Committee for Risk Assessment

RAC

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

Adopted

9 March 2018

9 March 2018

CLH-O-0000001412-86-202/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **2-methoxyethyl acrylate**

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

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

The proposal was submitted by **France** and received by RAC on **28 February 2017.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

France has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **14 March2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **28 April2017**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Sławomir Czerczak**

Co-Rapporteur, appointed by RAC: **Bogusław Barański**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **9 March** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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 current 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	GHS02 GHS05 GHS06 GHS08 Dgr	H226 H302 H331 H314 H317 H341 H360FD	EUH071		
RAC opinion	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	GHS02 GHS05 GHS06 GHS08 Dgr	H226 H302 H331 H314 H317 H341 H360FD	EUH071	oral; ATE = 404 mg/kg bw inhalation; ATE = 2.7 mg/L	
Resulting Annex VI entry if agreed by COM	TBD	2-methoxyethyl acrylate	221-499-3	3121-61-7	Flam. Liq. 3 Muta. 2 Repr. 1B Acute Tox. 3 Acute Tox. 4 Skin Corr. 1C Eye Dam. 1 Skin Sens. 1	H226 H341 H360FD H331 H302 H314 H318 H317	GHS02 GHS05 GHS06 GHS08 Dgr	H226 H341 H360FD H331 H302 H314 H317	EUH071	oral; ATE = 404 mg/kg bw inhalation; ATE = 2.7 mg/L	

GROUNDS FOR ADOPTION OF THE OPINION

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.

HUMAN HEALTH HAZARD EVALUATION

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. 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. 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.

An 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)**.

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**.

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. 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.

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.

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.

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 sufficient to fulfil the criteria for classification for repeated dose toxicity and therefore **no classification is warranted for STOT RE**.

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. 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.

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.

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	-

Histopathological findings in reproduction organs/endocrine organs	**Dose [mg/kg bw/day]**			
	0	**40**	**100**	**250/150**
- 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

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).