mutagenicity test on germ cells would be need to conclude if category 1B is fulfilled

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Category 2 for germ cell mutagenicity is warranted based on the positive *in vivo* data on somatic cells supported by the *in vitro* data.

RAC evaluation of germ cell mutagenicity
Summary of the Dossier Submitter's proposal ***In vitro*** Two OECD studies according to TG 476 and 473 were available. In the first study, 2-methoxyethyl acrylate was tested for its potential to cause gene mutations in the mouse lymphoma assay (MLA). The concentration range of the test substance was 0.63 to 40 µg/mL in the absence of metabolic activation and 20.25 to 648 µg/mL in the presence of metabolic activation, 4h treatment (-S9 mix and +S9 mix). The test substance induced toxicologically significant dose-related increases in the mutant frequency at the TK +/- locus in L5178Y cells both with and without metabolic activation. In the second study, the potential of 2-methoxyethyl acrylate to induce chromosomal aberrations was tested in cultured peripheral human lymphocytes. The lymphocytes were exposed to 2-methoxyethyl acrylate for 4h with or without metabolic activation followed by 20h culture in treatment-free media prior to cell harvest. The concentration range of the test substance was 10 to 40 µg/mL in the absence of S9 mix and 320 to 640 µg/mL in the presence of S9 mix. The test substance induced a statistically significant increase in the frequency of cells with aberrations, at a dose level of 640 µg/mL in the presence of metabolic activation but not without. The DS concluded that 2-methoxyethyl acrylate is considered to be genotoxic *in vitro* with and without metabolic activation.

In vivo

In a Comet assay performed with 2-methoxyethyl acrylate in male rats, according to OECD TG 489 and under GLP conditions, negative results were shown in the liver, equivocal results in the glandular stomach and positive effects in the forestomach (non-glandular stomach).

Male rats (7/dose) were administered 120, 240 and 480 mg/kg bw of the substance for 2 consecutive days (at 0 and 24h). The animals were sacrificed 4h after the second dose. In the high-dose group, 1/7 animals died within 24h (no reason for the mortality was given in the original report). In the liver, no significant change in the % tail intensity was observed between the treatment groups and control group. In the glandular stomach tissue, a dose-related significant increase in the mean of median % tail intensity was noted in all dose groups, and in the mean % tail intensity in the mid and high dose groups, compared with the control group. However, the increase fell within the range of the historical control data, which is composed by a limited dataset (only 11 animals) thus its adequacy is questionable. In the non-glandular stomach tissue, a significant increase in the % tail intensity was also observed of the mid and high dose groups (see table below).

Summary Table Comet Assay

Dose Level	Group Mean % Tail Intensity	Group Mean of Mean of Median % Tail Intensity per Animal
Glandular Stomach		
Vehicle	2.05 ± 0.62	0.69 ± 0.42
480 mg/kg bw	3.98 ± 1.71[a]	2.52 ± 1.61[b]
240 mg/kg bw	2.92 ± 0.79[b]	1.22 ± 0.63[c]
120 mg/kg bw	2.72 ± 0.99	1.18 ± 0.64[c]
Positive (MNU)	21.09 ± 1.81[a]	19.28 ± 1.88[a]
Non-Glandular Stomach		
Vehicle	6.68 ± 1.88	4.35 ± 1.74
480 mg/kg bw	11.42 ± 3.16[a]	9.30 ± 3.87[a]
240 mg/kg bw	11.92 ± 3.58[a]	10.29 ± 3.97[a]
120 mg/kg bw	7.92 ± 2.42	5.92 ± 2.42
Positive (MNU)	41.68 ± 3.60[a]	41.90 ± 4.21[a]

[a]= P < 0.001
[b]= P < 0.01
[c]= P < 0.05

At the site of contact: inflammation and degeneration of the glandular and non-glandular stomach tissues in the mid and high dose animals are considered to be the result of the corrosive properties of the substance and were more severe in non-glandular stomach than in glandular stomach.

A statistically significant increase in the mean % tail intensity in the non-glandular stomach was observed already at the lowest dose, showing only minimal concomitant histopathological findings in the non-glandular stomach. Moreover, in the non-glandular stomach, the increase in cytotoxicity was clearly dose-related, but did not correlate with an increased genotoxic response. The genotoxicity effects were higher at the mid dose. If the genotoxicity effects were only the result of a cytotoxic response, the highest % tail intensity in the Comet assay would have been expected to be in the highest dose group, but this is not the case. Marked cytotoxicity including ulceration and necrosis were only observed at 480 mg/kg bw (highest dose) and not at 240 mg/kg bw (mid dose) (see table below).

Summary of individual histopathological findings in the non-glandular stomach (forestomach)

Group	Animal No.	Findings
Control	1 to 7	No abnormalities detected
120 mg/kg bw	27, 28, 29, 31	No abnormalities detected
	30, 32, 33	Minimal vacuolisation of the limiting ridge
240 mg/kg bw	21, 25	No abnormalities detected
	23, 24	Minimal vacuolisation of the limiting ridge
	26	Minimal vacuolisation of the limiting ridge, slight epithelial hyperplasia
	20	Minimal vacuolisation of the limiting ridge, slight inflammation of submucosa, minimal myofibre degeneration
	22	Minimal focal ulceration of the limiting ridge
480 mg/kg bw	13, 16	Minimal to slight myofibre degeneration, submucosa inflammation, epithelium vacuolisation, slight mucosal necrosis
	15, 19	Minimal to moderate myofibre degeneration, submucosa inflammation and/or epithelium vacuolisation and/or moderate erosion and/or slight ulceration
	14, 18	Minimal to slight myofibre degeneration, inflammation and/or epithelium vacuolisation, and/or slight ulceration of the limiting ridge, and marked ulceration of the epithelium

According to the DS, these results suggest that the genotoxic response cannot only be explained by cytotoxicity . Therefore, the results in non-glandular stomach were considered to be an intrinsic genotoxic response.

Therefore, the DS proposed category 2 for germ cell mutagenicity for 2-methoxyethyl acrylate based on the positive *in vivo* data on somatic cells and supported by the *in vitro* data.

Comments received during public consultation

Two MSCAs supported the DS proposal to classify 2-methoxyethyl acrylate as Muta. category 2.

One Industry Association commented on the interpretation of the Comet assay mutagenicity data presented by the DS noting that increases in DNA migration in the clear evidence of cytotoxicity should be interpreted with caution.

The DS responded that histopathological analysis of the non-glandular stomach showed cytotoxic effects and more particularly at the high dose level that would suggest

genotoxicity due to cytotoxicity. However, the effects are higher at the mid dose, where the cytotoxic effect are lower, than in the high dose.

The DS's response was, that OECD TG 489 clearly stated that "conversely, low or moderate cytotoxicity is often seen with known genotoxins, showing that it is not possible to distinguish DNA migration induced by genotoxicity versus that induced by cytotoxicity in the C assay alone". The DS stated that this OECD TG 489 statement does not mean that the effects should be disregarded.

Assessment and comparison with the classification criteria

The two *in vitro* mutagenicity tests (MLA +/- metabolic activation) and chromosome aberration assay (+ metabolic activation) show a positive mutagenic effect of 2-methoxyethyl acrylate.

RAC noted that the *in vivo* Comet assay with 2-methoxyethyl acrylate indicates negative results in liver, equivocal results in the glandular stomach and positive effects in the forestomach in rats. When comets are seen at the initial site of contact (forestomach), but not at a distant site (here the liver), the classification as mutagenic needs to be carefully considered. On the one hand, classification as a germ cell mutagen is important, because in the absence of carcinogenicity data it indirectly highlights the potential for carcinogenicity. On the other hand, it is not appropriate to classify substances that are not germ cell mutagens. Where the data is limited, it can be difficult to judge whether a classification for germ cell mutagenicity is appropriate. For germ cell mutagenicity hazard assessment and classification purposes the study designs exposing the bone marrow are still considered to be the most informative. When a substance is known to be distributed around the body, and especially one that is toxic to reproduction, such tests are still the most logical choice for further evaluation of *in vitro* mutagens.

Nevertheless, taking the arguments presented by the DS (see above) into account and the fact that humans have comparable squamous epithelial tissues in the oral cavity and the upper two-thirds of the oesophagus as in the rat forestomach, the mutagenicity effect observed in the Comet assay are considered to be relevant for humans and should be regarded as positive e (CLP guidance 2017, page 381). Therefore, the substance is considered to have genotoxic potential that may also be expected in humans at the route of entry.

In the tables below, the following is shown:

a) comparison of genotoxicity and cytotoxicity in glandular and non-glandular for the individual animals in two groups of doses 480 mg/kg and 240 mg/kg and
b) comparison of genotoxicity and cytotoxicity expressed as "cytotoxicity score"

Comparison of genotoxicity and cytotoxicity for the individual animals in the group of 480 mg/kg

	GLANDULAR		NON GLANDULAR	
No of animal	Median % Tail Intensity*	Individual Histopathology	Median % Tail Intensity*	Individual Histopathology
13	1.97/0.97	erosion, glandular epithelium, slight, multifocal	3.19/4.42	mucosal necrosis, limiting ridge, slight vacuolation non-glandular

		inflammation, submucosa, minimal, neutrophilic myofibre degeneration, glandular epithelium, minimal		epithelium, minimal inflammation, submucosa, slight neutrophilic myofibre degeneration, non-glandular, minimal
14	4.11/2.38	erosion, glandular epithelium, slight focal inflammation, submucosa, minimal neutrophilic myofibre degeneration glandular epithelium, minimal	12.54/13.33	ulceration, limiting ridge, minimal focal ulceration, non-glandular epithelium, marked vacuolation non-glandular epithelium, minimal inflammation, submucosa, slight neutrophilic myofibre degeneration, non-glandular, slight
15	1.54/1.06	erosion, glandular epithelium, minimal focal inflammation, submucosa, minimal neutrophilic myofibre degeneration glandular epithelium, slight	5.52/10.64	ulceration, limiting ridge, slight focal ulceration non-glandular epithelium, slight erosion, non-glandular epithelium, moderate vacuolation non-glandular epithelium, minimal inflammation, submucosa, slight neutrophilic myofibre degeneration glandular epithelium, moderate
16	4.94/5.65	erosion, glandular epithelium, slight focal inflammation, submucosa, minimal neutrophilic myofibre degeneration glandular epithelium, minimal	5.86/8.41	mucosal necrosis, limiting ridge, slight erosion non-glandular epithelium, slight inflammation, submucosa, slight neutrophilic myofibre degeneration glandular epithelium, slight
18	0.80/1.33	ulceration, limiting ridge, slight focal ulceration, non-glandular epithelium, marked inflammation, submucosa, slight neutrophilic myofibre degeneration glandular epithelium, moderate	12.19/6.84	ulceration limiting ridge, slight focal ulceration, non-glandular epithelium, marked inflammation, submucosa, slight neutrophilic myofibre degeneration glandular epithelium, moderate
19	2.64/2.80	erosion, glandular epithelium, slight focal inflammation, submucosa, minimal neutrophilic	11.76/16.90	ulceration, limiting ridge, minimal focal inflammation, submucosa, slight neutrophilic myofibre degeneration non-glandular epithelium, minimal
	2.52		**9.30**	

Comparison of genotoxicity and cytotoxicity for the individual animals in the group of 240 mg/kg

	GLANDULAR		NON GLANDULAR	
No of animal	Median % Tail Intensity*	Individual Histopathology	Median % Tail Intensity*	Individual Histopathology
20	1.43/1.89	erosion, glandular epithelium, minimal multifocal inflammation, submucosa, minimal neutrophilic	13.97/16.59	ulceration, limiting ridge, slight focal vacuolation non-glandular epithelium, slight inflammation, submucosa, slight neutrophilic
21	1.67/2.26	inflammation, submucosa, minimal neutrophilic	11.27/13.86	no abnormalities detected
22	1.53/0.33	inflammation, submucosa, minimal neutrophilic	14.81/12.21	ulceration, limiting ridge, minimal focal
23	0.60/0.37	erosion, glandular epithelium, minimal focal	7.75/9.77	erosion, glandular epithelium, minimal focal
24	2.20/1.74	erosion, glandular epithelium, minimal focal	8.94/6.86	vacuolation non-glandular epithelium, limiting ridge, slight
25	1.01/0.91	no abnormalities detected	4.21/2.82	no abnormalities detected
26	0.71/0.51	erosion, glandular epithelium, minimal focal	10.68/10.34	erosion, glandular epithelium, minimal focal
	1.22	**10.29**		

**two replicate slides*

Comparison of genotoxicity and cytotoxicity expressed as "cytotoxicity score"

At the dose of 480 mg/kg:*

Animals' No	GLANDULAR Median % Tail Intensity	NON-GLANDULAR Median % Tail Intensity	CYTOTOXICITY SCORE
13	1.97	3.19	MINIMAL TO SLIGHT
13	0.97	4.42	MINIMAL TO SLIGHT
14	4.11	12.54	MINIMAL TO MARKED
14	2.38	13.33	MINIMAL TO SLIGHT
15	1.54	5.52	MINIMAL TO MODERATE
15	1.06	10.64	MINIMAL TO SLIGHT
16	4.94	5.86	SLIGHT

16	5.65	8.41	MINIMAL TO SLIGHT
18	0.80	12.19	SLIGHT TO MODERATE
18	1.33	6.84	SLIGHT TO MODERATE
19	2.64	11.76	MINIMAL TO SLIGHT
19	2.80	16.90	MINIMAL TO SLIGHT
mean	2.52	9.30	

At the dose of 240 mg/kg:*

Animals' No	GLANDULAR Median % Tail Intensity	NON-GLANDULAR Median % Tail Intensity	**CYTOTOXICITY SCORE**
20	1.43	13.97	SLIGHT
20	1.89	16.59	MINIMAL
21	1.67	11.27	No
21	2.26	13.86	MINIMAL
22	1.53	14.81	MINIMAL
22	0.33	12.21	MINIMAL
23	0.60	7.75	MINIMAL
23	0.37	9.77	MINIMAL
24	2.20	8.94	MINIMAL
24	1.74	6.86	MINIMAL
25	1.01	4.21	NO
25	0.91	2.82	NO
26	0.71	10.68	MINIMAL
26	0.51	10.34	MINIMAL
mean	1.22	10.29	

**green fields indicate above-average genotoxicity results, yellow fields indicate low cytotoxicity*

Comparison of genotoxicity and "cytotoxicity score" expressed as "NO", "MINIMAL", "MINIMAL TO SLIGHT", "SLIGHT", "MINIMAL TO MARKED", "MINIMAL TO MODERATE" for the individual animals showed that in the non-glandular stomach, the increase in cytotoxicity was not correlated with an increased genotoxic response. The genotoxicity effects are higher at the mid dose, but cytotoxicity was clearly dose-related. This result suggests that the genotoxic response cannot only be explained by a cytotoxic response. Therefore, the effects in non-glandular stomach are considered true intrinsic genotoxic response. In the rat No 21, dosed with 240 mg/kg, the "cytotoxicity score" is "NO" but the "Median % Tail Intensity" is 11.27, i.e. above median, and in the rat No 22, the "cytotoxicity score" is "MINIMAL" but "Median % Tail Intensity" is 12.21 and 14.81, i.e. above median. The same was observed in rat No 26.

Detoxification with regard to the genotoxic potential of the substance may occur in liver as shown by the negative result in this organ from the Comet assay *in vivo* and the decreased cytotoxicity in presence of metabolic activation *in vitro*. However, this is not supported by the *in vitro* assays as positive results were observed with and without metabolic activation

in the MLA. In addition, the test substance was positive with S9 and negative without S9 in the mammalian chromosomal aberration test. However, a positive result without S9 cannot be excluded since long-term treatment according to OECD guideline was not performed.

No human data are available with 2-methoxyethyl acrylate, therefore classification as Muta. 1A is not appropriate.

The classification in category 1B was considered. There is neither *in vivo* heritable germ cell mutagenicity test nor tests in human germ cells available with 2-methoxyethyl acrylate. However, in the combined oral (gavage) repeated dose toxicity study with the reproduction/developmental toxicity screening test (see above), the following histopathological findings in male reproduction organs were observed:

- Enlarged cells
- Chronic active inflammation
- Most stages of spermatogenesis missing
- Multiple acrosomes
- Individual cell necrosis
- Spermatidic giant cells

which shows the potential of the substance or its metabolite to reach the germ cells. However, the evidence from this test is not sufficient on its own to assess the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells. Therefore Muta 1B is not considered appropriate.

Overall, RAC considers that the classification criteria in CLP for Muta, category 1B, are not met, while the criteria for classification in category 2 (Table 3.5.1) are met based on "*Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from: (…) Other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays*".

In conclusion, RAC is of the opinion that 2-methoxyethyl acrylate should be classified to for **germ cell mutagenicity, category 2; H341 "Suspected of causing genetic defects"** based on the positive *in vitro* and *in vivo* data.

10.9 Carcinogenicity

Not evaluated. No data.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 25: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, Klimish score deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Combined repeated dose toxicity study with reproduction/developmental toxicity screening test OECD 422, GLP 1(reliable without restriction) Oral (gavage) Wistar rats 10/sex/group	2-MEA 0, 40, 100, 250/150 mg/kg bw (250 mg/kg bw/day: from Day 1 to 11; 150 mg/kg bw/day: from Day 12 to study termination) Males: 2 weeks prior to mating, during mating, and up to termination Females: during 2 weeks prior to mating, during mating, during post-coitum, and during at least 4 days of lactation Vehicle: propylene glycol	<u>Parental effects:</u> **250/150 mg/kg bw per day** 30% mortality in males (euthanised on days 2 and 8) Hunch posture, piloerection, pale and lean appearance (f/m) Red vagina or bleeding from vaginal in 2 females Bw loss (m/f) Hematology: ↓haemoglglobin, MCHC, MCH, Platelets (m+f), MCV (f), ↑prothrombin time (f) Reduced relative organ weight: thymus, prostate (m) Reduced absolute organ weight: testis, epididymides (m) Histopathology: degeneration of seminiferous tubular epithelium, edema, inflammation and enlarged ampholitic cells, impairment of the spermatogenetic cycle in testes. Sperm degeneration, atrophy and inflammation in epididymides. Hepatocellular necrosis in liver (m/f). Atrophy and haemorrhage in thymus (m/f) **100 mg/kg bw per day** 1 female died on study day 21 post-coitum Red vagina or bleeding from vaginal in 1 female Bw loss during gestation in females and reduced bw gain in males Hematology: ↓haemoglglobin, MCHC, MCH, Platelets, MCV, ↑ prothrombin time (f) Reduced relative organ weight: thymus, prostate (m) Reduced absolute organ weight: testis, epididymides (m) Histopathology: degeneration of seminiferous tubular epithelium, edema, necrosis, inflammation and enlarged ampholitic cells, impairment of the spermatogenetic cycle in testes. Sperm granuloma, degeneration, atrophy and inflammation in epididymides. Haemorrhage and apoptosis in thymus (m/f). **40 mg/kg bw per day** Histopathology: necrosis, enlarged ampholitic cells, impairment of the spermatogenetic cycle in testes. Sperm granuloma in one male in epididymides. Atrophy, haemorrhage and apoptosis in thymus. **A LOAEL for parental toxicity of 40 mg/kg bw was derived from this study.** <u>Reproductive effects:</u> **250/150 mg/kg bw per day** ↑precoital time ↓fertility index (20% vs 100% in control) ↓number of corporea lutea and implantation sites	Study report, 2012b

Method, guideline, Klimish score deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		100 mg/kg bw per day ↓fertility index (90%) ↓number of corporea lutea and implantation sites **40 mg/kg bw per day** ↑precoital time ↑ duration of gestation **A LOAEL for reproductive toxicity of 40 mg/kg bw was derived from this study.** <u>Developmental effects</u> **250/150 mg/kg bw per day** ↓ number of live pups (at day 1): 0% vs 100% in control **100 mg/kg bw per day** ↓ number of live pups (at day 1): 0% vs 100% in control **40 mg/kg bw per day** ↓ number of live pups (at day 1): 70% vs 100% in control ↓ viability index (66.7% vs 99% in control) Sligh decrease in the bw of pups Lean and pale appearances of surviving pups Absence of milk in the stomach and blue discoloration of the snout. In addition autolysis was noted for pups found dead. **A LOAEL for developmental toxicity of 40 mg/kg bw was derived from this study.**	

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

A combined oral repeated dose toxicity study with the reproduction/developmental toxicity screening test was performed with 2 -methoxyethyl acrylate (2 -MEA) according to OECD 422 (Study report, 2012b). The LOAEL for parental toxicity was 40 mg/kg bw based on histopathological changes on testis, epididymides and thymus at all dose levels. Mortality and severe bw effects occurred in parental animals at 100 mg/kg bw onward. At 100 and 250/150 mg/kg bw no live litters were observed. The LOAEL for reproductive effects was 40 mg/kg bw based on dose-related increase precoital time and reduced fertility at all dose levels. The LOAEL for developmental toxicity was 40 mg/kg bw based on decreased live litters and decrease viability index.

In addition, there are data available on effects on fertility for the expected primary metabolite 2-methoxyethanol (CAS no. 109-86-4) which showed effects in reproduction toxicity studies as observed for 2-MEA. Studies on 2-methoxyethanol with respect to effects on fertility show consistent toxicity to the male reproductive system in multiple species (mice, rats, guinea-pigs, rabbits and dogs) exposed by all routes of administration (subcutaneous, dermal, oral or inhalation) (CICAD, 2009). Effects on reproductive ability as well as reproductive organs have been observed, often from the lowest dose or concentration tested. Single

or repeated oral administration of 2-methoxyethanol induced adverse effects on the testes (including weight and histopathological changes or biochemical indicators of testicular damage, such as urinary creatinine) and/or various sperm parameters in every identified studies in which these endpoints were examined (CICAD, 2009).

10.10.3 Comparison with the CLP criteria

Reproductive toxicity category 1 in the CLP Regulation is dedicated to "substances which are known or presumed human reproductive toxicant". "Substances are classified in category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function an fertility or when there is evidence from animal studies possibly supplemented with other information, to provide as strong presumption that the substance has the capacity to interfere with reproduction with humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (category 1A) or from animal data (category 1B). "

Reproductive toxicity category 2 in the CLP Regulation is dedicated to substances which are "suspected human reproductive toxicants". "Substances are classified in category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function or fertility, and where the evidence is not sufficiently convincing to place the substance in category 1."

No human data were provided, therefore Repr. 1A is not appropriate.

In the reproductive/developmental screening study (Study report, 2012b), weight and histopathology effects on reproductive organs were observed (including testis, epididymis) from 40 mg/kg bw (oral gavage). In this study, fertility effects were observed at all dose levels including increase precoital time and dose-related decreased fertility index.

These effects may be considered secondary to the high parental toxicity observed at 100 and 250/150 mg/kg bw (body weight loss, mortality). However, at 40 mg/kg bw/d, no indication of marked general toxicity has been observed. Indeed, at this dose only changes in hematological parameters were observed in females (decreased MCV and MCH). The adversity of these findings is not clear as no change in haematocrit and haemoglobin was reported at this dose level.

In conclusion, the available data on reproductive toxicity present clear evidence of adverse effects on fertility. Because the effects are severe and not considered secondary to maternal or parental toxicity at the low dose level, the available data support classification for reproductive toxicity category 1B. There is no information that the effects may not be relevant to human and the quality of the study is good, therefore, category 2 according to the CLP criteria is not considered appropriate.

10.10.4 Adverse effects on development

Table 26: Summary table of animal studies on adverse effects on development

Method, guideline, Klimish score deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Combined repeated dose toxicity study with reproduction/developmental toxicity screening test OECD 422, GLP 1(reliable without restriction)	2-MEA 0, 40, 100, 250/150 mg/kg bw (250 mg/kg bw/day: from Day 1 to 11; 150 mg/kg bw/day: from Day 12 to study termination) Males: 2 weeks prior to	See results in table 25.	Study report, 2012b

Method, guideline, Klimish score deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Oral (gavage) Wistar rats 10/sex/group	mating, during mating, and up to termination Females: during 2 weeks prior to mating, during mating, during post-coitum, and during at least 4 days of lactation Vehicle: propylene glycol		
Non guideline Non-GLP 3 (unreliable) CD-1 mouse 50 mice/group Only one dose level, short treatment period, short reporting, dose above the maximum tolerable dose, pups were not examined for malformations	Oral : gavage GD6-13 (daily, 7 days/week) 0, 650 mg/kg bw Vehicle: distilled water	Maternal toxicity: 30% mortality in dams Developmental toxicity: 100% intrauterine death	Hardin et al., 1987

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

A developmental toxicity study evaluating 60 chemicals in mice including 2-methoxyethyl acrylate (2-MEA) was published by Hardin et al. (1987). Fifty pregnant mice were dosed by gavage with 650 mg/kg bw/day of the test substance on gestation days 6 -13. The mice were then permitted to deliver litters. The test substance produced 30% maternal mortality and 100% intrauterine death. Therefore, the test substance adversely affected all measures of reproductive success since no liveborn pups were recorded. Dead pups were not examined for malformations. However, it should be pointed out that maternal mortality was 30% and that the dose tested was too high to be suitable for evaluating developmental toxicity.

In the combined screening study (Confidential report, 2012b), implantation sites were only noted for nine females at 100 mg/kg bw/day and two females at 250/150 mg/kg bw/day. The remaining females were non pregnant or did not mate. No pups were born at 100 and 250/150 mg/kg bw/day. Out of the nine litters at 40 mg/kg bw/day, only six had live pups at first litter check. The number of pups per litter was decreased when compared to the control group. In addition, most of these pups did not survive the first days of lactation. At 40 mg/kg bw/day, lean and pale appearance was seen in the surviving pups and body weights were slightly, but not statistically significantly decreased when compared to the control. Macroscopic findings involved absence of milk in the stomach and blue discolouration of the snout. In addition, autolysis was noted for pups found dead. Based on the results of the study, the NOAEL for developmental toxicity in rats was considered to be lower than 40 mg/kg bw/day. High maternal toxicity was observed at 100 mg/kg bw and above.

In addition, there are data on developmental toxicity for the primary expected metabolite 2-methoxyethanol (CAS no.109-86-4) which showed similar effects in developmental toxicity studies as observed for 2-MEA. 2-methoxyethanol has consistently induced developmental toxicity in numerous oral studies in several species of laboratory animals, generally at doses lower than those that are maternally toxic, and often at the

lowest exposure level tested (CICAD, 2009). Decreased fetal body weights were noted in rats repeatedly exposed to 2-methoxyethanol doses of 16 mg/kg bw/day or more in the diet during gestation, with malformations being observed at doses of 31 mg/kg bw/day or greater, whereas maternal toxicity was present only at higher doses. Similar results were obtained in several other studies in rats exposed to 2 methoxyethanol in the diet or by gavage. In many of the studies, the cardiovascular system, kidney and skeletal system were the principal targets for 2-methoxyethanol-induced malformations; functional defects of the heart were also noted (CICAD, 2009).

10.10.6 Comparison with the CLP criteria

Reproductive toxicity category 1 in the CLP Regulation is dedicated to "substances which are known or presumed human reproductive toxicant". "Substances are classified in category 1 for reproductive toxicity when they are known to have produced an adverse effect on development in humans or when there is evidence from animal studies possibly supplemented with other information, to provide as strong presumption that the substance has the capacity to interfere with reproduction with humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (category 1A) or from animal data (category 1B)."

Reproductive toxicity category 2 in the CLP Regulation is dedicated to substances which are "suspected human reproductive toxicants". "Substances are classified in category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development, and where the evidence is not sufficiently convincing to place the substance in category 1."

No human data were available and therefore, Repr. 1A is not considered appropriate.

The developmental toxicity study published by Hardin et al., 1987 is not considered appropriate for classification as only one dose was tested and the dose was above the maximum tolerated dose.

In the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (Study report, 2012b) performed in rat, dose-related decrease in live birth and viability index was observed at all dose tested. At 100 and 150/250 mg/kg bw, where high maternal toxicity occured, no dam had live pups on day 1. At 40 mg/kg bw, decrease live birth index and viability index was observed without clear evidence of maternal toxicity.

As marked developmental effects were observed an OECD guideline developmental screening study, 2-MEA is considered to meet the criteria for classification as Repr. 1B (H360D) according to Regulation (EC) 1272/2008.

There are no information supporting that the effect could not be relevant for human and therefore Repr. 2 is not considered appropriate.

10.10.7 Adverse effects on or via lactation

No specific data available.

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

No specific data available.

10.10.9 Comparison with the CLP criteria

According to the CLP criteria classification for lactation is recommended when "absorption, metabolism, distribution and excretion studies indicate the likelihood that the substance is present at toxic levels in breast milk. In the reproductive screening toxicity study, no milk was present in the stomach of the dead pups. There is no data on the presence of 2-MEA in the breast milk. Since most of these pups did not survive the first days of lactation, the reason of death is probably not related to lactation.Therefore, there is no sufficient information to propose a classification for effects on or via lactation.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

In conclusion, 2-MEA has been found to induce both reproductive and developmental effects. These effects observed at 40 mg/kg bw could not be explained by maternotoxicity. Classification **Reproductive toxicity category 1B, H360FD "May damage fertility or the unborn child**" is thus warranted.

No specific concentration limit could be set for 2-MEA based on the available data as no NOAEL could be determined in the available screening study.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The combined oral repeated dose toxicity study with the reproduction/developmental toxicity screening test described above (see the STOT RE section) was used for assessing both fertility and developmental effects.

Adverse effects on sexual function and fertility

Implantation sites were only noted for nine females at 100 mg/kg bw/day and two females at 250/150 mg/kg bw/day. The remaining females were not pregnant or did not mate. No pups were born at 100 and 250/150 mg/kg bw/day. Out of the nine litters at 40 mg/kg bw/day, only six had live pups (less live pups as compared to the control group). In addition, most of the pups did not survive the first days of lactation.

The LOAEL for parental toxicity was 40 mg/kg bw based on histopathological changes in the testis, epididymis and thymus at all dose levels. Histopathology included: necrosis, enlarged ampholitic cells, impairment of the spermatogenetic cycle in testes, sperm granuloma in one male in epididymis.

Mortality and severe body weight effects (see tables below) occurred in parental animals at 100 mg/kg bw/day onward. At 100 and 250/150 mg/kg bw/day no live litters were observed. The LOAEL for reproductive effects was 40 mg/kg bw/day based on dose-related increase pre-coital time, reduced fertility index at all dose levels and reduced number of corpora lutea and implantation sites. Some reproductive effects appeared at a level of 40 mg/kg bw/day (see tables below) where parental toxicity was not marked. The DS proposed, in the light of these effects, a classification as Repr. 1B; H360F.

In addition, there are data available on effects on fertility for the expected primary metabolite, 2-methoxyethanol (CAS no. 109-86-4). Studies on 2-methoxyethanol with respect to effects on fertility showed consistent toxicity to the male reproductive system in multiple species (mice, rats, guinea-pigs, rabbits and dogs) exposed by all routes of administration (subcutaneous, dermal, oral or inhalation). Effects on reproductive ability

as well as reproductive organs have been observed, often from the lowest dose tested. Single or repeated oral administration of 2-methoxyethanol induced adverse effects on the testes (including weight and histopathological changes or biochemical indicators of testicular damage, such as urinary creatinine) and/or various sperm parameters in every identified studies in which these endpoints were examined.

Developmental toxicity

At 40 mg/kg bw/day, lean and pale appearance was seen in the surviving pups and body weights were slightly, but not statistically significantly decreased when compared to the control. Macroscopic findings involved absence of milk in the stomach and blue discolouration of the snout. In addition, autolysis was noted in pups found dead. Based on the results of the study, the NOAEL for developmental toxicity in rats was considered to be lower than 40 mg/kg bw/day. High maternal toxicity was observed at 100 mg/kg bw and above.

The LOAEL for developmental toxicity was 40 mg/kg bw/day based on decreased live litters and decrease viability index.

A developmental toxicity study evaluating 60 chemicals in mice including 2-methoxyethyl acrylate was published (Hardin *et al.*, 1987). Fifty pregnant mice were dosed by gavage with 650 mg/kg bw/day of the test substance on gestation days 6 -13. The mice were then permitted to deliver litters. The test substance produced 30% maternal mortality and 100% intrauterine death. Therefore, 2-methoxyethyl acrylate adversely affected all measures of reproductive success since no live born pups were recorded. Dead pups were not examined for malformations. However, it should be pointed out that maternal mortality was 30% and that the dose tested was too high to be suitable for evaluating developmental toxicity. This developmental toxicity study was not considered appropriate for classification as only one dose was tested and that was above the maximum tolerated dose.

In addition, there are data on developmental toxicity for the primary expected metabolite, 2-methoxyethanol which showed similar effects in developmental toxicity studies as observed for 2-methoxyethyl acrylate. The metabolite, 2-methoxyethanol, has consistently induced developmental toxicity in numerous oral studies in several species of laboratory animals, generally at doses lower than those that are maternally toxic, and often at the lowest exposure level tested. Decreased foetal body weights were noted in rats repeatedly exposed to 2-methoxyethanol doses of 16 mg/kg bw/day or more in the diet during gestation, with malformations being observed at doses of 31 mg/kg bw/day or greater, whereas maternal toxicity was present only at higher doses. Similar results were obtained in several other studies in rats exposed to 2-methoxyethanol in the diet or by gavage. In many of the studies, the cardiovascular system, kidney and skeletal systems were the principal targets for 2-methoxyethanol induced malformations; functional defects of the heart were also noted.

The DS proposed, in the light of these effects, a classification as Repr. 1B H360D "May damage the unborn child".

Adverse effects on or via lactation

No specific data available.

Comments received during public consultation

Two MSCAs supported the proposed classification for both sexual function and fertility and developmental effects as Repr. 1B; H360FD.

Assessment and comparison with the classification criteria

Sexual function and fertility

In this combined study described above, the LOAEL for parental toxicity was 40 mg/kg bw/day based on histopathological changes in the testes and epididymis as well as atrophy, haemorrhage and apoptosis in the thymus. The LOAEL for reproductive effects was 40 mg/kg bw/day based on histopathological changes in the testis and epididymis and a dose-related increase in pre-coital time and reduced fertility at all dose levels.

Body weight and histopathological effects on reproductive organs were observed in the testis and epididymis from 40 mg/kg bw as follows:

- Body weight loss: At 250 mg/kg bw/day, most male animals and a few female animals showed a body weight loss, which slightly recovered during treatment at 150 mg/kg bw/day. Reduced body weight gains were also noted for males at 100 mg/kg bw/day.

The reduced body weight gains for females of the 100 mg/kg bw/day dose group during the first two weeks of post-coitum was considered a cause of their pregnancy status (i.e. implantation sites only instead of live foetuses) and not toxicologically relevant. However at 40 mg/kg bw/day, no indication of marked general toxicity has been observed.

- Mortality: At 250 mg/kg bw/day: 2 males died on day 2 (no cause of death could be determined), 1 male was killed on day 8 (showed ulcerative inflammation in the stomach with resultant peritonitis); at 100 mg/kg bw/day: 1 female killed in extremis on day 21 post-coitum.

No changes in body weights and mortality were observed at 40 mg/kg bw/day. Indeed, at this dose only changes in haematological parameters were observed in females (decreased MCV and MCH). The adversity of these findings is not clear as no change in haematocrit and haemoglobin was reported.

- Histopathology effects on reproductive organs: At 100 mg/kg bw/day: degeneration of seminiferous tubular epithelium, oedema, necrosis inflammation and enlarged ampholitic cells in testes, impairment of the spermatogenetic cycle in testis, sperm granuloma, degeneration, atrophy and inflammation in epididymis. At 40 mg/kg bw/day: necrosis, enlarged ampholitic cells, impairment of the spermatogenetic cycle in testis. There were no statistically significant changes in histopathological observations based on Fisher's Exact test at 5% or 1% level and on Steel’s test at 5%.

A summary of mortality of parental animals, of the adverse general toxicity (besides mortality) and quantitative data on histopathological findings in reproduction organs/endocrine organs of males and females and changes in reproduction organ weights of males data is presented in the tables below.

Summary of mortality of parental animals

40 mg/kg	100 mg/kg	250 mg/kg (150 mg/kg from day 12)
not described	1 ♀ killed in extremis on day 21 post-coitum	- 2 ♂ on day 2 (no cause of death could be determined) - 1 ♂ was killed on day 8 (showed ulcerative inflammation in the stomach with resultant peritonitis) - marked atrophy of the thymus found in the dead animals (see above section of STOT RE).

Summary of adverse general toxicity (besides mortality)

Adverse general toxicity observed	40 mg/kg bw/day	100 mg/kg bw/day	250 mg/kg bw/day (150 mg/kg bw/day from day 12)
Clinical signs in animals found dead	not described	not described	Only at 250 mg/kg bw/day: - hunch posture (18 animals 1-5 days), salivation (3 animals 1 day) and piloerection (1♀ 2 days) - the findings disappeared during dosing 150 mg/kg bw/day No clinical symptoms can be attributed to 150 mg/kg bw/day.
Body weight	not described	bw loss during gestation (♀) and reduced bw gain (♂)	bw loss (♂, ♀)

Summary of histopathological findings in reproduction organs/endocrine organs of males and females

Histopathological findings in reproduction organs/endocrine organs	Dose [mg/kg bw/day]			
	0	40	100	250/150
Number of animals per group	10	10	10	10
Primary effects				
TESTES (males)	5	5	9	10
- Enlarged cells	-	-	-	2
- Degeneration of seminiferous tubular epithelium	1	2	9	8
- Oedema	4	3	7	7
- Chronic active inflammation	-	-	-	2
- Dilated rete	-	1	1	-
TESTES, PAS STAGING (males)	5	5	5	8
- All stages missing	-	-	1	5
- Enlarged cells	-	-	-	2
- Most stages missing	-	-	4	1
- Some stages missing	-	-	-	1
- Multiple acrosomes	-	-	-	1
- Asynchronous tubules	-	2	4	1
- Individual cell necrosis	-	3	3	-

- Reduced spermatagonia	-	1	5	6
- Spermatidic giant cells	-	1	3	1
- Vacuolation basilar	-	-	1	-
EPIDIDYMIS (males)	5	5	8	10
- Sperm granuloma	-	1	1	-
- Sperm degeneration	-	-	8	8
- Hypospermia	-	-	1	8
- Atrophy	-	-	7	8
- Chronic active inflammation	-	-	1	4
Secondary effects:				
UTERUS (female)	5	6	8	6
- Stromal hyperplasia	-	-	-	1
- Dilation cyclic	-	-	-	3
- Haemorrhage	2	6	3	-
- Inflammation supp	-	-	1	-
- Necrotic debris/neut	-	-	1	-
- Implant sites	5	6	3	1
- Throphoblasts/Necro	-	-	3	-
ADRENALS (females)	5	-	1	5
- Hypertrophy cortex	5	-	-	-
- Extra cortical nodule	1	-	-	-
- Extramed haematopoiesis	-	-	1	-
MAMMARY GLAND AREA (females)	5	4	5	5
- Hyperplasia	5	4	4	-
- Inactive gland	-	-	1	5
- Active gland	-	1	1	-
- Infiltrate lymphoid	-	1	-	-
THYMUS (females)	5	5	6	5
- Increased apoptosis	-	1	-	-
- Haemorrhage/Congestion	-	1	1	-
- Atrophy lymphoid	-	1	2	1
- Hyperplasia duct	-	1	-	-
THYMUS (males)	5	5	4	8
- Increased apoptosis	-	1	2	-
- Haemorrhage/Congestion	-	1	1	1
- Atrophy lymphoid	-	-	1	4

Summary of statistically significant changes in reproduction organ weights of males:

Organ weights	Dose [mg/kg bw/day]			
	Control animals	40	100	250/150
Testis, absolute [g]	3.31 ± 0.18	3.26 ± 0.31	1.86 ± 0.30**	1.46 ± 0.11**
Testis, relative [%]	1.02 ± 0.12	1.03 ± 0.07	0.62 ± 0.10**	0.51 ± 0.05**
Epididymis, absolute [g]	1.133 ± 0.106	1.104 ± 0.077	0.801 ± 0.091**	0.645 ± 0.068**
Epididymis, relative [%]	0.348 ± 0.043	0.349 ± 0.027	0.268 ± 0.040**	0.225 ± 0.027**
Seminal vesicles, absolute [g]	1.778 ± 0.222	1.460 ± 0.138	1.377 ± 0.201*	1.420 ± 0.213*
Seminal vesicles, relative [%]	0.543 ± 0.067	0.468 ± 0.049	0.471 ± 0.065	0.482 ± 0.065

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

Fertility effects were observed at all dose levels including increase pre-coital time of females treated at 40 mg/kg bw/day and 250/150 mg/kg bw/day and dose-related decreased fertility index: at 100 mg/kg bw/day reduction of fertility index (90% calculated as no. animals with implants/no. of mating x 100) and reduction of number of corpora lutea and implantation sites. In addition, a dose related decrease was noted for number of corpora lutea (control group: 14.5; 11.3 and 9.9 at 40 and 100 mg/kg bw/day, respectively) and implantation sites (control group: 11.1; 9.8 and 7.8 at 40 and 100 mg/kg bw/day, respectively). Steel's test significant at 1% level was observed only in "no. of corpora lutea" in the highest dose.

Summary of reproduction data is presented in the table below.

Parameter	**Dose [mg/kg bw/day]**			
	0	**40**	**100**	**250/150**
Mating index [%]	100	100	100	90
No. of females mated	10/10	10/10	10/10	9/10
Fertility index [%]	100	100	90	20
No. of implantation sites#	11.1 ± 2.0	9.8 ± 1.4	7.8 ± 4.3	3.5 ± 3.5
No. of *corpora lutea*#	14.5 ± 4.3	11.3 ± 1.8	9.9 ± 4.5	1.1 ± 3.0**
Duration of gestation [d]#	21.4 ± 0.5	23.1 ± 0.6	n.a.	n.a.
Conception index [%]	100	100	90	22.2
No. of pregnant females	10/10	10/10	9/10	2/10
No. of non-pregnant females	0/10	0/10	1/10	8/10
No. of females with live pups (day 1)	10/10	7/10	0/10	0/10
Gestation index [%]	100	70	0	0
Litter size	10	9	n.a.	n.a.

*/** Steel's test significant at 5% (*) or 1% (**) level, n.a. = not applicable; # mean value ± standard deviation

RAC concludes, that the available data on reproductive toxicity, dose-related fertility effects (increased precoital time, reduced fertility index, reduced number of corpora lutea

and implantation sites) at all dose levels, histopathological changes in the testis and epididymis at all dose levels and statistically significant changes in reproduction organ weights of males, represent clear evidence of adverse effects on sexual function and fertility.

Fertility effects were not considered to be secondary non-specific consequences to the high parental toxicity observed at 100 and 250/150 mg/kg bw/day (body weight loss, mortality) since they were present at 40 mg/kg bw/day, where no indication of marked general toxicity was observed.

The effects on fertility were also supported by data from the primary metabolite 2-methyoxyethanol.

The available animal data support classification for reproductive toxicity category 1B H360F. There is no information that the effects may not be relevant to human and the quality of the study is good, therefore, RAC considers that a classification as Repr. 1B; H360F "May damage fertility" is warranted.

Developmental toxicity

In the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test performed in rat, dose-related decrease in live births and viability index was observed at all dose tested.

The observed maternal effects included a body weight loss at 250 mg/kg bw/day in a few female, which slightly recovered during treatment at 150 mg/kg bw/day; a body weight loss at the end of post-coitum for females treated with 100 mg/kg bw/day; the death of one female on day 21 post-coitum at 100 mg/kg bw/day. It is noticed that at 40 mg/kg bw/day, where no indication of marked general maternal toxicity was observed, a decrease in live births and in viability index were observed. At 100 and 150/250 mg/kg bw/day, where high maternal toxicity occurred, no dam had live pups on day 1. Since limited maternal toxicity was reported in the low dose group, the effects on development is not considered to be a secondary non-specific consequence of maternal toxicity.

Summary of developmental data

	Dose [mg/kg bw/day]			
	0	**40**	**100**	**250/150**
Pub weight [g]#	6.0 ± 0.7	5.6 ± 0.5	n.p.	n.p.
Sex ratio [% males]	42	43	n.p.	n.p.
Viability index [%]	99	66.7**	n.p.	n.p.
Litter size	10	9	n.p.	n.p.
Dead pups at first litter check				
- Litters affected	0/10	6/10**	n.p.	n.p.
- Total	0	13	n.p.	n.p.
Living pups at first litter check	103	30	n.p.	n.p.
- Mean per litter	10.3 ± 2.4	3.3 ± 3.2++	n.p.	n.p.
Postnatal loss				
- Litters affected	1/10	1/9	n.p.	n.p.
- Total	1	10**	n.p.	n.p.
- % of living pups	1.0	33	n.p.	n.p.

*/** Fisher's Exact test significant at 5% (*) or 1% (**) level; +/++ Steel's test significant at 5% (+) or 1% (++) level; n.p. = no pups were born at 100 and 250/150 mg/kg bw/day; # mean values ± standard deviation.

In the opinion of RAC, due to marked developmental effects manifesting as dose-related

decreases in live births and viability index at all doses, as observed in an OECD guideline-compliant developmental screening study, 2-methoxyethyl acrylate was considered to meet the criteria for classification as Repr. 1B; H360D "May damage the unborn child". Since limited maternal toxicity was reported in the low dose group, the effects on development were not considered to be a secondary consequence of maternal toxicity.

The effects on development were also supported by data from the primary metabolite 2-methyoxyethanol.

There was no information that the effects could not be relevant for humans and therefore Repr. 2 was not considered appropriate.

Conclusion on fertility and development

The LOAEL for adverse effects on sexual function and fertility was 40 mg/kg bw/day based on:

- dose-related fertility effects (increase in precoital time, reduced fertility index, reduced number of corpora lutea and implantation sites) at all dose levels, but without clear dose-response relationships for all parameters - Steel's test statistically significant at 1% level was observed only at the highest dose,

- histopathological changes in the testis and epididymis at all dose levels (not statistically significant based on Fisher's Exact test at 5% or 1% level and on Steel's test at 5%) and

- statistically significant changes in reproductive organ weights of males (Dunnett's test).

Fertility effects were not considered to be secondary non-specific consequences at the high parental toxicity observed at 100 and 250/150 mg/kg bw/day (body weight loss, mortality) since at 40 mg/kg bw/day where the effects were also observed, no indication of marked general toxicity has been observed.

The LOAEL for developmental toxicity was 40 mg/kg bw/day based on statistically significant (Fisher's Exact test significant at 1% level) decreased live litters and decrease viability index.

In summary, the decreased live litters and viability index observed in the developmental screening study were considered sufficient effects for classification as Repr. 1B; H360D "May damage the unborn child", supported by evidence from the well documented reproductive toxicity data for the main metabolite, 2-methoxyethanol.In conclusion, based on marked fertility and developmental effects in animals, RAC is of the opinion that 2-methoxyethyl acrylate meets the criteria for classification as **Repr. 1B; H360FD "May damage fertility. May damage the unborn child".**

Specific concentration limit

No specific concentration limit could be set for 2-methoxyethyl acrylate based on the limited data available from the screening study OECD TG 422 as no ED_{10} (effective dose with a 10% effect level above the background) could be determined in the available screening study (Guidance p. 3.7.2.5.1.).

Adverse effects on or via lactation

There is no information to propose a classification for effects on or via lactation. In the reproductive screening toxicity study, no pups were born at 100 and 250/150 mg/kg

bw/day. Out of the nine litters at 40 mg/kg bw/day, only six had live pups. In addition, most of these pups did not survive the first days of lactation

10.11 Specific target organ toxicity-single exposure

Not evaluated.

10.12 Specific target organ toxicity-repeated exposure

In the screening 28-day study described in table 25, at 250 mg/kg bw/day, 2 males died on day 2 (no cause of death could be determined), 1 male was killed on day 8 (showed ulcerative inflammation in the stomach with resultant peritonitis) and at 100 mg/kg bw/day, one female was killed *in extremis* on day 21 *post-coitum* (Study report, 2012b).

In the prenatal developmental toxicity study in mouse (Hardin et al., 1987), a mortality rate of 30% was observed at 650 mg/kg bw per day.

Table 27: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Study report, 2012b	250	28-day	83 mg/kg bw	STOT RE 2
Hardin et al., 1987	650	8-day corresponding to exposure during GD 6-13	73 mg/kg bw	STOT RE 2

10.12.1 Comparison with the CLP criteria

According to Regulation (EC) No. 1272/2008 substances are classified as specific target organ toxicants following repeated exposure by the use of expert judgement on the basis of the weight of all available evidence. Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure are assigned to the STOT-RE categories.

Classification of 2-MEA as STOT RE 2 is justified by the following findings observed at dose values for STOT RE 2:

- In screening developmental toxicity studies in rats, after oral exposure during 1-month, high mortality of 30% was seen at doses of 250 mg/kg bw/d in males. These are within the guidance values of $30 < C \leq 300$ mg/kg bw for the 28 day repeated toxicity study for classification as STOT RE 2.
- In the prenatal developmental toxicity study in mice, a mortality rate of 30% was observed at 650 mg/kg bw per day. There are within guidance values of $100 < C \leq 1000$ mg/kg bw/d justifying classification as STOT RE 2.

2-MEA induces corrosive and acute effects. Furthermore, based on the hypothesized metabolism, it is not expected to be bioaccumulable. Furthermore, the factor between LD_{50} (404 mg/kg bw) and LOAEL (about 80 mg/kg bw/day) is about 5 supporting low cumulative potential. Moreover, lethality occurred during the 3 first days in the 28-day study suggesting that these effects are related to acute toxicity.

10.12.2 Conclusion on classification and labelling for STOT RE

Taking into account the low cumulative potential of 2-MEA, mortality observed in the sub-acute oral toxicity studies are considered to be related to acute toxicity. Thus, 2-MEA does not warrant classification as STOT RE for mortality.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS considered that 2-methoxyethyl acrylate does not warrant classification as STOT RE because in a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test in rats (Study report, 2012b) and a prenatal developmental toxicity gavage study in mice (Hardin et al., 1987), the results did not meet the classification criteria.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

There is quite limited information available on effects after repeated exposure to 2-methoxyethyl acrylate and no 90-day repeated-dose toxicity studies are available in the CLH report.

In the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422, GLP 1 - reliable without restriction) Wistar rats (10/sex/group) were exposed 7 days/week by gavage, 2 weeks prior to mating, during mating, and, for males, up to termination, for females, also during post-coitum at least 4 days of lactation. Dose levels were 0, 40, 100, 250/150mg/kg bw (250 mg from day 1 to 11; 150 mg from day 12 to study termination; dose reduced due to severe toxicity).

Sacrifice of all surviving males (after completion of the mating period) and females which delivered, on lactation days 5-7, and females which did not deliver on post-coitum days 25-27 (females with evidence of mating) or approximately 21 days after the last day of the mating period (females without evidence of mating).

Pronounced lethality of parental animals, about 30%, was observed at the beginning of exposure at 250 mg/kg bw/day (very close to acute oral LD_{50} of 404 mg/kg bw): two males died on day 2 and 1 male was killed on day 8 because of peritonitis. At 100 mg/kg bw/day: 1 female killed in extremis on day 21 post-coitum. Clinical signs in males and females found dead at both dose levels included hunched posture, piloerection, pale and lean appearance. Hepatocellular necrosis was observed, but only at the high dose level (250/150 mg/kg bw/day), i.e. very close to median lethal dose.

In addition, there were the following minimal or slight histopathological changes in the thymus in parental animals at all dose levels. In males, lymphoid cortical atrophy was

present in 1/4 at 100 mg/kg bw/day and 4/10 at 250/150 mg/kg bw/day. In females, these effects occurred in 1/5, 2/6 and 1/5 females at 40, 100 and 250 mg/kg bw/day, respectively. These changes were not statistically significant (Fisher's Exact test and the Steel's test was applied to frequency data).

Furthermore, corrosion in the forestomach was described in the Comet assay performed with 2-methoxyethyl acrylate on male rats after two days oral exposure to 240 mg/kg/day (see mutagenicity section below), but histological examination of the liver was not provided.

Taking to account all above available evidence, RAC considers that some level of toxicity after repeated dose exposure was observed, but not sufficientto fulfil the criteria for classification for repeated dose toxicity and therefore **no classification is warranted for STOT-RE**.

10.13 Aspiration hazard

Not evaluated.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated.

13 REFERENCES

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14 ANNEXES

See separated annex I file for detailed study summaries.