Committee for Risk Assessment

RAC

Annex 1

Background document

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

2,3-epoxypropyl methacrylate; glycidyl methacrylate

EC Number: 203-441-9
CAS Number: 106-91-2

CLH-O-0000001412-86-96/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

4 December 2015

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: 2,3-epoxypropyl methacrylate (Glycidyl methacrylate, GMA)

EC Number: 203-441-9

CAS Number: 106-91-2

Index Number: 607-123-00-4

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 2,3-EPOXYPROPYL METHACRYLATE; GLYCIDYL METHACRYLATE

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Part A.

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***2,3-epoxypropyl methacrylate; glycidyl methacrylate***
EC number:	***203-441-9***
CAS number:	***106-91-2***
Annex VI Index number:	***607-123-00-4***
Degree of purity:	***>= 99.5 — <= 100.0 % (w/w)***
Impurities and additives:	***None relevant for classification***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	Acute Tox. 4* H302 Acute Tox. 4* H312 Acute Tox. 4* H332 Eye Irrit. 2 H319 Skin Irrit. 2 H315 Skin Sens. 1 H317 Note D applies (Annex VI, Part 1, section 1.1.3.1) The * indicates a minimum classification (Annex VI, Part 1, section 1.2.1)	Xn; R20/21/22 Xi; R36/38 R43 Note D applies (Annex VI, Part 1, section 1.1.3.1) Specific concentration limits: Xi; R36/38: C ≥ 10 %
Current proposal for consideration by RAC	Acute Tox. 4 H302 Acute Tox. 3 H311 No classification for Acute Tox inhalation Eye Dam. 1 H318 Skin Corr. 1C H314 Skin Sens. 1 H317 Repr. 1B H360F Muta. 2 H341	Not relevant seen the repealing of this Directive

	Carc. 1B H350 STOT SE 1 H370 (affected organs: respiratory tract; route of exposure: inhalation)	
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 4 H302 Acute Tox. 3 H311 Eye Dam. 1 H318 Skin Corr. 1C H314 Skin Sens. 1 H317 Repr. 1B H360F Muta. 2 H341 Carc. 1B H350 STOT SE 1 H370 (affected organs: respiratory tract; route of exposure: inhalation) Note D	

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3a: Classification and labelling according to CLP / GHS for physicochemical properties

CLP Annex I reference and Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification	Reason for no classification
2.1.Explosives:			None	Not assessed
2.2 Flammable gases:			None	Not assessed
2.3 Flammable aerosols:			None	Not assessed
2.4 Oxidising gases:			None	Not assessed
2.5 Gases under pressure:			None	Not assessed
2.6 Flammable liquids:			None	Not assessed
2.7 Flammable solids:			None	Not assessed
2.8 Self-reactive substances and mixtures:			None	Not assessed
2.9 Pyrophoric liquids:			None	Not assessed
2.10 Pyrophoric solids:			None	Not assessed
2.11 Self-heating substances and mixtures:			None	Not assessed
2.12 Substances and mixtures which in contact with water emit flammable gases:			None	Not assessed
2.13 Oxidising liquids:			None	Not assessed

2.14 Oxidising solids:			None	Not assessed
2.15 Organic peroxides:			None	Not assessed
2.16 Corrosive to metals:			None	Not assessed

Table 3b: Classification and labelling according to CLP / GHS for health hazards

CLP Annex I reference and Hazard class	**Proposed classification**	**Proposed SCLs and/or M-factors**	**Current classification**	**Reason for no classification**
3.1 Acute toxicity - oral:	Acute Tox. 4 (H302)	.	Acute Tox. 4* (H302)	
3.1 Acute toxicity - dermal:	Acute Tox. 3 (H311)		Acute Tox. 4* (H312)	
3.1 Acute toxicity - inhalation:			Acute Tox. 4* (H332)	conclusive but not sufficient for classification
3.2 Skin corrosion / irritation:	Skin Corr. 1C (H314)		Skin Irrit. 2 (H315)	
3.3 Serious eye damage / eye irritation:	Eye Dam. 1 (H318)		Eye Irrit. 2 (H319)	
3.4 Respiration sensitisation:			None	No data
3.4 Skin sensitisation:	Skin Sens. 1 (H317)	.	Skin Sens. 1 (H317)	
3.5 Germ cell mutagenicity:	Muta. 2 (H341)		None	
3.6 Carcino-genicity:	Carc. 1B (H350)		None	
3.7 Reproductive Toxicity:	Repr. 1B (H360F)		None	
3.7 Reproductive Toxicity: Effects on or	None		None	data lacking

via lactation:				
3.8 Specific target organ toxicity - single:	STOT SE 1 (H370) Affected organs: respiratory tract Route of exposure: Inhalation		None	
3.9 Specific target organ toxicity - repeated:	None		None	conclusive but not sufficient for classification
3.10 Aspiration hazard:	None		None	conclusive but not sufficient for classification

The * indicates a minimum classification (Annex VI, Part 1, section 1.2.1)

Table 3c: Classification and labelling according to CLP / GHS for environmental hazards

CLP Annex I reference and Hazard class	**Proposed classification**	**Proposed SCLs and/or M-factors**	**Current classification**	**Reason for no classification**
4.1 Hazards to the aquatic environment (acute/short-term):			None	Not assessed
4.1 Hazards to the aquatic environment (long-term):			None	Not assessed
5.1 Hazardous to the ozone layer:			None	Not assessed

Labelling

Signal word: Danger

Hazard pictograms:

GHS05: corrosion

GHS06: skull and crossbones

GHS08: health hazard

Hazard statements:

H302: Harmful if swallowed.
H311: Toxic in contact with skin.
H314: Causes severe skin burns and eye damage.
H317: May cause an allergic skin reaction.
H341: Suspected of causing genetic defects
H350: May cause cancer
H360F: May damage fertility
H370: Causes damage to the respiratory tract by inhalation.

Precautionary statements:

(as applied by the registrants but not relevant for harmonisation)

P201: Obtain special instructions before use.
P202: Do not handle until all safety precautions have been read and understood.
P260: Do not breathe dust/fume/gas/mist/vapours/spray.
P264: Wash hands thoroughly after handling.
P270: Do not eat, drink or smoke when using this product.
P272: Contaminated work clothing should not be allowed out of the workplace.
P363: Wash contaminated clothing before reuse.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P304+P340: IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P301+P330+P331: IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
P303+P361+P353: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P310: Immediately call a POISON CENTER or doctor/physician.
P333+P313: If skin irritation or rash occurs: Get medical advice/attention.
P321: Specific treatment (see on this label).
P322: Specific measures (see on this label).
P307+P311: IF exposed: Call a POISON CENTER or doctor/physician.
P308+P313: IF exposed or concerned: Get medical advice/attention.
P405: Store locked up.
P501: Dispose of contents/container in accordance with local and national regulations

Proposed notes assigned to an entry: Note D

BACKGROUND TO THE CLH PROPOSAL

1.4 History of the previous classification and labelling

Much history is not available to the current submitters, but the substance has had the same harmonised classification for many years, initially since 1991 under the DSD Directive, now under the CLP Regulation, based on information such as for instance summarised in the OECD SIDS Initial Assessment Report of 1999.

However since then new studies have become available which required a revision within the framework of the substance evaluation as performed by its SIEF. This revised classification is now part of the most recent update (Quarter2 2013) of the REACH Registration dossier and consequently also the harmonised classification should be updated.

This CLH proposal was requested by the Lead Registrant for the substance (Stadex Nederland BV) who acts on behalf of the current 11 members of the SIEF. The draft of the proposed revision was performed by a sub-set of the SIEF and consisted of representatives of DOW, DuPont, Mitsubishi Rayon and Stadex. The proposed changes of the classification are agreed by the dossier submitter (The Netherlands). The proposal is submitted by the Netherlands as it contains changes of the harmonised classification (article 37.6).

1.5 Short summary of the scientific justification for the CLH proposal

Toxicokinetics

Results indicate that glycidyl methacrylate (GMA) is rapidly metabolized *in vivo* in rabbits as measured by disappearance of parent material (95% of intravenous administered dose of 200 mg/kg eliminated within 10 minutes). Maximum blood levels of GMA were increased by 10-fold in rabbits co-administered a carboxylesterase inhibitor indicating that GMA was likely metabolized by carboxylesterase to glycidol and methacrylic acid. Domoradzki (2004) observed similar findings in rat, rabbit and human liver homogenate, and rat and rabbit respiratory tract epithelial cell fractions *in vitro*. Glycidol was identified as the metabolite of GMA in this study.

Metabolism of GMA to glycidol has ramifications for hazard identification. Glycidol has a harmonised classification according to CLP (entry 603-063-00-8) as carcinogenic (category 1B), germ cell mutagenic (category 2) and toxic to reproduction (category 1B).

Acute toxicity

- Acute toxicity oral Category 4 (H302) (the LD50 values of all available studies are between 300 and 2000 mg/kg bw)
- Acute toxicity dermal Category 3 (H311) (the actual LD50 value of 480 mg/kg bw lies between 200 and 1000 mg/kg bw)
- No classification for Acute toxicity inhalation is required since the LC0 in rats exposed for 4 hours lies at 2394 mg/m^3, the highest practically attainable vapour concentration and highest dose tested.

STOT SE

The findings in the acute toxicity inhalation studies do warrant a classification for Specific Target

Organ Toxicity, Single Exposure Category 1 (damage to the respiratory tract after inhalation) (H370). Local respiratory tract tissue injury is also expected given the corrosiveness seen on skin and eyes, while with repeated inhalation exposure degenerative effects occur at the portal of entry in both rats and rabbits at similar concentrations (around 10 to 60 mg/m^3). These effects seem concentration related and are best addressed via a STOT SE Category 1.

Skin corrosion

In a DOT standard test (equivalent to OECD Test Guideline 404, key study), moderate necrosis occurred by 4 hours exposure in 2 out of 6 animals but not 1-hour exposure (only very slight or superficial).

GMA was corrosive to the skin of rabbits. The 0.1 ml applied area showed red, swelled and blistered after one or two days, subdermal bleeding and ulcers after three days, and hard, thicker, cracked, pigmentation after five days of exposure. The pathological changes were degeneration and necrosis of surface skin cells, disappearance of cellular boundaries, displaying pink staining material, bleeding in the corium cells and lymph cell infiltration with accompanying formation of abscesses.

A single covered topical application to the skin of albino rabbits for four hours induced moderate to severe skin irritation including necrosis with slight to moderate oedema. A 10% solution (aqueous) produced slight redness and oedema after 1 application (for 4 hours) and a moderate burn after 2 applications.

According to the available studies GMA must be classified according to CLP as: Skin corrosive Category 1C (H314)

Eye damage

GMA induced slight to moderate conjunctivitis in the unwashed eye where slight corneal injury cleared in one week in a study resembling the OECD eye irritation study but which was very limitedly reported.

In inhalation study using rats, eye irritation was also induced. Acute exposure for 4 hours induced eye irritation at 1563 mg/m^3 and 2394 mg/m^3. Corneal opacity was also observed slightly at 610 mg/m^3, and moderately at 1563 mg/m^3 and 2394 mg/m^3. These changes did not heal within 14 days post-exposure. In sub-acute study, rats were exposed at 58.2, 223 and 931 mg/m^3, 6 hours/day, 5 days/week for 2 weeks. As a result, eye irritation and corneal clouding were observed at 931 mg/m^3.

As GMA is corrosive to the skin, GMA is also considered to induce serious eye damage (CLP chapter 3.3.2.3).

Skin sensitisation

The substance is a rapid and moderate sensitiser in guinea pigs and humans.

According to the available studies GMA must be classified according to CLP for Skin sensitisation Category 1 (H317) in view of the results found both in humans and animals.

Respiratory sensitisation

Respiratory sensitisation was not observed in any of the acute and sub-acute inhalation studies. However, these studies are not developed to determine the respiratory sensitising potential of substances.

Repeated dose toxicity

The major toxicity was tissue damages in the first exposure sites such as forestomach by oral administration and respiratory tract by inhalation, due to the corrosiveness of the substance and NOAELs were 30 mg/kg for rat oral (systemic effects), 10 mg/kg bw/day for rat oral (local effects), 12 mg/m^3 for rat inhalation (local effects) and 2.91 mg/m^3 for rabbit inhalation (local effects).

Based on the available studies and based on the other classifications already presented, no further classification of GMA for this endpoint is required under CLP.

Mutagenicity

Genotoxicity studies on GMA *in vitro* showed positive results. In micronucleus tests *in vivo*, oral administration of GMA increased the frequency of micronucleated polychromatic erythrocytes at the highest dose only, while mostly negative results were shown in other *in vivo* genotoxicity studies including micronucleus tests by intraperitoneal administration and including the gene mutation study using transgenic Big Blue Fischer 344 rats. However, *in vitro* and *in vivo* studies indicate carboxylesterase mediated hydrolysis of GMA to glycidol. Glycidol, a metabolite of GMA, is classified as a Category 2 germ cell mutagen under CLP. Based on the available studies for GMA itself and read across data for glycidol, GMA is considered to be a substance with mutagenic potential for somatic cells. There is only very limited information regarding germ cell mutagenicity in which only a slight and not dose-related increase in unscheduled DNA synthesis was observed in the germ cells. This is not sufficient for category 1B.

According to the available studies GMA must be classified according to CLP for Germ Cell Mutagenicity Category 2 (H341).

Carcinogenicity

There are reports on chronic exposure studies with GMA, but each one has significant methodological deficiencies such that the conclusion is that there are no acceptable chronic studies with GMA. In a very limited one-year study (Hadidian et al., 1968), rats (3 males and 3 females) were dosed 5 days/week by gavage at 0.1 mg/kg. Groups of 15 male and 15 female rats were also dosed at 0.3 mg/kg. The authors concluded that no tissue effects related to the treatment were found. However the doses applied are considered to be too low. There was also a 26-weeks inhalation toxicity study at concentrations of 15.3 and 206 mg/m^3 in rats and rabbits (Ouyang Guoshun et al., 1990). A wide range of toxic effects were observed in both species at both concentrations. However, because of the low purity of the material used, the author suggested that the effects may have been caused by the impurities present. Therefore it is questionable if the systemic toxicity was caused by GMA itself. Consequently a Read Across for GMA was used. Rationale: although the kinetics of carboxylesterase-mediated hydrolysis of GMA appear to be species dependent, the primary metabolite of GMA found in humans, rats and rabbits is glycidol (see chapter 4.1). In terms of Annex XI of REACH, this means the similarity is based on the formation of a breakdown product (a metabolite) of GMA that is a known carcinogen (Cat 1B). Chronic bioassay data were located for glycidol in rats and mice and show carcinogenicity.

According to the available studies on glycidol GMA must be classified according to CLP as Carcinogenic Category 1B (H350).

Reproductive toxicity

Oral toxicity study was performed on GMA in SD (Crj: CD) rats by an OECD combined repeated dose and reproductive/developmental toxicity screening test (OECD TG 422). Administration was conducted by gavage at doses of 10, 30 and 100 mg/kg/day from 14 days before mating to 14 days after mating in males and from 14 days before mating to day 3 of lactation in females. The fertility index (number of delivered animals/ number of mated animals) decreased significantly at 100 mg/kg.

Male mice injected i. p. with 5 consecutive daily doses of 0, 25, 50 or 100 mg/kg/day of GMA showed an increase in the percentage of abnormal sperm and decrease in the number of sperm. These results were confirmed in a subsequent study where mice were dosed i. p. with 0, 5, 25 or 100 mg/kg for five consecutive days. At 100 mg/kg, mice had decreased caudal epididymal weights, slightly lower testicular weights, decreased sperm counts and increased abnormal sperm. Mice given 25 mg/kg/day showed decreased sperm counts and increased abnormal sperm. These results might support the decreased fertility index of the rat study at 100 mg/kg/day.

Three reliable developmental studies by two different routes, oral and inhalation, indicated no teratogenicity even at the highest doses which showed maternal toxicity. The significant increase in fetal resorption was considered for classification for developmental toxicity. However, as this effect was not observed in the comparable OECD 422 study (same route and dose levels), only observed in the presence of maternal toxicity and the main metabolite glycidol has no classification for development, no classification is proposed.

However based on the available studies the substance must be classified under the CLP Regulation as Toxic to Reproduction Category 1B (H360F). In addition, glycidol, a metabolite of GMA, is classified as a Category 1B reproductive toxicant under CLP. Based on the available studies for GMA itself and read across data for glycidol, GMA is considered to be a reproductive toxicant. The relevant differentiation is effects on sexual function and fertility.

Other effects

There was no evidence of neurotoxic effects at any exposure level.

1.6 Current harmonised classification and labelling

1.6.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

- Acute Tox. 4* H302
- Acute Tox. 4* H312
- Acute Tox. 4* H332
- Eye Irrit. 2 H319
- Skin Irrit. 2 H315
- Skin Sens. 1

Note D applies (Annex VI, Part 1, section 1.1.3.1)

The * indicates a minimum classification (Annex VI, Part 1, section 1.2.1)

1.7 Current self-classification and labelling

1.7.1 Current self-classification and labelling based on the CLP Regulation criteria

Self-classification as applied by the registrants

- Acute Tox. 4 oral
- Acute Tox. 3 dermal
- No classification for Acute Tox inhalation but instead STOT SE 1
- STOT SE 1 (affected organs: respiratory tract; route of exposure: inhalation)
- Eye Dam. 1
- Skin Corr. 1C
- Skin Sens. 1
- Repr. 1B H360F
- Muta. 2
- Carc. 1B

Note D applies (Annex VI, Part 1, section 1.1.3.1)

Pictogram and signal word: GHS05, GHS06 and GHS08 and "Danger"

See table 3a/3b/3c for further details

RAC general comment

The proposed classification of 2,3-epoxypropyl methacrylate or glycidyl methacrylate (GMA, used throughout this opinion) is partly based on glycidol, the *in vivo* metabolite of GMA. Its relevance for the hazardous properties of GMA is discussed below.

The toxicokinetic behaviour of GMA was investigated in rabbits. After an intravenous injection of 200 mg/kg bw, over 95% of the parent compound disappeared from the blood within 10 minutes. Following a subcutaneous injection at 800 mg/kg bw, the toxicokinetics appeared to fit with a one-compartment, open model with a first-order absorption. Subcutaneous co-administration of a carboxylesterase inhibitor resulted in about ten-fold higher maximum blood concentrations of GMA. The substance was metabolised in a first order process when incubated *in vitro* with whole blood, plasma, erythrocyte suspension and homogenates of various tissues (Shi Tao *et al.,* 1988).

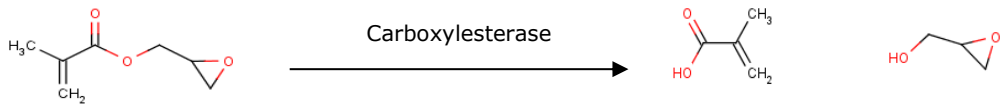
In vitro incubations of ^{14}C-GMA with nasal epithelial tissue preparations and liver homogenates from human, rat and rabbit resulted in all cases in the formation of only one metabolite tentatively identified as glycidol (Domoradzki, 2004). At an initial concentration of 2 mM of GMA, the half-live of GMA (via hydrolysis) was shorter in incubations with rat and rabbit tissues as compared to human tissues (biotransformation of GMA in liver homogenates was completed within approximately 30 minutes versus 2 hours, respectively).

Although the metabolic transformation is expected to be slower in humans than in rodents, GMA is expected to transform completely into glycidol and methacrylic acid (MAA) in rodents as well as in humans.

No metabolite resulting from the action of epoxide hydrolase was identified *in vitro*. Epoxide hydrolase was hypothesised to produce glycerol methacrylate from GMA as an alternative pathway to the action of carboxylesterase. Epoxide hydrolase has been shown to be active in liver, kidney and lung tissues of 9 tested species including man and rodents (Pacifici, 1991). However, the absence of corresponding GMA metabolites after

incubation with rat, rabbit or human liver tissues *in vitro*, provides evidence that the carboxylesterase pathway overrides the hypothetical epoxide hydrolase pathway for metabolism of GMA.

Figure 1: Proposed metabolism of GMA in mammals



GMA Methacrylic acid + glycidol

In addition, data were provided by the DS in order to compare the toxicological profile of GMA with glycidol and to support the use of glycidol data for the assessment of GMA. Relevant data for both GMA and glycidol are summarised in the table below. Only data/studies considered as key with regard to reliability and their relevance for the comparison of GMA with glyciod, are included in the table.

Status/endpoint	Glycidol	GMA
Harmonised classification for local effects	Skin Irrit. 2 – H315 Eye Irrit. 2 – H319 STOT SE 3 – H335	Skin Corr. 1C – H314* STOT SE 3 – H335* *Agreed by RAC, resulting in the future Annex VI entry if adopted by COM.
Repeated dose toxicity – oral	90-day study – gavage –rats (Irwin *et al.*, 1990) ≥ 100 mg/kg bw/day: decrease in sperm count and motility ≥ 200 mg/kg bw/day: mortality 400 mg/kg: complete mortality by week 2. Cerebellum necrosis, demyelination in brain medulla, tubular degeneration and/or kidney necrosis, thymus lymphoid necrosis, testicular atrophy and/or degeneration 90-day study – gavage – mice (Irwin *et al.*, 1990) ≥ 19 mg/kg bw/day: decrease in sperm count and motility; testicular atrophy ≥ 150 mg/kg bw/day: mortality; decreased body weight; brain demyelination 300 mg/kg bw/day: complete mortality by week 2; renal tubular cell degeneration	45-day study – gavage – rats (MHWJ[1], 1997) ≥ 30 mg/kg bw/day: salivation and squamous hyperplasia and cell infiltration in the forestomach in males 100 mg/kg bw/day: increase in kidney and adrenal weights in males and cell infiltration in the forestomach of females. Decrease in fertility index assumed to be due to decreased sperm motility.

[1] MHWJ: Ministry of Health and Welfare, Japan

Repeated dose toxicity – inhalation	50-day study – rats (Hine *et al,* 1956) 1.2 mg/L (single dose): 1/10 death of bronchopneumonia. Very slight irritation of the eyes, with slight lacrimation and encrustation of the eyelids and slight respiratory distress following the first few exposures. No significant gross or microscopic lesions	14-day study – rats (Landry *et al.*, 1991) 0.06 mg/L: very slight multifocal necrosis of respiratory epithelium in the nasal cavity 0.23 mg/L: slight to moderate multifocal necrosis and inflammation of the respiratory and olfactory nasal epithelium 0.93 mg/L: animals sacrificed at day 4: severe necrosis and inflammation in the nasal cavity. General debilitation with noisy and difficult respiration, eye irritation, corneal clouding and distended abdomen ≥ 0.23 mg/L: decreased body weight 14-day study – rabbits (Cieszlak *et al.*, 1996) 0.012 mg/L: degeneration of the nasal olfactory epithelium (reversible) ≥ 0.029 mg/L: olfactory epithelial degeneration, hyperplasia, erosions, ulcers and inflammation of the nasal epithelium (not fully reversible) 90-day study –rats (Landry *et al.*, 1996) ≥ 0.09 mg/L: hyperplasia of respiratory epithelium of nasal cavity in all animals
Mutagenicity	Positive micronucleus assay in mice after two intraperitoneal (IP) injections: 3 times higher micronuclei incidence in high dose group (150 mg/kg bw) vs controls (Irwin *et al*, NTP, 1990) Harmonised classification Muta. 2 – H341	Several positive assays *in vitro* Positive micronucleus assay in mice after a single gavage dose of 750/1000 mg/kg bw (MHWJ, 1997) Negative micronucleus assay in mice after single IP injection up to 300 mg/kg bw (Lick *et al.*, 1995)
Carcinogenicity	Induction of benign and malignant tumours in multiple organs in rats and mice of both sexes (Irwin *et al.*, NTP, 1990). Harmonised classification Carc. 1B; H350	No data
Reproductive toxicity	12-day study – oral – rats (Hahn, 1970) 15 mg/kg bw/day: infertility (reversible within 1 week) 90-day study – gavage – rats (Irwin *et al.*, 1990) ≥ 100 mg/kg bw/day: decrease in sperm count and motility 400 mg/kg bw/day: testicular atrophy and/or degeneration (general toxicity described above) 90-day study – gavage – mice (Irwin *et al.*, 1990) ≥ 19 mg/kg bw/day (lowest dose): decrease in sperm count and motility;	OECD TG 422 (45 days) – gavage – rats (MHWJ, 1997) 100 mg/kg bw/day: decrease in fertility index assumed to be due to decreased sperm motility

	testicular atrophy (general toxicity described above) Harmonised classification Repr. 1B – H360F	

A comparison of the toxicological profiles shows that GMA is skin corrosive whereas glycidol is irritant to the skin. In addition, GMA induces local toxicity by the oral and inhalation route that is either not observed with glycidol or is observed at higher doses and with less severity than with glycidol. This finding is consistent with the hydrolysis of GMA to glycidol and methacrylic acid by carboxylesterase at the site of contact. Local transformation of GMA to methacrylic acid is expected to induce corrosive lesions, with a severity which is proportionate to the relative tissue and species-specific carboxylesterase activity.

Due to the difference in local toxicity, the maximum doses that can be administered to animals are lower for GMA than for glycidol. This may explain why the systemic effects that are observed at the highest doses after repeated exposure to glycidol by the oral route (severe effects on brain, kidney, thymus) are not observed with GMA. The effect of glycidol on male reproductive function appears to be a sensitive adverse effect of glycidol which is observed at the LOAEL in both rats and mice (Irwin *et al.*,1990). Comparable effects are reported with GMA in studies investigating fertility.

Similarly, both glycidol and GMA induce mutagenicity. *In vivo*, the effect was clearly identified at high doses (high considering the respective toxicity and route of administration used) for both compounds. In particular, the induction of micronuclei in erythrocytes of GMA via the oral route provided support to the assumption that glycidol is formed from GMA.

Taken together, similar effects of GMA and glycidol as evidenced by alteration of male fertility and genotoxicity support the conclusion that glycidol is formed in vivo from GMA, as also evidenced by the toxicokinetic data.

RAC notes that the toxicity of MAA has not been further discussed by the DS in the CLH dossier. However, on the basis of available in vitro and in vivo data, considering the extensive hydrolysis of GMA into glycidol and the consistency of several systemic effects, GMA is generally expected to produce similar systemic effects as glycidol. This applies in particular to the most sensitive systemic effects of glycidol whereas the the corrosivity of GMA may prevent the possibility to reach high systemic doses of glycidol as a result of GMA metabolism. Glycidol is only considered to be irritant to skin.

2 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The current harmonised classification is deemed by the registrants and the dossier submitter to be inadequate since:

- A change in an existing entry is considered justified due to new data becoming available after the current harmonised classification was agreed
- The classification as CMR substance is entirely missing
- The classifications for eye damage/irritation and for skin corrosion/irritation should become more strict (for eye from Cat. 2 to Cat. 1; for skin from Cat. 2 to Cat. 1C)
- The classification for acute dermal toxicity should become more strict (from Cat. 4 to Cat. 3)

- The classification for acute inhalation toxicity (Cat. 4) should be replaced by a STOT SE 1 (affected organs: respiratory tract; route of exposure: inhalation)

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

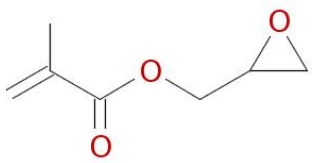
1.1 Name and other identifiers of the substance

The substance **2,3-epoxypropyl methacrylate** (GMA) is a mono constituent substance (origin: organic) having the following characteristics:

Table 4: Substance identity

EC number:	203-441-9
EC name:	2,3-epoxypropyl methacrylate
CAS number (EC inventory):	106-91-2
CAS number:	106-91-2
CAS name:	glycidyl methacrylate
IUPAC name:	oxiran-2-ylmethyl 2-methylprop-2-enoate
CLP Annex VI Index number:	607-123-00-4
Molecular formula:	C7H10O3
Molecular weight range:	142.1525

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
2,3-epoxypropyl methacrylate EC no.: 203-441-9	ca. 99.7 % (w/w)	>= 99.5 — <= 100.0 % (w/w)	

Current Annex VI entry: see chapter 1.7.1

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Water	<= 0.15 % (w/w)	>= 0.0 — <= 0.15 % (w/w)	impurity from the synthesis
sodium chloride EC no.: 231-598-3	<= 0.35 % (w/w)	>= 0.0 — <= 0.35 % (w/w)	impurity from the synthesis

Current Annex VI entry: none

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
Mequinol EC no.: 205-769-8	stabiliser	ca. 0.01 % (w/w)	>= 0.0050 — <= 0.015 % (w/w)	stabilizer to prevent hazardous polymerisation

Current Annex VI entry: 604-044-00-7:

Acute Tox. 4* H302

Skin Sens. 1 H317

Eye Irrit. 2 H319

1.2.1 Composition of test material

Information on the test material used in the different physic-chemical studies is given in each chapter when available while the information on the test material used in the toxicological studies is provided below for a global overview:

Table 8: Purity summary (blank if not available)

Study	Reference	Purity (%)
Toxicokinetic studies		
Intra-venous and subcutaneous (Rabbit)	Shi Tao et al. (1988)	
In vitro (rat, rabbit, human)	Domoradzki et al. (2004)	
Other	Bogdanffy (1987)	
	Dahl (1987)	
	Glatt (1984)	
	Mattes (1992)	
	Pacifici (1981)	
Acute toxicity studies		
Oral (rat, mouse, guinea pig)	Zdravko (1985)	
Oral (rat)	Olson (1960)	
Oral (rat, mouse)	Smyth (1969)	
Oral (rat)	EPA (1992)	97.8%
Inhalation (rat)	Nitschke (1990)	99.8%
Inhalation (rat) Dermal (rabbit)	Smyth (1969)	
Inhalation (rat, rabbit, guinea pig, dog)	Haag (1953)	
Intraperitoneal (rat, mouse)	Petrov (1973)	
Skin and/or eye irritation		
Skin (rabbit)	Ou-Yang (1988)	92%
Skin (rabbit)	Lockwood (1991)	
Skin and eye (rabbit)	Olson (1960)	
Skin (rabbit)	Smyth (1969)	

Table 8 cont: Purity summary (blank if not available)

Study	Reference	Purity (%)
Skin sensitisation studies		
Guinea pig	Dow (1990)	
Guinea pig	Ou-Yang (1988)	92%
Guinea pig	BIBRA (1988)	
Human	Dempsey (1982)	
Human	Matura (1995)	
Repeated dose toxicity		
Gavage (rat)	Ministry of Health and Welfare, Japan (1997)	99.93%
Oral (rat)	Hadidian (1968)	
Oral (rabbit)	Ou-Yang (1988)	92%
Inhalation (rat)	Landry (1996)	99.5%
Inhalation (rat)	Landry (1991)	99.61%
Inhalation (rat)	DuPont (1977)	
Inhalation (rat, rabbit)	Ouyang Guoshun (1990)	92%
Inhalation (rabbit)	Cieszlak (1996)	
In vitro mutagenicity studies		
	Ou-yang (1988)	92%
	Voogd (1981)	92%
	Linscombe (1995)	99.5%
	Ministry of Health and Welfare, Japan (1997)	99.93%
	Hude (1990)	97%
	Dorothy (1986)	98%
	Goodyear (1981)	
	Xie (1990a)	

Table 8: Purity summary (blank if not available)

Study	Reference	Purity (%)
	Hude (1991)	97%
	Xie (1990b)	
	Xie (1989)	
	Yang (1996)	
	Xie (1992)	
In vivo mutagenicity studies		
Intraperitoneal (mouse micronucleus assay)	Irwin (1990)	94%
Gavage (mouse micronucleus assay)	Ministry of Health and Welfare, Japan (1997)	99.93%
Inhalation (transgenic rodent mutation assay)	Gollapudi et al. (1999)	
Intraperitoneal (mouse micronucleus assay)	Ou-Yang (1988)	92%
Intraperitoneal (mouse micronucleus assay)	Lick (1995)	99.5%
Intraperitoneal (mouse micronucleus assay)	INBIFO (1979)	
(unscheduled DNA synthesis)	Xie (1990b)	
Carcinogenicity		
Gavage (rat)	Irwin et al. (1990)	94%
Reproductive toxicity studies		
Gavage (rat)	Ministry of Health and Welfare, Japan (1997)	99.93%
Intraperitoneal (mice)	Vedula (1994)	
Inhalation (rabbit)	Vedula (1995)	
Gavage (rat)	Ou-Yang (1988)	92%
Inhalation (rabbit)	Vedula (1996)	99.5%
Neurotoxicity studies		

Inhalation (rat)	Mattsson (1996)	

The available information regarding the purity of the tested substance is limited with many studies for which the purity could not be retrieved. Also for several studies the applied test substance had a purity clearly below the purity as put on the market. Some test batches (example acute oral study EPA/|OTS, 1992) contain epichlorohydrin and dichlorohydrin as impurities. Epichlorohydrin has a harmonised clasification as Carc. 1B and may also show mutagenic properties seen the presence of an epoxy group. Also dichlorohydrin is classified as Carc. 1B. The presence of these impurities may affect the results of the tests especially for mutagenicity and carcinogenicity. As the impurities were not known in many studies this could potentially make the test results less reliable. However, as indicated in the relevant summaries, most key studies were performed with a test substance with a high purity considered relevant for the substance as put on the market. Therefore, the test results are considered relevant for the substance as put on the market.

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Liquid	U.S. Coast Guard, Department of Transportation. CHRIS - Hazardous Chemical Data. Volume II. Washington, D.C.: U.S. Government Printing Office, 1984-5.	Measured
Melting/freezing point	-41.5 °C at 101.3 kPa	Lewis, R.J., Sr (Ed.). Hawley's Condensed Chemical Dictionary. 12th ed. New York, NY: Van Nostrand Rheinhold Co., 1993, p. 569	Measured
Boiling point	189 °C at 101.3 kPa	Dean, J.A. Handbook of Organic Chemistry. New York, NY: McGraw-Hill Book Co., 1987., p. 1-226	Measured
Relative density	1.07 at 20°C	Lide, D.R. (ed.). CRC Handbook of Chemistry and Physics. 76th ed. Boca Raton, FL: CRC Press Inc., 1995-1996., p. 3-291	Measured
Vapour pressure	420 Pa at 25 °C	OECD SIDS for Glycidyl methacrylate, 1999 (study by MITI, Japan)	Measured (98.2% purity)
Surface tension	25 mN/m at 20°C	U.S. Coast Guard, Department of Transportation. CHRIS - Hazardous Chemical Data. Volume II. Washington, D.C.: U.S. Government Printing Office, 1984-5.	Surface tension was measured for undiluted liquid
Water solubility	50 g/L at 25 °C	OECD SIDS for Glycidyl methacrylate, 1999 (study by MITI, Japan)	Measured

Partition coefficient n-octanol/water	Log Kow (Pow): 0.96 at 25 °C	OECD SIDS for Glycidyl methacrylate, 1999 (study by MITI, Japan)	Measured (98.2% purity)
Flash point	76 °C at 1013 hPa	Kirk-Othmer Encyclopedia of Chemical Technology. 3rd ed., Volumes 1-26. New York, NY: John Wiley and Sons, 1978-1984., p. 15(81) 347	Closed cup (open cup was 84 degrees C)
Flammability	Flammable		Estimated. With a flashpoint of 76 (closed cup) – 84 (open cup) deg C (170 – 185 deg F) and a low volatility (vapour pressure of 4.2 x 100 Pa @ 25 deg C) a separate flammability test is not needed. GMA is a combustible liquid class IIIA and should be kept away from heat, sparks, flame and any source of ignition.
Explosive properties	Non explosive		There are no chemical groups associated with explosive properties in the molecule
Self-ignition temperature	389 °C at 1013 hPa	Seamanship Hazardous Chemical Database version 4.2.29	Measured
Oxidising properties	None		This substance is incapable of reacting exothermally with combustible materials on the basis of its chemical structure
Granulometry			The particle size distribution cannot be measured as the substance is a liquid.
Stability in organic solvents and identity of relevant degradation products			The stability of the substance in organic solvents is not considered to be critical.
Dissociation constant			It is scientifically not possible to perform the test (the substance is neither an acid nor a base).
Viscosity	Viscosity at 21°C: 5.481 mPa · s (dynamic)	U.S. Coast Guard, Department of Transportation. CHRIS - Hazardous Chemical Data. Volume II. Washington, D.C.: U.S. Government Printing Office, 1984-5.	Measured

2 MANUFACTURE AND USES

2.1 Manufacture

This substance is produced, handled and used in closed system under very well controlled conditions as a monomer unit to produce polymers or as an intermediate to produce further non-polymeric chemical products. The substance is not included in consumer products.

2.2 Identified uses

Only to be handled and used under very well controlled conditions in closed systems as industrial feedstock for further synthesis.

The substance is only approved and registered for a very limited number of industrial uses (PROC1/2/3/4/8b/9/15 and ERC 1/2/6a/6c/6d). Professional and consumer uses are advised against.

PROC1	*Use in closed process, no likelihood of exposure*
PROC2	*Use in closed, continuous process with occasional controlled exposure*
PROC3	*Use in closed batch process (synthesis or formulation)*
PROC4	*Use in batch and other process (synthesis) where opportunity for exposure arises*
PROC8b	*Transfer of chemicals from/to vessels/large containers at dedicated facilities*
PROC9	*Transfer of chemicals into small containers (dedicated filling line)*
PROC15	*Use as laboratory reagent*
ERC1	*manufacture*
ERC2	*formulation*
ERC6a	*Industrial use as intermediate for further processing (not under strictly controlled conditions)*
ERC6c	*Industrial use of monomers for manufacture of thermoplastics*
ERC6d	*Industrial use of process regulators for polymerisation processes in production of resins, rubbers, polymers*

Note that all PROCs can be combined with all ERCs

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Not relevant	Not relevant	Not relevant	Not relevant

3.1 *Hazard classes for physico-chemical properties*

Not relevant for the substance

3.1.1 Summary and discussion of classification for physico-chemical properties

Not relevant for the substance

3.1.2 Comparison with criteria

Not relevant for the substance

3.1.3 Conclusions on classification and labelling

Note D is warranted (see Table 8) because GMA contains a stabiliser (polymerisation inhibitor) to prevent spontaneous polymerisation.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The results of studies on absorption, metabolism, distribution and elimination are summarised in the following table:

Table 11a: Summary of toxicokinetic studies

Method	Results	Remarks	Reference
Rabbit intra-venous and subcutaneous Doses/conc.: intravenous: 200 mg/kg subcutaneous: 800 mg/kg		2 (reliable with restrictions) key study experimental result **Test material: GMA**	Shi Tao et al. (1988) OECD (1999)
in vitro study	Main ADME results:	2 (reliable with	Domoradzki et al.

Method	Results	Remarks	Reference
rat, rabbit and human direct addition to liver homogenate and nasal epithelial tissue Exposure regime: 6 hours Doses/conc.: 2 mM and 40 mM no data	metabolism: Under all circumstances within 30-120 minutes only one metabolite appeared, tentatively identified as glycidol Metabolites identified: yes Details on metabolites: Only glycidol (EINECS 209-128-3) At an initial starting concentration of GMA at 2 mM, half-lives of GMA hydrolysis were faster in incubations with rat and rabbit (30 min) tissue in comparison to humans (2 hrs). Carboxylesterease activity was lower in humans than in rats and rabbits.	restrictions) key study experimental result **Test material: GMA** Form: liquid	(2004)
in vitro study	General information on the distribution of carboxylesterase and epoxy hydratase in different tissues and species.	2 (reliable with restrictions) supporting study experimental result **Test material: GMA**	Bogdanffy (1987) Dahl (1987) Glatt (1984) Mattes (1992) Pacifici (1981) OECD (1999)

Key study summaries

Shi Tao et al. (1988)

Toxicokinetics of GMA was investigated in rabbits. After an intravenous injection at 200 mg/kg, the concentration-time curve of this substance could exactly fit the two-compartment open model, and over 95 % of the parent compound had disappeared from the blood within 10 minutes. Following a subcutaneous injection at 800 mg/kg, the toxicokinetics appeared to fit a first-order absorption one-compartment open model. This substance was metabolized by a first-order process in incubation with whole blood, plasma, erythrocyte suspension, and homogenates of brain, heart, liver, lung, spleen, kidney, small intestine, and muscle. The highest rate of elimination had been found in blood and liver homogenate. The subcutaneous co-administration of tri-o-cresyl-phosphate (a carboxylesterase inhibitor) with this substance resulted in about a tenfold increase in the maximum blood concentrations of this substance, compared to those of animals dosed with this substance alone. *In vitro*, metabolism rate could be also decreased by tri-o-cresyl phosphate.

Domoradzki et al. (2004)

The purpose of this investigation was to study the metabolism of GMA *in vitro* using tissues from humans, rats, and rabbits. Differences in carboxylesterase and epoxide hydrolase activities in tissues from these species may result in differences in formation of glycidol, methacrylic acid, GMA-diol and glycerol. Results from this study may provide a basis to judge the relative sensitivity of humans to rabbits and rats for the generation of toxic effects with GMA.

In vitro incubations of GMA with nasal tissue preparations and liver homogenate from human, rat and rabbit resulted in the formation of only one metabolite. This metabolite was tentatively identified as glycidol based on retention time match with ^{14}C glycidol. No other metabolites were found. At an initial starting concentration of GMA at 2 mM, half-lives of GMA hydrolysis were faster in incubations with rat and rabbit tissue than with human tissue (completed within 30 minutes for rat and rabbit versus 2 hours for humans). At a concentration of 2 mM, GMA metabolism in liver from the fastest to the slowest was rabbit >rat>>human (Table 11b). Even though cellular fractions were not tested for epoxide hydrolase activity, carboxylesterase activities using 4-nitrophenyl acetate as a substrate were measured in human, rat and rabbit nasal tissue homogenates and human nasal tissues used in this study were found to be lower in this activity than in the other species (Table 11c). Based on *in vitro* results obtained in this study, it appears that GMA is principally metabolized to one metabolite (glycidol) and that glycerol and GMA-diol were not formed. Since glycerol or GMA-diol were not observed as metabolites, it appears that epoxide hydrolysis was not a major *in vitro* route of metabolism for GMA using rat, rabbit and human tissue homogenate preparations in the incubations, although epoxide hydrolase activity in these preparations was not determined.

Figure 1: Carboxylesterase activity in nasal tissues (Domoradzki et al. 2004)

Carboxylesterase Activity in Nasal (Respiratory and Olfactory) Tissue of Human, Rabbit and Rat Using the Substrate, 4-Nitrophenyl Acetate.

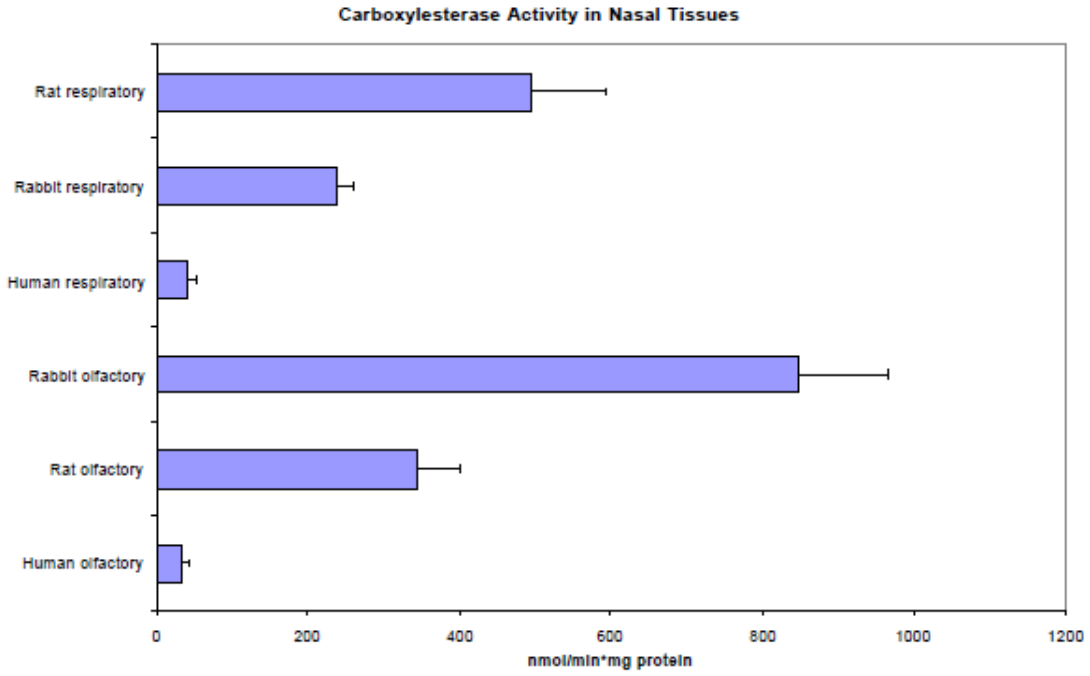


Table 11b:Metabolism of GMA in nasal epithelium and liver (Domoradzki et al. 2004)

METABOLISM OF GLYCIDYL METHACRYLATE IN NASAL EPITHELIUM AND LIVER OF FISCHER 344 RATS, NEW ZEALAND RABBITS, AND HUMANS

Table 1. Glycidyl Methacrylate and a Metabolite Present in *In Vitro* Preparations of Human, Rabbit and Rat Liver Incubated for Varying Times Following the Addition of ^{14}C-Glycidyl Methacrylate at a 2 mM Concentration.

Sample Name	Incubation Time (minutes)	Metabolite[a]	GMA[a]
Rat Liver Control (Denatured)[c]	60	0	100
Rat Liver	1	4	96
	2	10	90
	5	26	74
	10	43	57
	30	89	11
	45	97	3
	60	99	1
Rabbit Liver Control (Thermal)[b]	60	0	100
Rabbit Liver Control (Denatured)[c]	60	0	100
Rabbit Liver	1	10	90
	2	22	78
	5	55	45
	10	78	22
	30	98	2
	45	100	0
	60	100	0
Human Liver Control (Thermal)[b]	360	0	100
Human Liver Control (Denatured)[c]	360	NA[e]	NA[e]
Human Liver 221981	1	2	98
	2	2	98
	5	5	95
	10	9	91
	30	21	79
	45	25	75
	60	39	61
	120	79	21
	240	87	13
	360	85	15
Human Liver Control (Denatured)[c]	360	0	100
Human Liver 624971	1	3	97
	2	4	96
	5	8	92
	10	19	81
	30	36	64
	45	51	49
	60	68	32
	120	96	4
	240	99	1
	360	99	1

[a] Data are peak area percent response of ^{14}C-RAM
[b] ^{14}C-GMA in buffer at 37 degrees centigrade for 60 minutes
[c] denatured by heating at 100°C for 10 minutes and then incubated at 37°C for 60 minutes
[d] sample lost
[e] not applicable

Table 11c: Carboxylesterase activity measure (Domoradzki et al. 2004)

METABOLISM OF GLYCIDYL METHACRYLATEIN NASAL EPITHELIUM AND LIVER OF FISCHER 344 RATS, NEW ZEALAND RABBITS AND HUMANS

Table 11. Carboxylesterase Activity Measured with the Substrate, 4-Nitrophenyl Acetate, in Rat, Rabbit, and Human Nasal Tissues

Tissue Homogenate	mg protein	mOD/min	OD/min	nmoles/min	nmol/min*mg protein	Mean	SD
Rat respiratory	0.001178	30.2	0.0302	0.46037	390.803	493.602	98.08
		49.43	0.04943	0.75351	639.649		
	0.003534	136.12	0.13612	2.07500	587.153		
		110.42	0.11042	1.68323	476.296		
	0.00589	168.48	0.16848	2.56829	436.043		
		166.79	0.16679	2.54253	431.669		
Rat olfactory	0.003095	55.69	0.05569	0.84893	274.292	343.899	56.28
		89.81	0.08981	1.36905	442.344		
	0.009285	205.31	0.20531	3.12973	337.073		
		215.49	0.21549	3.28491	353.787		
	0.015475	314.69	0.31469	4.79710	309.991		
		351.15	0.35115	5.35290	345.906		
Rabbit respiratory	0.00393	67.51	0.06751	1.02912	261.862	237.146	23.70
		68.71	0.06871	1.04741	266.516		
	0.01179	171.46	0.17146	2.61372	221.690		
		188.68	0.18868	2.87622	243.954		
	0.01965	272.61	0.27261	4.15564	211.483		
		280.2	0.2802	4.27134	217.371		
Rabbit olfactory	0.003946	265.67	0.26567	4.04985	1026.317	846.955	120.13
		216.41	0.21641	3.29893	836.019		
	0.011838	682.14	0.68214	10.39848	878.398		
		702.48	0.70248	10.70854	904.590		
	0.01973	884.71	0.88471	13.48643	683.550		
		974.41	0.97441	14.85381	752.854		
Human respiratory	0.03069	90.666	0.090666	1.38210	45.034	41.727	13.00
		128.198	0.128198	1.95424	63.677		
	0.06138	115.981	0.115981	1.76800	28.804		
		180.364	0.180364	2.74945	44.794		
	0.09207	237.876	0.237876	3.62616	39.385		
		173.134	0.173134	2.63924	28.666		
Human olfactory	0.00298	8.629	0.008629	0.13154	44.141	32.802	11.00
		9.714	0.009714	0.14808	49.691		
	0.00596	7.014	0.007014	0.10692	17.940		
		8.094	0.008094	0.12338	20.702		
	0.00894	21.511	0.021511	0.32791	36.679		
		21.355	0.021355	0.32553	36.413		
	0.0149	27.751	0.027751	0.42303	28.392		
		27.819	0.027819	0.42407	28.461		

Supporting studies

Bogdanffy, 1987
Inhalation exposure of rats and mice to glycol ether acetates and acrylate esters causes degeneration of the olfactory epithelium but not of the respiratory epithelium. Since these compounds are metabolized via carboxylesterase to acids that are toxic to the olfactory epithelium, the activity and cellular distribution of carboxylesterase in the nasal passages of rats and mice were studied. Olfactory mucosal carboxylesterase in both rats and mice was found to have a Vmax value for the hydrolysis of p-nitrophenyl butyrate approximately 3 to 6 times larger than that for respiratory mucosa. Similarly, the second-order rate constant for binding and catalysis, was approximately four

times greater in olfactory mucosa than in respiratory mucosa of both rats and mice. These data demonstrate that the olfactory mucosa of rats and mice hydrolyze carboxylesters more efficiently than the respiratory mucosae. Enzyme histochemistry was employed to identify the individual cells within the respiratory and olfactory mucosae which contain carboxylesterase activity. All cell types of the respiratory epithelium had some carboxylesterase activity, with varying intensities between individual cell populations. Ciliated and cuboidal epithelial cells were most active in this region. In the olfactory mucosa, however, Bowman's glands stained most intensely, sustentacular cells demonstrated moderate activity, and no activity was detectable in olfactory sensory cells. Together, these data quantitate carboxylesterase activity in nasal mucosal homogenates and localize the enzyme in individual cell types. The data suggest that olfactory mucosa may metabolize carboxylesters to acids more readily than respiratory mucosa. However, such metabolism does not occur in the target cell population, the olfactory sensory neurons, raising the possibility of intercellular migration of toxic acid metabolites.

Dahl, 1986

Esters are a widespread class of organic compounds found both in industry and the environment. Because esters are often volatile and, therefore, readily inhaled, the capacity of respiratory tract tissues as well as liver S-9 homogenates from rats, rabbits, and Syrian hamsters to hydrolyze a variety of esters was investigated. A new technique to determine hydrolysis rates by measuring carboxylic acid residues using ion chromatography was proven effective. The results indicated that esters, including potentially carcinogenic β-lactones, are readily hydrolyzed by respiratory tract enzymes. Species and tissue differences were apparent. The nasal ethmoturbinates had especially high levels of esterase activity with tissue weight-normalized activities from rabbits and hamsters for most substrates exceeding all other tissues tested, including liver. Phenyl acetate was the most rapidly hydrolyzed by ethmoturbinate tissue of the esters tested. Among straight chain aliphatic alcohol acetates, hydrolysis rates increased with carbon number up to pentyl alcohol and then decreased. Branched 4-carbon alcohol acetates were less rapidly hydrolyzed than n-butyl acetate. Correlation of hydrophobicity constants with hydrolysis rates indicated that, for the straight chain aliphatic acetates, a bilinear model best fit the data.

Mattes, 1992

Carboxylesterase activity in the rat nasal mucosa plays an important role in the response of this tissue to certain toxic inhaled esters. We have examined this activity in extracts of both Fischer-344 rat and human nasal tissue using the substrate α-naphthyl butyrate, the same substrate as that used for histochemical analysis of this activity. We find substantially higher activity in rat nasal extracts than in human nasal extracts. The Michaelis constant (Km), however, is approximately the same for both activities and is significantly less than that reported for rat nasal carboxylesterase activity using dibasic esters as a substrate. p-Nitrophenyl butyrate is a competitive inhibitor of the rat α-naphthyl butyrate esterase, but surprisingly has no effect on the human activity. The assay reported here should prove a powerful tool in the development of a valid in vitro nasal toxicity assay system that uses cultured rat or human cells expressing carboxylesterase activity.

Pacifici, 1981

1. Glutathione transferase and epoxide hydratase activities were determined in liver, lung and kidney of nine species including man with [7-^{3}H]styrene oxide as substrate.

2. Activity was detectable in all tissues. The activities of both enzymes were higher in the liver of all species than in either kidney or lung.

3. The baboon had the highest hepatic epoxide hydratase activity, 31 \pm 2nmol/mg per min, while the mouse had the lowest hepatic activity, 1.9 \pm 0.1 nmol/mg per min.

4. Rodent species had higher glutathione transferase activity than non-rodent species, mouse liver having the highest activity, 143\pm13nmol conjugate/mg per min.

5. Taking all the data into account, it is concluded that no single species of those studied is a suitable model for the disposition of epoxides in man.

4.1.2 Human information

See above mentioned Domoradzki et al. (2004) study.

4.1.3 Summary and discussion on toxicokinetics

Limited toxicokinetic and metabolism data are available for GMA. The metabolism of GMA in mammals is hypothesized to proceed by at least two different and competing enzyme systems, epoxide hydrolase and non-specific carboxylesterases. The two metabolic routes are shown in the Figure 2. Metabolism of GMA by carboxylesterase would result in formation of glycidol and methacrylic acid as metabolites, while initial metabolism by epoxide hydrolase would result in the formation of glycerol methacrylate. The relative speed at which the two competing metabolic reactions occur in different tissues and species is likely to be important for understanding the toxicity of GMA.

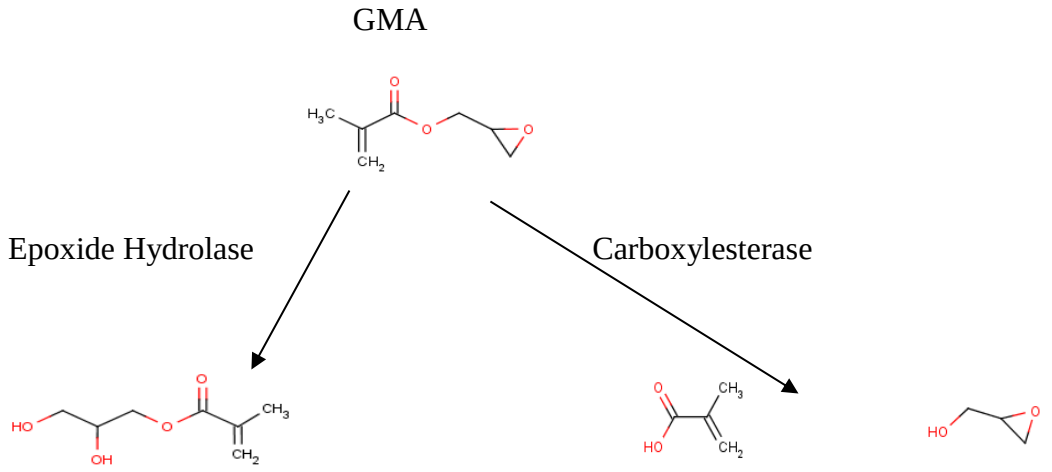


Figure 2: Hypothesized metabolism of GMA in mammals

Species differences in the activity of these enzymes suggest that the carboxylesterase route of metabolism may predominate in the nasal tissue of rabbits (yielding glycidol and methacrylic acid) while the epoxide hydrolase route was hypothesized to predominate in rats and humans (producing

glycerol methacrylate, then glycerol and methacrylic acid by carboxylesterase) (Bogdanffy et al.: 1987, Dahl et al.: 1987, Glatt et al.: 1984, Mattes and Mattes: 1992, Pacifici et al.: 1981).

Toxicokinetics of GMA were investigated in rabbits. After an intravenous injection at 200 mg/kg, over 95 % of the parent compound disappeared from the blood within 10 minutes according to a two-compartment open model. Following a subcutaneous injection at 800 mg/kg, the toxicokinetics appeared to fit a first-order absorption one-compartment open model. This substance was metabolized by incubation with whole blood, plasma, erythrocyte suspension, and homogenates of various tissues. The subcutaneous co-administration of tri-*o*-cresyl-phosphate (a carboxylesterase inhibitor) with this substance resulted in about a ten-fold increase in the maximum blood concentrations, compared to those of animals dosed with this substance alone (Shi Tao et al. 1988). This finding suggests a key role of carboxylesterase in GMA metabolism and although metabolites were not specifically measured, could implicate glycidol as that metabolite.

More definitive work on the metabolism of GMA was studied by Domoradzki et al. (2004) *in vitro* using liver homogenate and nasal epithelial tissues from humans, rats and rabbits. Radiolabeled GMA [^{14}C 1,3- glycidyl] was used in this study and was 92% radio chemically pure. *In vitro* incubations of ^{14} C GMA with tissue preparations from human, rat and rabbit resulted in the formation of only one metabolite. This metabolite was tentatively identified as glycidol based on retention time match with ^{14}C-glycidol. At an initial starting concentration of GMA at 2 mM, half-lives of GMA hydrolysis were faster in incubations with rat and rabbit tissue. Although the biotransformation was faster in rats and rabbits as compared to humans (completed within 30 minutes versus 2 hours), under all circumstances only one metabolite appeared which was tentatively identified as glycidol (EINECS 209 -128 -3).

Overall, the available studies show that GMA is metabolised into glycidol. Metabolism of GMA to glycidol has ramifications for hazard identification. Glycidol has a harmonised classification according to CLP as carcinogenic (category 1B), germ cell mutagenic (category 2) and toxic to reproduction (category 1B).

4.2 Acute toxicity

4.2.1 Non human information

4.2.1.1 Acute toxicity: oral

Table 12a: Summary table of relevant acute toxicity studies (oral)

Method	Results	Remarks	Reference
Rat oral: unspecified exposure regimen Dose: no data	LD50: 597 mg/kg bw	4 (not assignable) supporting study experimental result **Test material: GMA**	Zdravko (1985) OECD (1999)
Rat (n=2 per dose) oral: 10% in corn oil Dose: 126, 252, 500 and 1000 mg/kg bw	LD50: ca. 700 mg/kg bw	3 (not reliable) supporting study experimental result **Test material : GMA**	Olson (1960) Smyth (1969) OECD (1999)
Rat (albino) oral: unspecified dose	LD50: 451 mg/kg bw	4 (not assignable) supporting study experimental result **Test material: GMA (purity 97.8%)** **Mixture: 0.3% epichlorohydrin + 0.6% dichlorohydrin**	EPA (1992) OECD (1999)
Mouse oral: unspecified dose	LD50: 390 mg/kg bw	4 (not assignable) supporting study experimental result **Test material: GMA**	Zdravko (1985) OECD (1999)
Rat oral: unspecified dose	LD50: 1050 mg/kg bw	4 (not assignable) supporting study experimental result **Test material: GMA**	Smyth (1969) OECD (1999)
Guinea pig oral: unspecified dose	LD50: 697 mg/kg bw	2 (reliable with restrictions) supporting study experimental result **Test material: GMA**	Zdravko (1985) OECD (1999)

All available acute oral studies are old and have limitations either in reporting (score 4) or in the conduct of the study (score 3). The OECD has adopted an oral LD50 value for GMA of 597 mg/kg bw for rat (Zdravko et al.: 1985). All other acute oral studies resulted in LD50 values in the same range.

4.2.1.2 Acute toxicity: inhalation

Table 12b: Summary table of relevant acute toxicity studies (inhalation)

Method	**Results**	**Remarks**	**Reference**
Rat (Fischer 344) 5 male+ 5 female/group inhalation: vapour (whole body), single 4-hour period, 14-day post-exposure observation period Analytical concentration: 105, 269, 412 ppm (310, 1563, 2394 mg/m^3) OECD Guideline 403 (Acute Inhalation Toxicity)	LC0 (4 h): > 2394 mg/m^3 (412 ppm) (male/female): no mortality	2 (reliable with restrictions) key study experimental result **Test material: GMA purity 99.8%**	Nitschke (1990) OECD (1999) Nitschke (1999)
Rat 6 per group inhalation: vapour Concentration: saturated	Concentrated vapour inhalation by rats maximum for no deaths = 2 hours There was no recorded inhalation metered vapour concentration mortality in rats.	4 (not assignable) supporting study experimental result **Test material: GMA**	Smyth (1969) OECD (1999)
Rat inhalation: vapour Concentration: 1400 mg/m^3 air	LCL_0 (6 h): 1400 mg/m^3 air Changes in lungs, thorax, respiration, etc.	3 (not reliable) supporting study experimental result **Test material: GMA**	Haag (1953) OECD (1999)
Rabbit inhalation: vapour Concentration: 1400 mg/m^3 air	LCL_0 (6 h): 1400 mg/m^3 air Changes in lungs, thorax, respiration, etc.	3 (not reliable) supporting study experimental result **Test material: GMA**	Haag (1953) OECD (1999)
Guinea pig inhalation: vapour Concentration: 1400 mg/m^3 air	LCL_0 (6 h): 1400 mg/m^3 air Changes in lungs, thorax, respiration, etc.	3 (not reliable) supporting study experimental result **Test material: GMA**	Haag (1953) OECD (1999)
Dog inhalation: vapour Concentration: 1400 mg/m^3 air	LCL_0 (6 h): 1400 mg/m^3 air Changes in lungs, thorax, respiration, etc.	3 (not reliable) supporting study experimental result **Test material: GMA**	Haag (1953) OECD (1999)

LCL_0= (lethal concentration low): The lowest concentration of a substance, in air, that causes death to mammals in acute (<24h) or sub-acute or chronic (>24 h) exposure.

Nitschke (1990)

The purpose of this study was to determine the acute toxicity of inhaled glycidyl methacrylate vapors. Groups of 5 male and 5 female rats were exposed to nominal concentrations of 105, 255 or 412 ppm (0.61, 1.48 or 2.30 mg/l, respectively) of the test material for a single 4-hour period. The highest concentration was the maximum practically attainable vapor concentration.

All animals survived the exposure and 14-day post-exposure observation period. At 412 ppm, labored breathing was noted clinically at the end of the exposure period and eye irritation was noted in a few animals for several days following exposure. Body weights of animals exposed to 412 ppm were decreased as much as 15% from pre-exposure values but thereafter the animals achieved expected body weight gains. Similar but less severe effects were noted in animals exposed to 269 ppm. At the lowest concentration, 105 ppm, a very slight transitory body weight loss of 3% was noted only on the day following exposure to the test material, but thereafter the animals achieved expected body weight gains. Corneal opacities were noted in all but one rat exposed to the various concentrations of glycidyl methacrylate. While these lesions were consistent with corneal dystrophy, a spontaneous lesion in Fischer 344 rats, the high frequency of the corneal opacity is considered to be exposure-related.

In another inhalation toxicity study, acute exposure to rats with saturated vapour of this substance for 2 hours resulted in no deaths (Smyth et al.: 1969). It was reported that saturated vapour of GMA at 20 °C was 474 ppm (2754 mg/m^3) (No reference in Draft Workplace Environmental Exposure Level Guide: 1999). Higher concentrations including the testing of aerosols were not performed. No other additional information or further details were provided.

4.2.1.3 Acute toxicity: dermal

Table 12c: Summary table of relevant acute toxicity studies (dermal)

Method	**Results**	**Remarks**	**Reference**
Rabbit Dermal, no data Dose: no data	LD50: 480 mg/kg bw	4 (not assignable) key study experimental result **Test material: GMA**	Smyth (1969) OECD (1999)

The available information on the only available study is very limited and would normally not be usable for classification. However, there is already a harmonised minimum classification of GMA for acute dermal toxicity. It is likely that this study was the basis for the current harmonised classification. Therefore, this study can be used to adapt the current minimum classification.

4.2.1.4 Acute toxicity: other routes

Table 12d: Summary table of relevant acute toxicity studies (other routes)

Method	Results	Remarks	Reference
Rat Intraperitoneal Dose:	LD50: 290 mg/kg bw	4 (not assignable) supporting study experimental result **Test material: GMA**	Petrov (1973) OECD (1999)
Mouse Intraperitoneal Dose:	LD50: 350 mg/kg bw	4 (not assignable) supporting study experimental result **Test material: GMA**	Petrov (1973) OECD (1999)

4.2.2 Human information

No relevant information available

4.2.3 Summary and discussion of acute toxicity

All available acute oral studies are old and have limitations either in reporting (score 4) or in the conduct of the study (score 3). A key study can therefore not be determined. The OECD has adopted an oral LD50 value for GMA of 597 mg/kg bw for rat (Zdravko et al.: 1985). Although the available studies are only limitedly reported, all studies provide the same range of LD50 values of 390 – 1050 mg/kg bw. Therefore, overall the level of evidence is considered sufficient to allow classification for acute oral toxicity.

In an inhalation toxicity study by OECD TG 403 (key study), there was no mortality observed in rats exposed for 4 hours at 2394 mg/m^3, the highest practically attainable vapour concentration. Change of respiration (laboured breathing) and eyes (irritation and corneal opacity), and decrease in body weight were induced even at the lowest concentration of 1563 mg/m^3 (Nitschke et al.: 1990).

In another inhalation toxicity study, acute exposure to rats with saturated vapour of this chemical substance for 2 hours resulted in no deaths (Smyth et al.: 1969). It was reported that saturated vapour of glycidyl methacrylate at 20 °C was 474 ppm (2754 mg/m^3) (No reference in Workplace Environmental Exposure Level Guide: 1999). Overall, higher concentrations including the testing of aerosols were not performed. This study is considered as supportive only because only very limited information is available and especially information on the mortality at exposure duration longer than 2 hours is missing.

The available information on the only available acute dermal study is very limited and would normally not be usable for classification. However, there is already a harmonised minimum

classification of GMA for acute dermal toxicity. It is likely that this study was the basis for the current harmonised classification. Therefore, this study can be used to adapt the current minimum classification. The dermal LD50 for rabbits in this study was 480 mg/kg bw (Smyth et al.: 1969).

Table 13: Summary of LD50s

Routes	Strain	Type	Values	Reference
Oral	Rats	LD50	597 mg/kg	Zdravko, 1985
	Rats	LD50	about 700 mg/kg	Olson, 1960; Smyth, 1969
	Rats*	LD50	451 mg/kg	EPA/OTS, 1992
	Mice	LD50	390 mg/kg	Zdravko, 1985
	Mice	LD50	1050 mg/kg	Smyth, 1969
	Guinea pigs	LD50	697 mg/kg	Zdravko, 1985
Inhalation	Rats	LC0	2394 mg/m^3/4hr	Nitschke, 1990
	Rats**	LCL_0	1400 mg/m^3/6hr	Haag, 1953
	Rabbits**	LCL_0	1400 mg/m^3/6hr	Haag, 1953
	Guinea pigs**	LCL_0	1400 mg/m^3/6hr	Haag, 1953
	Dogs**	LCL_0	1400 mg/m^3/6hr	Haag, 1953
	Rats	LCL_0	saturated/2 hr	Smyth, 1969
Dermal	Rabbits	LD50	480 mg/kg	Smyth, 1969

LC0 = lethal concentration 0%, the calculated concentration at which none of the population is expected to die.
LCL_0= (lethal concentration low): The lowest concentration of a substance, in air, that causes death to mammals in acute (<24h) or sub-acute or chronic (>24 h) exposure.
* as a mixture of GMA (97.8 %), epichlorohydrin (0.3 %) and dichlorohydrin (0.6 %).
** Changes in lungs, thorax, respiration, etc. were observed.

4.2.4 Comparison with criteria

The oral LD50 of all studies are all between the borders of Acute oral category 4 of 300 to 2000 mg/kg bw.

The dermal LD50 of the only study (Smith, 1969) of 480 mg/kg bw is between the borders of Acute dermal category 3 of 200 to 1000 mg/kg bw.

The available inhalation studies indicate no mortality up to the saturated vapour pressure. Aerosols were not tested.

4.2.5 Conclusions on classification and labelling

Based on the available data GMA must be classified according to CLP as

- Acute toxicity oral Category 4 (H302) (the LD50 values of all available studies are between the Category 4 limits of 300 and 2000 mg/kg bw)

- Acute toxicity dermal Category 3 (H311) (the actual LD50 value of 480 mg/kg bw lies between the Category 3 limits of 200 and 1000 mg/kg bw)
- No classification for Acute toxicity inhalation is required since the LC0 in rats exposed for 4 hours lies at 2394 mg/m^3, the highest practically attainable vapour concentration. As higher concentrations including aerosols were not tested, it is concluded that no classification for acute inhalation toxicity is based on absence of data.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity- oral

Several acute oral studies were available, but all had limitations either in reporting or in the conduct of the study. A key study could therefore not be determined. The OECD has adopted (OECD, 1999) an oral LD_{50} value for GMA of 597 mg/kg bw in the rat, based on a study by Zdravko *et al.* (1985). Although the available studies were not reported in detail, all studies provided LD_{50} values within the same range (390 to 1050 mg/kg bw). Thus, the DS proposed to classify GMA for acute oral toxicity in category 4 (H302) as the LD_{50} values of all available studies were within the limits for classification in category 4 (300 - 2000 mg/kg bw).

Acute toxicity - inhalation

In an inhalation toxicity study performed according to OECD Test Guideline (TG) 403 (key study), there were no mortalities observed in rats exposed for 4 hours up to 2394 mg/m^3 GMA, the highest practically attainable vapour concentration. Laboured breathing and eye irritation were observed at the 2394 mg/m^3. Corneal opacity and decreased body weights were induced in all groups, even at the lowest concentration of 1563 mg/m^3 (Nitschke *et al.*, 1990).

In another inhalation toxicity study, acute exposure of rats with saturated vapour of GMA for 2 hours did not result in any deaths (Smyth *et al.*, 1969). It was reported that the saturated vapour concentration of GMA at 20°C was 474 ppm (2754 mg/m^3) (quoted in Workplace Environmental Exposure Level Guide 1999). Tests with higher concentrations of GMA, including aerosols were not performed. This study was considered as supportive because only very limited information was available. For example, information on mortalities at exposure durations longer than 2 hours was lacking.

Another study (Haag, 1953) reported that the lowest concentration that caused deaths was 1400 mg/m^3 for 6 h in rats, rabbits, guinea pigs and dogs. However, the study was not considered reliable by the DS.

Overall, no classification for acute inhalation toxicity was proposed by the DS, since the LC_0 in rats exposed for 4 hours was considered to be 2394 mg/m^3, the highest practically attainable vapour concentration. However, higher concentrations including aerosols were not tested and the DS concluded that no classification for acute inhalation toxicity was warranted for GMA, based on the absence of data.

Acute toxicity - dermal

The available information was very limited in the only available acute dermal toxicity study (Smyth *et al.*, 1969) and would normally not be usable for classification, according to the DS. However, GMA has an existing entry in Annex VI to the CLP with a minimum classification for acute dermal toxicity (category 4). It was considered likely by the DS that this study was the basis for the existing harmonised classification. Therefore, this

study could be used according to the DS to adopt the existing minimum classification. The dermal LD_{50} for rabbits in this study was 480 mg/kg bw and the DS proposed to classify GMA for dermal acute toxicity in category 3 (H311).

Comments received during public consultation

One Member State (MS) requested clarifications on the reliability of some studies on acute toxicity by oral and dermal routes. Another MS raised doubts on whether the data were sufficiently reliable to remove the existing classification for acute inhalation toxicity.

Assessment and comparison with the classification criteria

Acute toxicity - oral

RAC agrees with the assessment made by the DS that all six results from the four available studies indicated acute oral toxicity within a similar range of doses and are altogether sufficient to conclude on the classification of GMA for acute oral toxicity. All available LD_{50} values in rats, mice and guinea pigs are within the range of 300 - 2000 mg/kg bw and a classification for **Acute Tox. 4; H302** is therefore warranted for GMA, according to CLP.

Acute toxicity - inhalation

A study performed according to OECD TG 403 (Nitschke *et al.*, 1990) reported no mortality in rats at the maximum achievable vapour concentration of 2394 mg/m^3 for 4 h. Two other studies were performed on vapours but both studies have limitations in their reporting (Smyth *et al.*, 1969) and/or in their reliability (Haag, 1953) and none of them provided a result that allows RAC to conclude on the LC_{50} value.
RAC concludes that none of the available studies, including a guideline study (Nitschke *et al.*, 1990), provides evidence of an LC_{50} value within the range of values for classification. **No classification for acute inhalation toxicity** is warranted.

Acute toxicity - dermal

An LD_{50} value of 480 mg/kg bw is available from an acute dermal toxicity study in rabbits which is within the range of 300 - 2000 mg/kg bw corresponding to a classification in category 3. Information on this study is very limited and its reliability cannot be assessed in detail by RAC. RAC notes however that the LD_{50} values obtained in the same publication (Smyth *et al.*, 1969) for acute oral toxicity are within a similar range of values as in other available oral studies providing some support to the reliability of the study. The LD_{50} obtained via the dermal route in rabbit is within a similar range as the LD_{50} values obtained via the oral route in rats, mice and guinea pigs. A higher LD_{50} is generally expected by the dermal route compared to the oral route but no information is available on the relative absorption of GMA by the different routes. Also, the studies of longer duration by oral route suggest that the rabbit is a more sensitive species to toxicity of GMA than the rat.
Considering these elements, RAC concludes that the LD_{50} of 480 mg/kg bw in rabbit by the dermal route is plausible. On this basis, RAC agrees with the DS proposal to classify GMA as **Acute Tox. 3; H311.**

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

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

This assessment focusses on the inhalation route as a classification for acute oral and dermal toxicity is already proposed based on mortality. An additional classification for STOT SE for these routes is considered a double classification.

Key Studies

Laboured breathing was induced in rats by acute inhalation exposure for 4 hours at 1563 mg/m^3 and 2394 mg/m^3 (Nitscke et al. 1990). In another acute inhalation study, changes in lungs, thorax, respiration, etc. were observed in rats, rabbits, guinea pigs and dogs (Haag, 1953). In this study, exposure was conducted at 1400 mg/m^3 for 6 hours. No further details are available. These changes may have resulted from respiratory irritation of this substance. The criteria for STOT SE Category 1 is Inhalation (rat) vapour mg/L/4h Concentration $\leq$ 10 mg/l = 10000 mg/m^3.

Supporting Studies

In inhalation repeated dose toxicity studies, there were many changes in the respiratory tract, such as noisy and difficult respiration (mouth breathing), and hyperplasia, necrosis and inflammation in nasal tissues. In one sub-acute toxicity study, rabbits were exposed at 2.9, 12, 29 or 60 mg/m^3 (6 hours/day, 5 days/week) for 13 consecutive days. Treatment-related degeneration of the nasal olfactory epithelium was observed at 12 mg/m^3. At 29 and 60 mg/m^3, there were olfactory epithelial degeneration, and hyperplasia, erosion, ulcers and inflammation of the nasal epithelium. After a 4-week recovery period, there was reversibility of these changes except for olfactory epithelial degeneration observed at 29 and 60 mg/m^3, which showed only partial reversibility. At 12 mg/m^3, nasal tissue was indistinguishable from controls at one-month post-exposure (Cieszlak et al., 1996).

Rats exposed to GMA at concentrations of 58.2, 233 or 931 mg/m^3 (7.09, 28.4 or 113 mg/kg/day, respectively) for 2 weeks (6 hours/day, 5 days/week) (Landry et al.: 1991) showed general debilitation with noisy and difficult respiration (mouth breathing) was observed at 931 mg/m^3 in day 4. The animals at 931mg/m^3 were terminated early on day 4 because of the severity of the respiratory effects. Microscopically, there was severe multifocal necrosis and inflammation of the olfactory and respiratory epithelium in the nasal cavity and also effects on the lungs including congestions, inflammation and necrosis. At 233 mg/m^3, there were slight to moderate multifocal necrosis, and inflammation of the respiratory and olfactory nasal epithelium. At 58.2 mg/m^3, microscopically there was very slight multifocal necrosis of individual respiratory epithelial cells in 3 of 5 males and in 2 of 5 females. These changes in respiratory tract were considered due to irritation of GMA. There were no histopathological changes in any other tissues. Therefore, 58.2 mg/m^3 (7.09 mg/kg/day) was considered to be LOAEL because of tissue damages in respiratory tract.

Rabbits exposed to 2.91, 11.6, 29.1, 58.2 mg/m^3 (6 hours/day, 5 days/week) (Cieszlak et al., 1996) showed treatment-related degeneration of the nasal olfactory epithelium was at 11.6 mg/m^3. Olfactory epithelial degeneration, and hyperplasia, erosions, ulcers and inflammation of the nasal epithelium were observed at 29.1 and 58.2 mg/m^3. After 4-week recovery period, there was complete reversibility of these changes except for olfactory epithelial degeneration observed at 29.1 and 58.2 mg/m^3, which showed only partial reversibility. At 11.6 mg/m^3, nasal tissue was indistinguishable from controls at one month post-exposure. 2.91 mg/m^3 (0.26 mg/kg/day) was considered to be NOAEL. Unfortunately purity of chemical and GLP were not mentioned.

A sub-chronic inhalation toxicity study in in rats at concentrations of 2.9, 12 or 87 mg/m^3 (0.35, 1.46 or 10.6 mg/kg/day, respectively) for 13 weeks (6 hours/day, 5 days/week) (Landry et al.: 1996). There were no treatment related in-life observations, and no significant treatment-related effects on body weight, urinalysis, clinical chemistry or hematology parameters, as well as gross pathologic changes or organ weights at any exposure level. Treatment-related effects were limited to hyperplasia of respiratory epithelium of the nasal tissues in all animals at 87 mg/m^3. In all affected animals, the hyperplastic respiratory epithelium was approximately two to three times as thick as in control animals, and was located in the anterior portions of the nasal passages, involving the tips of the turbinates and the lateral walls of the nasal passages. These changes were considered to result from respiratory irritation. Therefore, NOAEL was considered 12 mg/m^3 (1.46 mg/kg/day) for both sexes.

4.3.2 Comparison with criteria

Specific target organ toxicity (single exposure) is defined in the CLP Regulation as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed and not specifically addressed in other sections are to be covered.

Specific target organ toxicity following a repeated exposure is classified as described in Specific target organ toxicity — Repeated exposure and is therefore excluded here.

The adverse health effects produced by a single exposure include consistent and identifiable toxic effects in humans, or, in experimental animals, toxicologically significant changes which have affected the function or morphology of a tissue/organ, or have produced serious changes to the biochemistry or haematology of the organism, and these changes are relevant for human health.

STOT SE recognizes three categories of which the most severe category 1 is described in the CLP Regulation as:

> "*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 single exposure. Substances are classified in Category 1 for specific target organ toxicity (single exposure) on the basis of:*
> *(a) reliable and good quality evidence from human cases or epidemiological studies; or*
> *(b) observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations. Guidance dose/concentration values are provided below (see 3.8.2.1.9) to be used as part of weight-of-evidence evaluation*".

The CLP Guidance concentration value for single dose exposures for placing a substance in Category 1 is C ≤ 10 mg/l/4h for vapours (rat). The experimental value found for GMA in an acute inhalation toxicity study was at 1.563 mg/l/4h (laboured breathing was observed at this concentration of 269 ppm or 1.563 mg/l/4h by Nitschke et al. (1990) and thus this substance must be classified in STOT SE Category 1 (damage to the respiratory tract after inhalation). This classification is also justified because of the severe multifocal necroses and inflammation of the lung after 4 days of exposure to 0.931 mg/L for 6 hours. Although the exposure was repeated for 4 days and the exposure period per day was somewhat longer, the exposure concentration was clearly below the guidance value of 10 mg/L for category 1. Therefore it is very likely that comparable respiratory tract irritation would have occurred after single exposure. These effects have probably occurred in the available acute inhalation study. However, they were probably not observed due to

the limited necropsy and the long post-exposure observation period in an acute study. Local respiratory tract tissue injury is also expected given the corrosiveness seen on skin and eyes.

As STOT SE effects are only observed after inhalation exposure and are due to local irritation, it is not expected that acute oral and dermal exposure will result in comparable effects. Further, for the oral route, GMA is already classified as acute toxic Cat. 4. For the dermal route the substance is already classified as acute toxic cat. 3. An additional classification for these routes with STOT SE would be a double classification.

4.3.3 Conclusions on classification and labelling

Classification with STOT SE in category 1 is required with the respiratory tract as target organ and the inhalation route.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

Laboured breathing was induced in rats by acute inhalation exposure for 4 hours at 1563 mg/m^3 and 2394 mg/m^3 (Nitscke *et al.*, 1990). In another acute inhalation study, changes in e.g. lungs, thorax and respiration were observed in rats, rabbits, guinea pigs and dogs (Haag, 1953). In this study, exposure was conducted at 1400 mg/m^3 for 6 hours. No further details were available. The DS considered that these changes may have resulted from respiratory irritation of GMA.

According to the CLP criteria, the guidance values for placing a substance in category 1 after a single-dose exposure are ≤ 10 mg/L/4h for vapours (rat). Laboured breathing was found in the acute inhalation study at 1.563 mg/L/4h (Nitschke *et al.*, 1990) and thus the DS proposed to classify GMA as STOT SE 1 (damage to the respiratory tract after inhalation). According to the DS, this classification was also justified because severe multifocal necrosis and inflammation of the olfactory and respiratory nasal epithelium and congestion, inflammation and necrosis in the lung were observed in rats after 4 days (6-hours/day) of exposure to 0.931 mg/L of GMA (Landry *et al.*, 1991). Although the exposure was repeated for 4 days and the daily exposure period was somewhat longer than 4 hours as referred to in the CLP Table 3.8.2 for single exposures, the exposure concentration was clearly below the guidance limit value of 10 mg/L for category 1. Therefore it was considered very likely that comparable respiratory tract irritation would have occurred also after a single exposure. According to the DS, it was probable that these effects had occurred in the available acute inhalation study, but that they were not observed due to the limited necropsy performed and the long post-exposure observation period in the acute study. Local respiratory tract tissue injury was also expected given the corrosive effect on skin and eyes.

The DS concluded that a classification with STOT SE 1 was required with the respiratory tract as a target organ and the inhalation route as the route of exposure.

Comments received during public consultation

Two MS noted that the CLP guidance does not recommend classification for STOT SE 1 or 2 if the effects are consequences of a corrosive mode of action and therefore did not support the proposed classification. One of them recommended a classification for STOT SE 3 (H335) instead, considering the respiratory effects observed in the acute inhalation studies. Both MS also proposed to consider classification for specific target organ toxicity

after repeated exposure.

Assessment and comparison with the classification criteria

RAC considers that the effects observed on the respiratory tract after 4 days of a 6-hour daily exposure to 0.931 mg/l of GMA vapours demonstrate corrosivity of GMA vapours to the respiratory tract under these experimental conditions, which is consistent with the corrosive properties of GMA on skin and eyes (see section below). The severity of the effects (inflammation and necrosis) and the effect concentration (threshold value of 10 mg/L for category 1) are consistent with the criteria for STOT SE 1 but the effects were observed after a total of 24 h cumulated exposure. Thus, it is not possible to conclude that effects of similar severity would have occurred also after a single 4-hour exposure. However, the result supports the assumption that the clinical signs observed in the acute inhalation toxicity study can be attributable to respiratory tract irritation.

Significant functional changes, e.g. in the respiratory system, which are more than transient in nature, are considered to support classification as STOT SE 1 and 2. However, due to limited information, it is not possible to determine whether the clinical signs were transient or not.

RAC therefore considers that the data are not sufficiently robust to classify GMA for STOT SE in category 1 or 2.

However, laboured breathing and changes in the respiratory tract in the acute inhalation studies, in combination with the corrosive findings in the 4-day inhalation study and the skin corrosive properties, are considered by the RAC to be signs of at least transient respiratory tract irritation. Therefore, RAC supports classification of GMA as STOT SE 3; H335.

RAC is aware that the CLP guidance recommends that "an additional classification as specific target organ toxicant (single exposure, Category 1 or 2) is not indicated if the severe toxicological effect is the consequence of the local (i.e. corrosive) mode of action" while "the additional Category 3 is considered to be superfluous, although it can be assigned at the discretion of the classifier". However, RAC notes that the hazard statement H314 ("Causes severe skin burns and eye damage") does not include a reference to the respiratory tract. Thus, RAC recommends the additional classification of GMA as **STOT SE 3; H335**.

4.4 Irritation

4.4.1 Skin irritation

4.4.1.1 Non-human information

Table 14a: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Rabbit (New Zealand White) OECD Guideline 404 (Acute Dermal Irritation /	Overall irritation score: 5 of max. 6 Corrosiveness occurred in 2 out of 6 animals (Time point: 4 hours)	2 (reliable with restrictions) key study	Lockwood (1991) OECD (1999)

Method	Results	Remarks	Reference
Corrosion)		experimental result **Test material: GMA**	
Rabbit (albino) Undiluted GMA	Overall irritation score: 4 of max. 6 Single rabbit died after 1st application (Time point: 4 hours)	4 (not assignable) supporting study experimental result **Test material: GMA**	Olson (1960) OECD (1999)
Rabbit 10% solution in propylene glycol	Overall irritation score: 2 of max. 6 Single rabbit showed hypermia and edema after 1st application and moderate burn after 2nd application (Time point: 4 hours)	4 (not assignable) supporting study experimental result **Test material: GMA**	Olson (1960) OECD (1999)
Rabbit (domestic) 0.1 mL (100 mg) no data	Overall irritation score: 4 of max. 6 (Time point: 5 days)	3 (not reliable due to low amount of substance, absence of information on exposure duration and repeated exposure) experimental result **Test material: GMA purity 92%**	Ou-Yang (1988) OECD (1999)

4.4.1.2 Human information

No relevant information available

4.4.1.3 Summary and discussion of skin irritation

Key studies

Lockwood (1991)
A sample of GMA was tested for evaluation of corrosiveness to the skin by the DOT (Department of Transportation) test. A 4-hour exposure of the test material (0.5 ml on a one by one inch gauze pad covered with plastic) to the clipped backs of 6 New Zealand White rabbits resulted in moderate to severe erythema and slight to moderate edema at 4, 24 or 48 hours. Moderate necrosis was observed in 2 out of 6 rabbits. A 1-hour exposure resulted in slight erythema and edema, and superficial necrosis (2 rabbits) to the skin of 6 rabbits. The scoring system deviated from the Draize score with grades ranging from 1 (no effect) to 6. No information is available after 48 hours.

Table 14b: Summary of the Lockwood (1991) severity scores

GRADE	ERYTHEMA	EDEMA	NECROSIS
1	NONE	NONE	NONE
2	very slight	very slight	very slight
3	SLIGHT PINK	SLIGHT	SUPERFICIAL
4	MODERATE PINK	MODERATE	MODERATE
5	RED	MARKED	DEEP
6	VERY RED	SEVERE	VERY DEEP

Table 14c: Summary of the Lockwood (1991) study with respective severity scores after 1-hour exposure

	Rabbit: 1			2			3			4			5			6		
ON BACK INTACT	OBSERVATION TIME (HOURS) →	1/4	24	48	1/4	24	48	1/4	24	48	1/4	24	48	1/4	24	48	1/4	24	48
	ERYTHEMA	1	2	1	1	1	1	2	3	3	1	3	3	1	3	3	1	4	4
	EDEMA	1	1	1	1	1	1	1	3	2	1	2	2	1	1	1	1	1	1
	NECROSIS	1	1	1	1	1	1	1	1	3	1	1	2	1	1	1	1	1	1

Table 14d: Summary of the Lockwood (1991) study with respective severity scores after 4-hour exposure

	Rabbit: 1			2			3			4			5			6		
ON BACK INTACT	OBSERVATION TIME (HOURS) →	4	24	48	4	24	48	4	24	48	4	24	48	4	24	48	4	24	48
	ERYTHEMA	3	3	3	3	3	2	4	4	4	5	5	5	4	4	4	4	4	4
	EDEMA	3	1	1	3	3	2	4	3	3	4	4	2	4	3	2	3	3	2
	NECROSIS	1	1	1	1	1	1	1	3	4	1	3	4	1	1	1	1	3	2

Supporting studies

Olson (1960)

A single covered topical application to the skin of an albino rabbit for four hours induced moderate to severe skin irritation including necrosis with slight to moderate edema and mortality (Olson: 1960). A 10% solution produced slight redness and edema after 1 application (for 4 hours) and a moderate burn after 2 applications (Olson: 1960). No additional information was available with regards to this study.

Ou-Yang (1988)

One piece of undamaged skin two by two centimetres on either side of the spine was chosen for the testing, one side for testing and the other for control (an equal amount of tap water). 0.1 ml of original concentration of GMA was applied for five days. The localized skin reaction was observed daily. After the experiments, the skin was removed for microscopic examination. GMA induced high irritation to the skin of rabbits. The 0.1ml applied area showed red, swelled and blistered after one or two days, subdermal bleeding and ulcers after three days, and hard, thicker, cracked, pigmentation after five days. The pathological changes were degeneration and necrosis of surface skin cells, disappearance of cellular boundaries, displaying pink staining material, bleeding in the corium cells and lymph cell infiltration with accompanying formation of abscesses.

4.4.1.4 Comparison with criteria

The CLP Regulation recognises for the skin corrosive category 1 three subcategories 1A, 1B and 1C. Category 1A shows corrosiveness within an exposure of 3 minutes or less (observation after max 1 hour), category 1B shows corrosiveness after 3 minutes but within 1 hour or less (observation after max 14 days), and category 1C shows corrosiveness after 1 hour but within 4 hours or less (observation after max 14 days).

Corrosive adverse effects for GMA were observed in 2 out of 6 rabbits after 4 hours exposure but not after 1 hour and thus this substance must be classified as 1C (Lockwood: 1991).

4.4.1.5 Conclusions on classification and labelling

According to the available studies GMA must be classified according to CLP as: Skin corrosive Category 1C (H314).

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

In an OECD TG 404 study (Lockwood, 1991), rabbits were examined 4, 24 and 48 h after a 4-hour exposure to 0.5 ml of GMA under occluve conditions. Oedema and/or erythema were observed at all time points in all rabbits. They were accompanied by moderate necrosis (score 4) in 2/6 animals at the last observation point of 48 h after exposure. When animals were exposed for 1 h, slight erythema and moderate erythema were observed in some animals. Necrosis was identified in 2 animals at 48 h and described as very slight in one (score 2) and superficial in the other (score 3). Reversibility or worsening of the lesions in this study could not be further assessed as no data was available later than 48 h after exposure.

Two other studies were considered by the DS as supportive despite their limitations. In a poorly reported study, a single covered topical application to the skin of an albino rabbit for 4 h induced moderate to severe skin irritation including necrosis with slight to moderate oedema and mortality (Olson, 1960). Necrosis was also reported after skin exposure to GMA under non-guideline conditions (0.1 ml, 5-day exposure) (Ou-Yang *et al.*, 1988).

The DS concluded that corrosive effects of GMA were observed in 2/6 rabbits after a 4-h exposure but not after 1 h (Lockwood, 1991). Thus, the DS proposed to classify GMA as Skin Corr. 1C.

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

Necrosis was reported after a 4-h exposure to GMA both in the study by Lockwood (1991) and in the study by Olson (1960). In the former study, which was performed in accordance with OECD TG 404, necrosis was observed in 2/6 animals and was described as moderate 48 h after exposure. RAC considers that this result fulfils the criteria for classification as corrosive as the substance produces destruction of skin tissue in at least 1 tested animal after 4-h exposure. After a 1-h exposure, necrosis was observed in 2/6 animals and described as very slight to superficial 48 h after exposure. Although reversibility or worsening after 48 h is not known, RAC considers that superficial necrosis does not fulfill the definition of destruction of skin tissue and that the criteria for

classification in subcategory 1B are not met.

RAC therefore concludes that GMA should be classified as **Skin Corr. 1C; H314**.

4.4.2 Eye irritation

4.4.2.1 Non-human information

Table 15a: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Rabbit (albino) Direct instillation Undiluted GMA directly instilled in eye; 10% GMA in propylene glycol	Overall irritation score: 4 of max. 6 (Time point: 7 days post-exposure) Undiluted/unwashed eye: slight to moderate conjunctivitis, slight corneal injury cleared in one week. Undiluted/washed eye: slight conjunctivitis, cleared in 48 hrs. 10% GMA in propylene glycol/unwashed eye: slight conjunctivitis, cleared in one hour. 10% GMA in propylene glycol/washed eye: slight conjunctivitis, cleared in 24 hrs.	4 (not assignable) supporting study experimental result **Test material: GMA**	Olson (1960) Smyth (1969) OECD (1999)
Rat Inhalation single 4-hour Concentration: 610, 1563 and 2394 mg/m^3	Overall irritation score: 4 of max. 6 (Time point: 4 hour treatment, 14-day post-exposure) Eye irritation, corneal opacity Changes did not heal within 14 days	2 (reliable with restrictions) supporting study experimental result **Test material: GMA purity 99.8%**	Nitschke (1990)
Rat Inhalation Concentration: 58.2, 223 and 931 mg/m^3 6 hours/day, 5 days/week for 2 weeks	Overall irritation score: 4 of max. 6 (Time point: 2-weeks treatment, no post-exposure time evaluation) Eye irritation, corneal clouding	4 (not assignable) Supporting study experimental result **Test material: GMA purity 99.61%**	Landry (1991)

Supporting studies

Olson (1960)

GMA was applied to both eyes of the rabbit and within 30 seconds, one eye was washed with tap water for two minutes. GMA was allowed to remain in the other eye. Results showed that with undiluted GMA, slight to moderate conjunctivitis was observed in the unwashed eye where slight corneal injury cleared in one week. In the washed eye, slight conjunctivitis was observed which cleared in one hour. In a solution of 10% GMA in propylene glycol, slight conjunctivitis was observed in the unwashed eye which cleared after 48 hours. In the washed eye, slight conjunctivitis was observed which cleared within 24 hours.

In the OECS SIDS (1999) and the registration file, it is reported that direct instillation of undiluted GMA to the eye of albino rabbits induced severe irritation and corneal damage. The corneal damage did not heal within 7-days post-dosing, which is contradictory to what study report findings (Table 15b). This ocular damage was prevented by washing with water within 30 seconds (Olson: 1960, Smyth: 1969).

Table 15b: Summary of Olson (1960) study

EYE CONTACT IRRITATION IN RABBITS

Material	Conc.	Eye Contact Irritation Unwashed eye	Washed eye Ø
Glycidyl methacrylate	Undiluted	Sl. to mod. conjunctivitis; sl. corneal injury cleared in one week	Sl. conjunctivitis cleaned in 48 hours
Glycidyl methacrylate	10% in propylene glycol	Sl. conjunctivitis cleared in one hour	Sl. conjunctivitis cleared in 24 hours.

Toxicity Test	Glycidyl Acrylate	Glycidyl Methacrylate
Acute Oral	LD_{50} approximately 0.06 g/kg in rats.	LD_{50} approximately 0.7 g/kg in rats.
Eye Irritation	Undiluted and 10% in propylene glycol likely to produce permanent impairment of vision. Reacts rapidly with eye tissue. Chemical workers goggles recommended.	Undiluted: Moderately irritating. Side shield glasses recommended. 10% in propylene glycol: Slightly irritating, no corneal damage. Minimal eye protection satisfactory.

In inhalation study using rats, eye irritation was also induced. Acute exposure for 4 hours induced eye irritation at 1563 mg/m^3 and 2394 mg/m^3. Slight corneal opacity was also observed at 610 mg/m^3, and moderately at 1563 mg/m^3 and 2394 mg/m^3. These changes did not heal within 14 days post-exposure (Nitschke et al.: 1990). In a sub-acute study, rats were exposed at 58.2, 223 and 931 mg/m^3, 6 hours/day, 5 days/week for 2 weeks. As a result, eye irritation and corneal clouding were observed at 931 mg/m^3 (Landry et al., 1991).

4.4.2.2 Human information

No relevant information available

4.4.2.3 Summary and discussion of eye irritation

The only eye irritation study has some resemblance to the OECD eye irritation study but is limitedly reported and there are differences in the described effects between the available report from Olson (1960) and the summary in the registration file and in the OECD summary. Therefore, there are no reliable results from relevant studies although the results indicate that GMA has potential to induce

eye irritation. The supporting studies are performed using inhalation exposure and result in clear irritating effects on the eye. However, this is due to exposure of the vapour phase and not the liquid as required for classification for eye irritation.

4.4.2.4 Comparison with criteria

According to chapter 3.2.3 of CLP skin corrosive substances shall be considered as leading to serious eye damage. Seen the skin corrosivity of GMA as described in chapter 4.3, GMA must be considered as Eye damage category 1.

4.4.2.5 Conclusions on classification and labelling

According to the available studies GMA must be classified according to CLP as Eye damage Category 1 (H318).

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

There was only one eye irritation study available (Olson, 1960) which had some resemblance to the OECD TG on eye irritation but the study was limitedly reported. Furthermore, the effects were differently described in the study report available to the DS, REACH registration dossier and OECD SIDS summary (1999). In the unwashed eye, undiluted GMA induced slight to moderate conjunctivitis. Slight corneal injury that cleared within one week was described in the study report while corneal damage that did not heal within 7 days was reported in the OECD SIDS summary (1999) and REACH registration dossier. Therefore, the DS considered that the results were not reliable although they indicated that GMA had the potential to induce eye irritation.

Two supporting studies performed using inhalation exposure, reported corneal opacity from 610 mg/m^3. In the acute toxicity study (Nitschke *et al.*, 1990), this effect did not heal within 14 days post-exposure. Despite that this indicated a clear irritating effects on the eye, this was considered by the DS to be due to exposure to the vapour and not to the liquid.

Comments received during public consultation

One MS noted that the hazard statement H318 shall not be included in the labelling to avoid redundancy with H314.

Assessment and comparison with the classification criteria

Section 3.3.2.4 in the Guidance on the application of the CLP criteria (version 4.1) states that "A skin corrosive substance is considered to also cause serious eye damage which is indicated in the hazard statement for skin corrosion (H314: Causes severe skin burns and eye damage). Thus, in this case both classifications (Skin Corr. 1 and Eye Dam. 1) are required but the hazard statement H318 'Causes serious eye damage' is not indicated on the label because of redundancy (CLP Article 27)."

RAC concluded that GMA should be classified as **Skin Corr. 1C; H314.** Classification as **Eye Dam. 1** should also be added, but **without labelling with hazard statement H318**.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

See section 4.4.3.3 below

4.4.3.2 Human information

No relevant information available

4.4.3.3 Summary and discussion of respiratory tract irritation

Not relevant for CLP as this effect is covered under STOT SE (chapter 4.3).

4.5 Corrosivity

Table 16: Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference
Deliberately left empty			

4.5.1 Non-human information

In experimental data (see chapter 4.4.1, 4.4.2 and 4.4.3) the substance appeared corrosive to skin.

4.5.2 Human information

No relevant information available

4.5.3 Summary and discussion of corrosivity

The substance appears to be corrosive to skin.

4.5.4 Comparison with criteria

See section 4.4.1.

4.5.5 Conclusions on classification and labelling

See section 4.4.1.

4.6 Sensitisation

4.6.1 Skin sensititsation

4.6.1.1 Non-human information

Table 17a: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Guinea pig (Hartley) male Buehler test Delayed allergy reaction test Induction dose 25% reduced to 10%	No. with positive reactions: 7 out of 10 (test group); dose: 1% 1st reading: 8 out of 10 (positive control); dose: 5% Observations: slight erythema	2 (reliable with restrictions) key study experimental result **Test material: GMA**	Dow (1990) OECD (1999)
Guinea pig	No. with positive reactions: 1st reading: 6 out of 6 (test group); dose: unknown Observations: skin sensitisation (eg contact dermatitis)	4 (not assignable) supporting study experimental result **Test material: GMA**	BIBRA (1988) OECD (1999)
Guinea pig delayed allergy reaction test delayed allergy reaction test	No. with positive reactions: 1st reading: 7 out of 10 (test group); induction dose: 0.1 ml of 1% GMA in acetone, skin smear application 1st reading: 6 out of 10 (test group); induction dose: 0.1 ml of 1% GMA in acetone, intradermal injection Observations: Hyperemia, edema, scleroma and necrosis were observed on the treated area and these changes reached a peak on the fourth day. But no obvious change was observed on the control area.	4 (not assignable) experimental result **Test material: GMA purity 92%**	Ou-Yang (1988) OECD (1999)
Guinea pig rapid allergic reactive test with active stimulation rapid allergic reaction test (active stimulation)	No. with positive reactions: 1st reading: 5 out of 5 (test group); dose: i.v. 0.5% GMA solution in homologous serum albumin 1st reading: 0 out of 3 (negative control); dose: zero Observations: Breathing difficulties, wheezing, increased mouth and nose secretions, spasms and death were observed in the test group, but no	4 (not assignable) experimental result **Test material: GMA purity 92%**	Ou-Yang (1988) OECD (1999)

Method	Results	Remarks	Reference
	obvious changes in the control group.		
Guinea pig rapid allergic reaction test with passive stimulation rapid allergic reaction test (passive stimulation) (injection of serum of allergic guinea pigs)	No. with positive reactions: 1st reading: 5 out of 5 (test group); dose: see details on study design Observations: Blue circles or spots were observed most markedly in the one to three areas, followed by the one to ten area and in the one to thirty areas, and there were a few scattered blue spots. This showed that the reaction is related to the dosage.	4 (not assignable) experimental result **Test material: GMA purity 92%**	Ou-Yang (1988) OECD (1999)

Guinea pigs received three topical applications with 0.4 ml of 10 % (third application) or 25 % (first and second application) GMA in dipropylene glycol monomethyl ether during the three-week induction phase. The single challenge application with 1% GMA induced slight erythema in these animals (7/10). (The Dow Chemical Company: 1990) This study deviates from the OECD TG 406 as no control animals were included which were only exposed to the solvent during induction and to the same concentration of the substance during the challenge.

Regarding the Bibra study (1988), no additional information is available than that it induced a positive reaction in first reading in 6 out of 6 guinea pigs.

Ou-Yang et al. (1988) reported on delayed and rapid allergy reaction tests in guinea pigs. In delayed allergy reaction test, induction with localized smear applications or intradermal injection with 0.1 ml of 1 % GMA in acetone for 10 days and challenge with an unknown concentration induced hyperaemia, oedema, scleroma and necrosis. Those changes belong to the strong allergenic category. As for rapid allergic reaction test, two tests by active and passive stimulation were conducted. In the active stimulation, 0.5 % GMA with homologous serum albumin was injected intradermally and the challenge was conducted intravenously. Breathing difficulties, wheezing, increased mouth and nose secretions, spasms and death were observed, belonging to the strong allergic category. In the passive stimulation, firstly, the diluted serum given from the sensitized guinea pig was injected subcutaneously to other animals and one hour later, 0.5 ml of 0.1 % GMA with homologous serum albumin was injected intravenously to the same animals. Blue circles or spots observed belonged to the strong allergic category. Both the delayed and rapid allergy test results showed that GMA was a moderate sensitiser. The author reported that this might be the reason that the epoxy radical of GMA easily combined with protein.

4.6.1.2 Human information

Table 17b: Summary of human patch tests

Method	Results	Remarks	Reference
Human Vehicle: petrolatum human patch test	No. with positive reactions: 1st reading: 3 out of 3 (test group); dose: 1% Observations: erythema, oedema and	2 (reliable with restrictions) supporting study	Dempsey (1982) OECD (1999)

Method	Results	Remarks	Reference
	vesiculation	experimental result **Test material: GMA**	
human female human patch test	No. with positive reactions: 1st reading: 1 out of 1 (test group); dose: 0.01% and 0.05% Observations: A patch test conducted on a 31-year-old non-atopic woman showed a reaction to GMA (0.01 and 0.05 % acetone)	2 (reliable with restrictions) supporting study experimental result **Test material: GMA**	Matura (1995) OECD (1999)

There were two studies on human patch tests.

Three cases of allergic contact hypersensitivity to GMA used in adhesive sealant manufacturing were reported. Both closed and open patch testing with 1 % GMA solution in petrolatum was positive in all 3 cases. Symptoms included erythema, oedema, and vesiculation and a strong 2^+ reaction as scored according to the International Contact Dermatitis Research Group classification. (Dempsey: 1982).

Patch test was conducted for a 31-year-old non-atopic woman, who had worked as a chemist and mixed emulsions used to impregnate paper and textile materials to make them oil and water resistant. In this work, she had been in contact with acrylate derivatives (GMA, ethoxyethyl acrylate, etc.). In relation to this work, she had a history of recurrent acute vesiculopapular hand dermatitis with severe itching and burning mainly on the fingertips, palmar and dorsal aspects of the fingers, and both palms. As a result of patch test, she reacted only to nickel, GMA (0.01 and 0.05 % acet.) and ethoxyethyl acrylate among the European standard series and (meth) acrylate series. This reaction to nickel was relevant to her jewelry intolerance. (Matura et al.: 1995).

4.6.1.3 Summary and discussion of skin sensitisation

The key study (Dow, 1990) showed erythema in 7 out of 10 guinea pigs dermally induced with 25% GMA (reduced to 10% for the third induction) and dermally challenged with 1% GMA. The study resembles the Buehler study; however, a negative control group was missing. The induction dose of 10% induced some local effects. However, seen the strong reduction in concentration of the challenge dose it is expected that the observed effects are sensitisation and not irritation. The key study is supported by some other test with limited study information or using a different, non-standard, approach. Although the predictive value of these types of studies is not known, the results were considered positive. There were also a limited number of human cases.

4.6.1.4 Comparison with criteria

The CLP Regulation states that substances shall be classified as skin sensitisers (Category 1) where data are not sufficient for sub-categorisation in accordance with the following criteria:
"(i) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons, or
(ii) if there are positive results from an appropriate animal test"

For a non-adjuvant Guinea pig test, the criteria for a positive result is the induction of a response 15% of the animals or more. Although, the key study was not in line with the OECD requirements for a Buehler study, the positive response in 70% of the guinea pigs supported by positive results in other studies and the presence of a small number of human cases warrant classification for skin sensitisation. However, due to the changes during the study of the induction concentration in the key study from 25% to 10% it is not possible to conclude whether also the criteria for sub-category 1A for a Buehler are fulfilled because these require a response in more than 60% of the guinea pigs (fulfilled) after topical induction between 0.2 and 20% (unclear whether fulfilled). Therefore, the available data do not allow sub-categorisation and category 1 is proposed.

4.6.1.5 Conclusions on classification and labelling

According to the available studies GMA must be classified according to CLP for Skin sensitisation Category 1 (H317) in view of the results found both in humans and animals.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Several animal studies and human data as presented by the DS in the CLH report, are summarised in the tables below.

Table: Summary of animal data

Test	Induction	Challenge	Results	Klimisch score	Limitation	Reference
Buehler test	25% reduced to 10% at 3rd application in DPGME*	1%	7/10 positive (slight erythema)	2 (reliable with restrictions)	No control group	Dow 1990
Test on guinea pigs	No data	No data	6/6 positive	4 (not assignable)	Very limited information	BIBRA 1988
Delayed allergic reaction test	0.1 ml of 1% GMA in acetone for 10 days – skin smear	No data	7/10 positive (hyperaemia, oedema, scleroma, necrosis)	4 (not assignable)	Limited information	Ou-Yang *et al.,* 1988
Delayed allergic reaction test	0.1 ml of 1% GMA in acetone for 10 days – intradermal injection	No data	6/10 positive (hyperaemia, oedema, scleroma, necrosis)	4 (not assignable)	Limited information	Ou-Yang *et al.,* 1988
Rapid allergic reaction test	0.5% GMA with homologus	0.5% GMA with	5/5 positive (breathing	4 (not	Limited	Ou-Yang *et al.,*

(active stimulation)	serum albumin – intradermal injection	homologus serum albumin – intravenous	difficulties, wheezing, increased mouth and nose secretions, spasms, death)	assignable)	information	1988
Rapid allergic reaction test (passive stimulation)	Diluted serum from sensitised animal injected subcutaneously 1 h before challenge	0.5 ml of 0.1% GMA with homologus serum albumin – intravenous	5/5 positive (blue circles or spots)	4 (not assignable)	Limited information	Ou-Yang *et al.,* 1988

* Dipropylene glycol monomethyl ether

Table: Summary of human data

Test	Subjects	Conditions	Results	Klimisch score	Reference
Human patch test	3 cases of allergic contact hypersensitivity to GMA used in adhesive sealant manufacturing	Closed and open patch test with 1% GMA in petrolatum	3/3 positive (erythema, oedema, vesiculation)	2 (reliable with restrictions)	Dempsey, 1982
Human patch test	A 31-year-old non-atopic woman, who had worked in contact with acrylate derivatives including GMA	0.01% and 0.05% in acetone	Positive to GMA and ethoxyethyl acrylate in the European (meth)acrylate series	2 (reliable with restrictions)	Matura, 1995

The key study (Dow, 1990) showed erythema in 7 out of 10 guinea pigs dermally induced with 25% GMA (reduced to 10% for the third induction) and dermally challenged with 1% GMA. The study resembled the Buehler test, but was lacking a negative control group. The induction dose of 10% induced some local effects. However, according to the DS, the significant reduction in the challenge dose suggested that the observed effects were the result of sensitisation and not irritation. The results of the key study was supported by other tests which were reported with limited details or were conducted using a different, non-standard, approach. Although the predictive value of these types of studies was considered to be largely unknown, the results were considered positive. There were also a limited number of human cases reported.

Based on the available studies, the DS proposed to classify GMA for Skin Sens. 1: H317.

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

70% of positive reactions were obtained in the key Buehler study (Dow, 1990), which did not include a negative control group. GMA is corrosive for skin and produced slight erythema in some animals after the second induction application with a concentration of 25%. The third induction dose was reduced to 10%. The reaction to the 1% challenge dose was slight erythema. Since the challenge concentration is considered sufficiently

lower than 25%, irritant effects are unlikely and positive reactions are expected to be the result of sensitisation. The incidence of 70% of positive reactions exceeds the minimum level of 15% of responding animals according to the classification criteria for sub-category 1B in case the induction dose is above 20%. However, the induction concentration was changed from 25% to 10% GMA during the assay and it is therefore not possible to establish whether an incidence of 60% of positive reactions would have been obtained at a topical induction dose between 0.2% and 20% which would lead to classification in sub-category 1A according to the criteria for classification. No sub-categorisation is therefore proposed by RAC for GMA.

RAC notes that the results from other animal studies were of limited reliability due to the absence of detailed information and the use of non-standard protocols. However, all showed positive reactions. In addition, although positive for skin sensitisation, the human data addressed a very small number of cases. Altogether, RAC is of the opinion that these additional studies support the conclusion that GMA is a skin sensitiser.

RAC agrees with the DS that, taken together, the data support the existing classification of GMA as **Skin Sens. 1; H317**.

4.6.2 Respiratory sensitisation

Table 18: Summary table of relevant respiratory sensitisation studies

Method	Results	Remarks	Reference
Deliberately left empty			

4.6.2.1 Non-human information

In none of the acute and sub-acute inhalation studies respiratory sensitisation was observed.

4.6.2.2 Human information

No relevant information available. However, there are no reports found in public literature.

4.6.2.3 Summary and discussion of respiratory sensitisation

In none of the acute and sub-acute inhalation studies respiratory sensitisation was observed. However, these studies are not developed to determine the respiratory sensitising potential of substances.

4.6.2.4 Comparison with criteria

Not relevant.

4.6.2.5 Conclusions on classification and labelling

No classification for respiratory sensitisation is required based on absence of data.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

Respiratory sensitisation was not reported in any of the acute and sub-acute inhalation studies. However, these tests were not developed to determine the respiratory sensitising potential of substances. No classification for respiratory sensitisation was warranted because of the absence of data.

Comments received during public consultation

One MS noted that asthma is a common finding associated with exposure to methacrylates and made a request for potential human cases related to GMA exposure. The DS had not identified any such human cases.

Assessment and comparison with the classification criteria

The association of exposure to methacrylates, in particular to methyl methacrylate (Savonius, 1993, Borak, 2011), with cases of asthma raises a concern that GMA has a potential for respiratory sensitisation. However, in the absence of animal or human data showing respiratory sensitisation of GMA, and since data necessary in order to get a clear understanding of the sensitising and/or irritant properties of members within the group of methacrylate are currently not available to the RAC, RAC recommends **not to classify GMA for respiratory sensitisation**.

4.7 Repeated dose toxicity

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Table 19a: Summary table of relevant repeated dose toxicity studies (oral)

Method	Results	Remarks	Reference
Rat (Crj: CD(SD)) 12 male/12 female combined repeated dose and reproduction / developmental screening (oral: gavage) 10, 30, 100 mg/kg bw/day (nominal) Vehicle: corn oil Exposure: Duration of test: Males: 46 days Females: 40-47 days Post exposure observation period: 1 day	NOAEL: 10 mg/kg bw/day (nominal) (male) based on: test mat. (local adverse effect: squamous hyperplasia in forestomach) NOAEL: 30 mg/kg bw/day (nominal) (female) based on: test mat. (local adverse effect: squamous hyperplasia in forestomach) NOAEL: 30 mg/kg bw/day (nominal) (male/female) based on: test mat. (systemic adverse effect: increase in absolute and relative kidney and adrenal weights; increase in total protein and albumin) LOAEL: 30 mg/kg bw/day	1 (reliable without restriction) key study experimental result **Test material: GMA purity 99.93%**	Ministry of Health and Welfare, Japan (1997) OECD (1999)

Method	Results	Remarks	Reference
Duration of exposure: Males: 45 days Females: from 14 days before mating to day 3 of lactation (daily) OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test)	(nominal) (male) based on: test mat. (local adverse effect: squamous hyperplasia in forestomach) LOAEL: 100 mg/kg bw/day (nominal) (female) based on: test mat. (local adverse effect: squamous hyperplasia in forestomach) LOAEL: 100 mg/kg bw/day (nominal) (male/female) based on: test mat. (systemic adverse effect: increase in absolute and relative kidney and adrenal weights; increase in total protein and albumin)		
Rabbit Sub-acute (oral: unspecified) 50 mg/kg/day Exposure: 15 days (daily)	no NOAEL identified: 50 mg/kg bw/day (nominal) (Fifteen days after treatment, slow reactions and some head shaking was observed in the treated group. In addition, 2 of 10 animals succumbed to prostration and died. There were decrease in body weight, increase in relative weights of heart, liver and kidneys, and some haematological changes (increase in white blood cells, platelets and lymph cells, decrease in leukoplasts and intermediate cells). In pathological examination, there was heart bleeding, deterioration of the heart muscle fibres, widespread fatty changes in the liver with focal necrosis. In kidneys, extravasated blood and haemorrhaging were observed, and the upper part of the renal tubules was swollen. There was also red stained protein in the tubular cavity, cranial haemorrhaging, small focal necrosis of the grey matter, and ulceration and necrosis of the mucous membrane of the stomach. However, incidence of these changes was not reported.	3 not (reliable) supporting study experimental result **Test material: GMA purity 92%**	Ou-Yang (1988) OECD (1999)
Rat male/female	NOAEL: > 0.3 mg/kg bw/day (nominal) (male/female) (No effects in all tissues were	4 (not assignable); experimental result	Hadidian (1968)

Method	Results	Remarks	Reference
chronic (oral: gavage) 0.1 and 0.3 mg/kg/day Exposure: 1 year (5 days/week)	found, which could clearly be related to treatment. However, there was no more information provided)	**Test material: GMA**	OECD (1999)

Key studies

Oral toxicity study of GMA was performed in SD (Crj: CD) rats (n=12) by an OECD combined repeated dose and reproductive/developmental toxicity screening test (OECD TG 422). Administration was conducted at doses of 10, 30 and 100 mg/kg/day by gavage for 45 days in males and from 14 days before mating to day 3 of lactation in females (Ministry of Health and Welfare, Japan: 1997).

Salivation was observed at 30 mg/kg (5/12) and 100 mg/kg (12/12) in males. In males, there was an increase in absolute and relative kidney and adrenal weights at 100 mg/kg. In blood chemistry of males, increase in total protein and albumin was observed. These changes were not considered as adverse effects. In histological examination, squamous hyperplasia in forestomach was observed at 30 and 100 mg/kg in males and cellular infiltration in forestomach at 100 mg/kg in females (see Table 19b). These histological changes were considered to be due to the irritation of GMA. NOAEL (including local effects) for oral repeat toxicity was considered to be 10 mg/kg/day for males and 30 mg/kg/day for females. The NOAEL for repeated dose toxicity (systemic effects) was considered to be 30 mg/kg/day (both sexes).

Table 19b: GMA-induced histological changes in forestomach in rats as reported by the Ministry of Health and Welfare, Japan (1997, pg. 46)

	Male				Female			
	0	10	30	100 mg/kg/day	0	10	30	100
DIGESTIVE SYSTEM								
forestomach	(12)	(11)	(12)	(11)	(12)	(12)	(12)	(12)
edema	4	7	12**	7	0	0	0	0
microgranuloma	0	0	1	0	0	0	0	0
cellular infiltration	6	2	8	9	0	2	3	4*
cellular infiltration, lymphocyte	0	0	0	1	0	0	0	0
hyperkeratosis	0	0	1	0	0	0	0	1
squamous hyperplasia	1	1	11**	11**	3	2	1	5

Two other orally repeated toxicity studies were reported.

One-year study is very limited (Hadidian et al., 1968). Rats (3 males and 3 female) were dosed 5 days/week by gavage at 0.1 mg/kg. Groups of 15 male and 15 female rats were also dosed at 0.3 mg/kg. The authors concluded that no tissue effects related to the treatment were found. These dosages are considered to be too low.

In another study, five male and female rabbits were given orally at 50 mg/kg daily for 15 days (Ou-Yang et al.: 1988). Some animals showed slow reactions, head shaking and prostration, and two animals died. There were several haematological and pathological changes including bleeding, necrosis and so on in heart, liver, kidneys and stomach.

These studies cannot be accepted for hazard assessment because of unreliability such as no Test Guideline, no GLP, unlikely severe systemic toxicity compared to the key study, and insufficient information on protocol and data analysis including purity of substance and pathological data.

4.7.1.2 Repeated dose toxicity: inhalation

Table 19c: Summary table of relevant repeated dose toxicity studies (inhalation)

Method	Results	Remarks	Reference
Rat (Fischer 344) male/female Sub-chronic (inhalation: vapour) 0.5, 2 and 15 ppm (2.9, 12 and 87 mg/m^3, calculated daily dose 0.35, 1.46 and 10.59 mg/kg/day) (nominal conc.) Exposure: 13 weeks (6 hours per day, 5 days per week	NOAEC: 12 mg/m³ air (nominal) (male/female) (Hyperplasia of respiratory epithelium of the nasal tissues, graded as very slight, in all animals.) LOAEC: 87 mg/m³ air (nominal) (male/female) (Hyperplasia of respiratory epithelium of the nasal tissues, graded as very slight, in all animals).	2 (reliable with restrictions) key study experimental result **Test material: GMA purity 99.5%**	Landry (1996) OECD (1999)
Rat (Fischer 344) male/female sub-acute (inhalation: vapour) 0, 10, 40 and 160 ppm (0, 58.2, 233, and 931 mg/m^3, calculated daily dose 0, 7.09, 28.4 and 113 mg/kg/day) (nominal conc.) Exposure: 2 weeks (6 hours per day, 5 days per week)	LOAEC: 58.2 mg/m³ air (nominal) (male/female) (Very slight effects in the nasal cavity in 5 of the 10 rats exposed).	2 (reliable with restrictions) key study experimental result **Test material: GMA purity 99.61%**	Landry (1991) OECD (1999)
Rat Sub-acute (inhalation: vapour) 35 ppm (204 mg/m^3, calculated daily dose 24.9 mg/kg/day) (nominal conc.) Exposure: 2 weeks (6 hours/day, 5 days/week)	LOAEC: 204 mg/m³ air (Decrease in body weight gain, respiratory symptoms, and higher red blood cell count than that of control were observed. There were no histopathologic effects. No remaining exposure-related effects were observed at two weeks after exposure.)	2 (reliable with restrictions) supporting study experimental result **Test material: GMA**	DuPont (1977) OECD (1999)
Rat chronic (inhalation: vapour) 15.3 and 206 mg/m^3 (calculated daily dose: 2.24 and 30.1 mg/kg/day) (nominal conc.) Exposure: 26 weeks (6 hours/day, 6 days/week)	LOAEL: 15.3 mg/m³ air (nominal) (A wide range of chronic toxic effects, such as changes of liver and spleen weight, and enzyme (transaminase) levels in blood or tissue, and lesion in central nervous system, cardiovascular system, liver and kidney, were observed. At 206 mg/m^3, all the changes were more pronounced and the pathological lesions only got worsened after the exposure was ceased. On the other hands, the changes at 15.3 mg/m^3 were sparse and	2 (reliable with restrictions) supporting study experimental result **Test material: GMA purity 92%**	Ouyang Guoshun (1990) OECD (1999)

Method	Results	Remarks	Reference
	slight, and almost all vanished one month after the exposure was ended. Because of the higher vapour pressure and lower purity, the author suggested that the test material used in this study contained components other than GMA, which may have contributed to the toxicity observed.)		
Rabbit (New Zealand White) female Sub-acute (inhalation: vapour) 2, 5 and 10 ppm (11.6, 29.1, 58.2 mg/m^3, calculated daily dose 1.04, 2.62, 5.24 mg/kg/day) (nominal conc.) Exposure: 13 days (6 hours/day, daily)	LOAEC: 11.6 mg/m³ air (nominal) (Olfactory epithelial degeneration)	2 (reliable with restrictions) supporting study experimental result **Test material: GMA**	Cieszlak (1996) OECD (1999)
Rabbit chronic (inhalation: vapour) 15.3 and 206 mg/m^3 (calculated daily dose 1.18 and 15.9 mg/kg/day) Exposure: 26 weeks (6 hours/day, 6 days/week)	LOAEC: 15.3 mg/m³ air (nominal) (A wide range of chronic toxic effects, such as lesion in central nervous system, cardiovascular system, liver and kidney, and other degenerative changes in brain and coverings, were observed. Moreover, there were changes of cardiac EKG (not diagnostic of specified effects) and erythrocyte count. At 206 mg/m^3, all the changes were more pronounced and the pathological lesions only got worsened after the exposure of GMA was ceased. On the other hands, the changes at 15.3 mg/m^3 were sparse and slight, and almost all vanished one month after the exposure was ended. Because of the higher vapour pressure and lower purity, the author suggested that the test material used in this study contained components other than GMA, which may have contributed to the toxicity observed.)	2 (reliable with restrictions) supporting study experimental result **Test material: GMA purity 92%**	Ouyang Guoshun (1990) OECD (1999)

Key study: Landry (1996)

Sub-chronic inhalation toxicity study was conducted in rats at concentrations of 2.9, 12 or 87 mg/m^3 for 13 weeks (6 hours/day, 5 days/week) (Landry et al.: 1996). These three doses were calculated as approximately 0.35, 1.46 or 10.6 mg/kg/day, respectively. There were no treatment related in-life observations, and no significant treatment-related effects on body weight, urinalysis, clinical chemistry or haematology parameters, as well as gross pathologic changes or organ weights at any exposure level. Treatment-related effects were limited to hyperplasia of respiratory epithelium of the nasal tissues in all animals at 87 mg/m^3. In all affected animals, the hyperplastic respiratory epithelium was approximately two to three times as thick as in control animals, and was located in the anterior portions of the nasal passages, involving the tips of the turbinates and the lateral walls of the nasal passages. These changes were considered to have resulted from respiratory irritation. Therefore, NOAEC was considered 12 mg/m^3 (1.46 mg/kg/day) for both sexes.

Supportive studies

Sub-acute inhalation toxicity studies were performed in rats and rabbits. Rats were exposed to GMA at concentrations of 58.2, 233 or 931 mg/m^3 for 2 weeks (6 hours/day, 5 days/week) (Landry et al.: 1991). These three concentrations were calculated as 7.09, 28.4 or 113 mg/kg/day. Decrease in body weight was observed at 233 and 931 mg/m^3. At 931 mg/m^3, general debilitation with noisy and difficult respiration (mouth breathing), eye irritation, corneal clouding and distended abdomen (day 4) were observed. The animals at 931 mg/m^3 were terminated early on day 4 because of the severity of the respiratory and ocular effects. Microscopically, there was severe multifocal necrosis and inflammation of the olfactory epithelium in the nasal cavity. At 233 mg/m^3, there were slight to moderate multifocal necrosis, and inflammation of the respiratory and olfactory nasal epithelium. At 58.2 mg/m^3, microscopically there was very slight multifocal necrosis of individual respiratory epithelial cells in 3 of 5 males and in 2 of 5 females. These changes in respiratory tract were considered due to irritation of GMA. There were no histopathological changes in any other tissues. Therefore, 58.2 mg/m^3 (7.09 mg/kg/day) was considered to be LOAEC because of tissue damages in respiratory tract.

Rabbits were exposed at 2.91, 11.6, 29.1, 58.2 mg/m^3, 6 or 7 hours/day, daily for 13 consecutive days. (Cieszlak et al., 1996, Vedula et al., 1996. Two separate studies). Treatment-related degeneration of the nasal olfactory epithelium was observed at 11.6 mg/m^3. At 29.1 and 58.2 mg/m^3, there were olfactory epithelial degeneration, and the hyperplasia, erosions, ulcers and inflammation of the nasal epithelium. After 4-week recovery period, there was complete reversibility of these changes except for olfactory epithelial degeneration observed at 29.1 and 58.2 mg/m^3, which showed only partial reversibility. At 11.6 mg/m^3, nasal tissue was indistinguishable from controls at one month post-exposure. 2.91 mg/m^3 (0.26 mg/kg/day) was considered to be NOAEC.

In a 26-week inhalation toxicity study at concentrations of 15.3 and 206 mg/m^3 in rats and rabbits (Ouyang Guoshun et al.: 1990), a wide range of toxic effects, such as lesion in central nervous system, cardiovascular system, liver and kidney, were observed in both species at the low and high doses. However, because of the higher vapour pressure and lower purity, the author suggested that the test material used in this study contained components other than GMA, which may have contributed to the toxicity observed. Therefore these systemic toxicities observed in the studies are questionable.

4.7.1.3 Repeated dose toxicity: dermal

No relevant information available on GMA.

4.7.1.4 Repeated dose toxicity: other routes

No relevant information available.

4.7.1.5 Human information

No relevant information available

4.7.1.6 Other relevant information

Several oral and inhalation studies were available for glycidol. No dermal studies for glycidol were available.

Glycidol

Table 19d: Oral repeated dose toxicity studies with glycidol copied from the C&L proposal of glycidol (ECBI-92/95-add.3).

Species/strain	Dose (mg/kg bw)	Duration of treatment	Observations and remarks (specify group size, NOAEL, effects of major toxicological significance)
Rat (F344/N) 5 males and 5 females per dose group	0, 37.5, 75, 150, 300, 600	14 days	All rats receiving 600mg/kg died before end of study period. No other deaths occurred. Final mean body weights of males dosed at 150 or 300mg/kg were respectively 10% and 21% lower than that of vehicle controls. Final mean body weights of dosed and vehicle control female rats were similar. Oedema and degeneration of epididymal stroma was observed in 4/5 males given 300mg/kg. Atrophy of the testis and granulomatous inflammation of the epididymis were seen in the fifth animal (NTP, 1990).
Mouse ($B6C3F_1$) 5 males and 5 females per dose group	0, 37.5, 75, 150, 300, 600	14 days	All mice receiving 600mg/kg died within 4 days. 3/5 males and 2/5 females given 300mg/kg died before end of study. Other deaths occurring in lower dose groups considered to be due to mis-dosing. Final mean body weights of dosed males were similar to the controls. Final female mean body weights in groups dosed at 150 or 300mg/kg were respectively, 7% and 8% lower than that of controls. Females receiving 600mg/kg and males and females receiving 150mg/kg had diarrhoea. Inactivity and ruffled hair coats were observed for 2/5 males and 2/5 females dosed at 600mg/kg and 3/5 males and 2/5 females dosed at 300mg/kg. Focal demyelination in the medulla and thalamus of the brain was present in all female mice given 300mg/kg (NTP, 1990).

Table 19d: Oral repeated dose toxicity studies with glycidol copied from the C&L proposal of glycidol (ECBI-92/95-add.3).

Species/ strain	Dose (mg/kg bw)	Duration of treatment	Observations and remarks (specify group size, NOAEL, effects of major toxicological significance)
Rat (F344/N) 10 males and 10 females per dose group	0, 25, 50, 100, 200, 400	5 days per week, for 13 weeks	All rats dosed at 400mg/kg died by week 2. 3/10 males and 1/10 females given 200mg/kg died before end of study. Final mean body weights of rats in 50, 100 or 200mg/kg groups were respectively, 9%, 4% and 15% lower than that of the vehicle controls for males, and 6%, 7% and 11% lower for females. The frequencies of non-neoplastic lesions in 0, 100, 200 and 400mg/kg groups were respectively as follows: Cerebellar necrosis (males: 0/10, 0/10, 2/10, 10/10; females: 0/10, 0/10, 4/10, 9/10), brain demyelination (males: 0/10, 0/10, 5/10, 6/10; females: 0/10, not reported, 0/10, 6/10), renal tubular cell degeneration/necrosis (males: 0/6, not reported, 0/8, 6/10; females: 0/6, not reported, 0/6, 10/10), thymic lymphoid necrosis (males: 0/6, not reported, 0/8, 2/9; females: 0/6, not reported, 1/7, 9/10), testicular atrophy (2/10, 3/9, 10/10, 9/10) (NTP, 1990).
Mouse ($B6C3F_1$) 10 males and 10 females per dose group	0, 19, 38, 75, 150, 300	5 days per week, for 13 weeks	Due to an error, mice were given 125% of the nominal doses during week 2. All mice given 300mg/kg died by week 2. 4/10 males and 3/10 females given 150mg/kg died before end of study. Final mean body weights of treated animals were 6-10% lower than vehicle controls except for males dosed at 38mg/kg when the value was 5% greater than that for the control group. The frequencies of non-neoplastic lesions observed at 0, 75, 150 and 300mg/kg were respectively as follows: Brain demyelination (males: 0/10, 0/10, 5/10, 1/10; females: 0/10, 0/10, 3/10, 6/10), renal tubular cell degeneration (males: 0/10, not reported, 0/10, 4/10; females: 0/10, not reported, 0/10, 0/10), testicular atrophy/degeneration (2/10, 3/9, 5/10, 1/10) (NTP, 1990). **Data do not support classification with R48 (see Annex 1)**

Glycidol- Inhalation studies

Table 19e: Inhalation repeated dose toxicity studies with glycidol copied from the C&L proposal of glycidol (ECBI-92/95-add.3).

Species	conc. mg/l	Exposure time	Duration of treatment	Observations and remarks (specify group size, NOAEL, effects of major toxicological significance)
Rat (Long-Evans) (10 males)	0, 1.2	7 hours	Daily for 50 days	One rat died of bronchopneumonia between days 49 and 50, but there were no other deaths. Very slight irritation of the eyes, with slight lacrimation and encrustation of the eyelids and slight respiratory distress were observed following the first few exposures to glycidol. These signs of toxicity did not increase in severity with subsequent exposures. Necropsy at the end of dosing revealed no significant gross or microscopic lesions (Hine *et al*, 1956).

Table 19f: Comparison between GMA and glycidol repeated dose-toxicity studies

GMA	Glycidol
Rat: Males: 46 days; Females: 40-47 days Ministry of Health and Welfare, Japan (1997) NOAEL: 10 mg/kg bw/day (nominal) (male) based on: test mat. (local adverse effect: squamous hyperplasia in forestomach) NOAEL: 30 mg/kg bw/day (nominal) (female) based on: test mat. (local adverse effect: squamous hyperplasia in forestomach) NOAEL: 30 mg/kg bw/day (nominal) (male/female) based on: test mat. (systemic adverse effect: increase in absolute and relative kidney and adrenal weights; increase in total protein and albumin) LOAEL: 30 mg/kg bw/day (nominal) (male) based on: test mat. (local adverse effect: squamous hyperplasia in forestomach) LOAEL: 100 mg/kg bw/day (nominal) (female) based on: test mat. (local adverse effect: squamous hyperplasia in forestomach) LOAEL: 100 mg/kg bw/day (nominal) (male/female) based on: test mat. (systemic adverse effect: increase in absolute and relative kidney and adrenal weights; increase in total protein and albumin). The fertility index decreased significantly at 100 mg.kg group due to low sperm motility.	Rat oral 91-day study All rats that received 400 mg/kg died by week 2; three males and one female that received 200 mg/kg died during weeks 11-12. Sperm count and sperm motility were reduced in male rats that received 100 or 200 mg/kg. Necrosis of the cerebellum, demyelineation in the medulla of the brain, tubular degeneration and/or necrosis of the kidney, lymphoid necrosis of the thymus, and testicular atrophy and/or degeneration occurred in rats that received 400 mg/kg. Mouse oral 91-day study All mice that received 300 mg/kg died by week 2; deaths of mice that received 150 mg/kg occurred during weeks 4-8 for males and weeks 1-5 for females. Sperm count and sperm motility were reduced in dosed male mice. Compound-related histopathologic lesions included demyelination of the brain in males and females that received 150 or 300 mg/kg, testicular atrophy in males at all doses, and renal tubular cell degeneration in male mice that received 300 mg/kg. Based on reduced survival, reduced weight gain, and histopathologic lesions in the brain and kidney in rats that received 200 or 400 mg/kg and on reduced survival and histopathologic lesions of the brain in mice that received 150 or 300 mg/kg, doses selected for the 2-year studies of glycidol were 37.5 and 75 mg/kg for rats and 25 and 50 mg/kg for mice.
Rat 90-day Inhalation Landry (1996) NOAEC: 12 mg/m³ air (nominal) (male/female) (Hyperplasia of respiratory epithelium of the nasal tissues, graded as very slight, in all animals.) LOAEC: 87 mg/m³ air (nominal) (male/female) (Hyperplasia of respiratory epithelium of the nasal tissues, graded as very slight, in all animals). Rat 14-day inhalation study DuPont (1977) LOAEC: 204 mg/m³ air (Decrease in body weight gain, respiratory symptoms, and higher red blood cell count than that of control were observed. There were no histopathologic effects. No remaining exposure-related effects were observed at two weeks after exposure).	Rat 50-day Inhalation study (1.2 mg/L; 1200 mg/m^3)) One rat died of bronchopneumonia between days 49 and 50, but there were no other deaths. Very slight irritation of the eyes, with slight lacrimation and encrustation of the eyelids and slight respiratory distress were observed following the first few exposures to glycidol. These signs of toxicity did not increase in severity with subsequent exposures. Necropsy at the end of dosing revealed no significant gross or microscopic lesions.

Rat 182-day inhalation study Ouyang Guoshun (1990) LOAEL: 15.3 mg/m³ air (nominal) (A wide range of chronic toxic effects, such as changes of liver and spleen weight, and enzyme (transaminase) levels in blood or tissue, and lesion in central nervous system, cardiovascular system, liver and kidney, were observed. At 206 mg/m^3, all the changes were more pronounced and the pathological lesions only got worsened after the exposure was ceased. On the other hands, the changes at 15.3 mg/m^3 were sparse and slight, and almost all vanished one month after the exposure was ended. Rabbit 13-day inhalation study Cieszlak (1996) LOAEC: 11.6 mg/m³ air (nominal) (Olfactory epithelial degeneration). Rabbit 182-day study Ouyang Guoshun (1990) LOAEC: 15.3 mg/m³ air (nominal) (A wide range of chronic toxic effects, such as lesion in central nervous system, cardiovascular system, liver and kidney, and other degenerative changes in brain and coverings, were observed. Moreover, there were changes of cardiac EKG (not diagnostic of specified effects) and erythrocyte count. At 206 mg/m^3, all the changes were more pronounced and the pathological lesions only got worsened after the exposure of GMA was ceased. On the other hands, the changes at 15.3 mg/m^3 were sparse and slight, and almost all vanished one month after the exposure was ended.	

4.7.1.7 Summary and discussion of repeated dose toxicity

See chapter 4.8.1.

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

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

The observed effects in the available oral and inhalation repeated dose studies indicate mainly local effects at the port of entry. Systemic effects were absent or limited and are not severe enough for classification. The local effects are due to the irritating/corrosive properties of GMA and are expected to be much more acute than repeated dose effects. Partially different toxicity effects were observed with repeated dose toxicity studies with glycidol probably because GMA is corrosive whereas glycidol is irritant (Table 19f). GMA induced more severe local effects at lower doses/concentrations (oral: squamous hyperplasia in forestomach; inhalation: hyperplasia of

respiratory epithelium of the nasal tissues), in comparison to glycidol (oral: no reported local effects; inhalation: Very slight irritation of the eyes, with slight lacrimation and encrustation of the eyelids and slight respiratory distress were observed following the first few exposures to glycidol). In terms of systemic effects, both compounds induced kidney and male reproductive (GMA: reduction in sperm motility; glycidol: testicular atrophy) toxic effects at comparable doses. However, glycidol induced effects on the brains that were not observed with GMA at an external dose level that was (or could) not be tested with GMA.

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

According to Annex I: 3.9.1.1. of the CLP, '*specific target organ toxicity (repeated exposure) means specific, target organ toxicity arising from a repeated exposure to a substance or mixture. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included.*' In addition, Annex I: 3.9.1.6. of the CLP states that *'Non-lethal toxic effects observed after a single-event exposure are classified as described in Specific target organ toxicity — Single exposure (section 3.8) and are therefore excluded from section 3.9.* '. The effects observed after repeated oral and dermal exposure were limited to local effects on the port of entry and were caused by the irritating/corrosive properties of GMA. It is considered likely that these effects are concentration dependent and not on dose per kg bw and occur already after single exposure at concentrations not so different from the dose levels at which these effects were observed in the repeated dose study. Such effects are considered more relevant for acute classification. Therefore, based on the available studies and based on the other classifications already presented (STOT-SE Category 1 for inhalation and Acute Tox. category 3 and 4 for dermal and oral exposure)), no further classification of GMA for this endpoint is required under CLP (see also sections 4.4.3.3 and 4.4.3.5).

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

Based on the available studies and based on the other classifications already presented, no further classification of GMA for this endpoint is required under CLP (see also sections 4.4.3.3 and 4.4.3.5).

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

Summary of the Dossier Submitter's proposal

According to the DS, the effects observed after repeated oral and dermal exposure were limited to local effects and were caused by the irritating/corrosive properties of GMA. The DS considered it probable that these effects were concentration-dependent and would occur already after a single exposure at concentrations not so different from the dose levels at which these effects were actually observed in the repeated dose study. However, such effects were considered more relevant for consideration of an acute toxicity classification. Therefore, based on the available studies and the other classifications proposed by the DS, i.e. STOT SE 1 (with the respiratory tract as a target organ and the inhalation route as the route of exposure) and Acute Tox. 3 and 4 for the dermal and oral route, respectively, no classification of GMA for this hazard class was proposed by the DS.

Comments received during public consultation

Two MS proposed a classification for STOT RE 1 for the inhalation route, on the basis of local effects observed in the respiratory tract at concentrations lower than in acute inhalation exposure studies. One of them also pointed out the systemic effects observed in the 26-week inhalation studies (Ouyang Guoshun *et al.*, 1990).

Additional key elements

Available studies on GMA were conducted via the oral route (a 2-week study in rabbits and 7-week and 1-year studies in rats) and via the inhalation route (2-week, 13-week and 26-week studies in rats and 2-week and 26-week studies in rabbits).

Oral route

Subacute oral studies (effect levels to be compared with a range of guidance values of 60-600 mg/kg bw/day for category 2 according to CLP, as extrapolated for a 2-week study based on Haber's law)

In a 15-day oral study (Ou-Yang *et al.,* 1988), 10 rabbits were exposed to a dose of 50 mg/kg bw/day. Some animals showed slow reactivity, head shaking and prostration. Two animals died during the study. Decreases in body weight and increases in relative weights of heart, liver and kidney and haematological changes of unknown magnitude were reported. Severe pathological changes were described in the heart (bleeding, deterioration of muscle fibres), liver (fatty changes and focal necrosis), kidney (haemorrhaging, swollen renal tubules and presence of red stained protein), brain (haemorrhaging, focal necrosis of grey matter) and stomach (ulceration and necrosis of mucuous membrane). However, the incidences of these effects were not reported. The purity of the test substance was 92%.

Subchronic oral studies (effect levels to be compared with the range of guidance values of 20-200 mg/kg bw/day for category 2 according to CLP as extrapolated for a 45-day study based on Haber's law)

In a combined repeated dose and reproductive toxicity screening study (OECD TG 422) (MHWJ[2], 1997), rats were exposed to 10, 30 or 100 mg/kg bw/day by gavage for 46 days (males) or 40-47 days (females). At 30 mg/kg bw/day and above, salivation and squamous hyperplasia and cell infiltration in the forestomach were observed in males. At 100 mg/kg bw/day, an increase in kidney and adrenal weights was observed in males and cell infiltration was observed in the forestomach of females.

1-year oral study (effect levels to be compared with the range of guidance values of 2.5-25 mg/kg bw/day for category 2 according to CLP as extrapolated for a 1-year study)

In a 1-year oral study (Hadidian *et al.*, 1968), rats were exposed to a dose of 0.1 (n=3 animals/sex/dose) or 0.3 (n=15 animals/sex/dose) mg/kg bw/day by gavage. No tissue effects related to the treatment were observed. The information available is very limited.

Inhalation route

Subacute inhalation studies (effect concentrations to be compared with the range of guidance values of 1.2 - 6 mg/L/6h/day for vapours for category 2 according to CLP as extrapolated for a 2-week study based on Haber's law)

In a two-week study (Landry *et al.*, 1991), 5 rats/dose/sex were exposed to 0.06, 0.23 or 0.93 mg/L of GMA vapours 5 days/week, 6h/day. At 0.06 mg/L, 5/10 animals exhibited microscopically very slight multifocal necrosis of individual respiratory epithelial cells in the nasal cavity. At 0.23 mg/L, slight to moderate multifocal necrosis and inflammation of the respiratory and olfactory nasal epithelium were observed. At 0.93

[2] Ministry of Health and Welfare, Japan

mg/L, animals were sacrificed at day 4 because of severe respiratory and ocular effects. Necrosis and inflammation in the nasal cavity were severe. General debilitation with noisy and difficult respiration, eye irritation, corneal clouding and distended abdomen were reported. Decreased body weights were observed from 0.23 mg/L.

In another two-week study (Dupont, 1977), rats were exposed to 0.20 mg/L of GMA vapour 5 days/week, 6h/day. Decreased body weight gain, respiratory symptoms and higher red blood cell count but no histopathological effects were observed. No effects were reported two weeks after the end of exposure.

A two-week rabbit study is also available (Cieszlak *et al.*, 1996). Animals were exposed to 0.0036, 0.012, 0.029 or 0.058 mg/L of GMA vapours 6 or 7 h/day for 13 consecutive days. At 0.012 mg/L, degeneration of the nasal olfactory epithelium was observed at the end of the exposure period but could not be observed in the animals after a 4-week recovery period. From 0.029 mg/L, olfactory epithelial degeneration, hyperplasia, erosions, ulcers and inflammation of the nasal epithelium were reported. Some changes persisted after a 4-week recovery period.

<u>90-day inhalation studies (effect concentrations to be compared with the range of guidance values of 0.2 - 1 mg/L/6h/day for vapours for category 2 according to CLP)</u>

Rats were exposed to 0.003, 0.01 or 0.09 mg/L of GMA vapours 5 days/week, 6 h/day (Landry *et al.*, 1996). Hyperplasia of respiratory epithelium of the nasal cavity was observed in all animals at 0.09 mg/L and respiratory epithelium was 2 to 3 times as thick as in controls animals. No other treatment related changes were reported.

<u>26-week inhalation studies (effect concentrations to be compared with the range of guidance values of 0.1 – 0.5 mg/L/6h/day for vapours for category 2 under CLP as extrapolated for a 26-week inhalation study based on Haber's law)</u>

Rats and rabbits were exposed to 0.015 or 0.206 mg/L of GMA vapours 6 days/week, 6h/day (Ouyang Guoshun *et al.*, 1990). Lesions in the central nervous system, cardiovascular system, liver and kidney and other degenerative changes in the brain were observed at both concentration in rats and rabbits. At 0.206 mg/L, all changes were more pronounced after the inhalation exposure of GMA had ended. Almost all changes at 0.015 mg/L vanished one month after the exposure had ended. Because of the higher vapour pressure and lower purity, the author of the study suggested that the test material used in this study contained components other than GMA, which may have contributed to the toxicity observed.

Assessment and comparison with the classification criteria

By the oral route, severe toxicity including 20% deaths was described in rabbits after 15 days of exposure to 50 mg/kg bw/day (Ou-Yang *et al.*, 1988). The reporting of the study was limited, the purity of the test substance was low (92%) and the effects were not consistent with the effects observed in the acute toxicity studies, in which the LD_{50} values ranged from 390 to 1050 mg/kg bw in rats, mice and guinea pigs. The result was also not consistent with the results in the combined repated dose and reproductive toxicity screening study (MHWJ, 1997) in which severe effects were restricted to the gastrointestinal tract of rats exposed to 100 mg/kg bw/day for 45 days. It is therefore uncertain whether the severe effects in rabbits should be attributed to higher sensitivity of rabbits as compared to rats and/or to other potential factors such as toxicity of impurities. The combined study (MHWJ, 1997) is considered to be the only study sufficiently reliable upon which a discussion on classification for repeated dose toxicity via the oral route can be based upon. At doses relevant for classification for STOT RE 2, salivation, forestomach hyperplasia and cell infiltration were observed. Considering the corrosivity of the test substance, these finding are considered adaptative to repeated irritation, in particular in relation to administration by gavage. RAC therefore agrees with the DS that GMA does not warrant classification for repeated toxicity via the oral route.

By inhalation, systemic toxicity in several target organs was observed only in the 26-

week study in rats and rabbits. No systemic effects were reported in other available studies, in particular at the high dose of the 13-week study which was 6 times higher than the low dose in the 26-week study. Considering also the uncertainties raised by the study author on the purity of the test substance, this study was not considered to form a reliable basis for classification.

Local effects in the upper respiratory tract were observed in all 2-week and 13-week studies. At doses relevant for classification for STOT RE 1, multifocal necrosis and inflammation of the nasal epithelium were observed after 2 weeks of exposure in rats and rabbits. Necrosis was slight to moderate in rats at 0.23 mg/L and erosions, ulcers and changes, partially reversible after 4 weeks of recovery, were reported in rabbits from 0.012 mg/L. Hyperplasia of the respiratory epithelium was observed in rats at 0.09 mg/L in the 13-day study. These effects are consistent with the corrosive effects of GMA. The corrosive effects of GMA on the respiratory tract are the basis for the agreed classification for STOT SE 3 for respiratory irritation (see section above). However, RAC considers that significant local effects occurred in repeated dose toxicity studies at doses lower than the effective doses after acute exposure: effective doses of 1.4 to 2.4 mg/L were reported after a single exposure and of 0.9 mg/L after a 4-day exposure whereas the effective doses after repeated exposure were 0.23 and 0.029 mg/L in the two-week studies in rats and rabbits, respectively.

According to the Guidance on the Application of the CLP criteria (version 4.1) (section 3.9.2.5.1) repeated dose effects which occur at doses more than half an order of magnitude lower than the dose that mediates the evident acute toxicity effects (in this case, corrosivity) could be considered to be a repeated dose effect distinct from the acute toxicity. On this basis, RAC concludes that classification of GMA as **STOT RE 1 (respiratory tract) (inhalation)** is justified.

4.9 Germ cell mutagenicity (Mutagenicity)

4.9.1 Non-human information

4.9.1.1 *In vitro* data

Table 20a: Summary table of relevant *in vitro* mutagenicity studies

Method	Results	Remarks	Reference
Bacterial gene mutation assay (gene mutation) Salmonella typhimurium TA95, TA100 (met. act.: with and without) Test concentrations: 112, 224, 448, 896 µg/plate Positive control substance(s): MMS, 2-aminofluorene, 2-amine anthracene	Test results: positive for gene mutations in Salmonella typhimurium TA100 but not in TA95 ; met. act.: with and without ; cytotoxicity: unknown	2 (reliable with restrictions) supporting study experimental result **Test material: GMA purity 92%**	Ouyang (1988) OECD (1999)
Bacterial gene mutation assay	Test results:	2 (reliable with	Voogd (1981)

Method	Results	Remarks	Reference
(gene mutation) bacteria, other: Klebsiella pneumoniae (met. act.: without) Test concentrations: 0.05, 0.1, 0.2, 0.5, 1.0 mmol/L	positive for gene mutations in bacteria, other: Klebsiella pneumoniae ; met. act.: with and without ; cytotoxicity: unknown	restrictions) supporting study experimental result **Test material: GMA purity 92%**	OECD (1999)
mammalian cell gene mutation assay (gene mutation) Chinese hamster Ovary (CHO) (met. act.: with and without) Test concentrations: With metabolic activation: 25-600 microgram/ml Without metabolic activation: 5-80 microgram/ml Positive control substance(s): With metabolic activation: 20-methylcholantrene. Without metabolic activation: ethyl methanesulfonate chinese hamster ovary cell/hypoxantine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward gene mutation assay	Test results: Positive for gene mutations (Positive with metabolic activation, negative without metabolic activation) for Chinese hamster Ovary (CHO) (all strains/cell types tested) ; met. act.: with and without ; cytotoxicity: yes (With metabolic activation: 500 microgram/ml and more. Without metabolic activation: 50 microgram/ml and more)	2 (reliable with restrictions) supporting study experimental result **Test material: GMA purity 99.5%**	Linscombe (1995) OECD (1999)
in vitro mammalian chromosome aberration test (chromosome aberration) CHL/IU cell (met. act.: with and without); plates/test: 3; replicates: 2 Test concentrations: -S9 (continuous treatment): 0.0031, 0.0063, 0.013, 0.025, 0.050 mg/ml -S9 (short-term treatment): 0.0055, 0.011, 0.022, 0.044, 0.088 mg/ml +S9 (short-term treatment): 0.022, 0.044, 0.088, 0.18, 0.35 mg/ml Positive control substance(s): mitomycine C for continuous treatment, cyclophosphamide for	Test results: Positive for chromosomal aberrations (Clastogenicity positive with and without metabolic activation. Polyploidy positive without metabolic activation, ambiguous with metabolic activation) for CHL/IU cell (all strains/cell types tested) ; met. act.: with and without ; cytotoxicity: yes (only without metabolic activation)	1 (reliable without restriction) key study experimental result **Test material: GMA purity 99.93%**	Ministry of Health and Welfare, Japan (1997) OECD (1999)

Method	Results	Remarks	Reference
short term treatment OECD Guideline 473 (*In vitro* Mammalian Chromosome Aberration Test)			
SOS/umu test (SOS-Chromotest) E. coli, other: PQ37 (met. act.: with and without) Test concentrations: 0.1, 0.3 and 1.0 mmol/L	Test results: positive for genotoxic damage in E. coli, other: PQ37 ; met. act.: with and without ; cytotoxicity: unknown	2 (reliable with restrictions) supporting study experimental result **Test material: GMA purity 97%**	Hude (1990) OECD (1999)
bacterial reverse mutation assay (e.g. Ames test) Salmonella typhimurium TA97, TA98, TA100, TA1535 (met. act.: with and without) Test concentrations: 0, 10, 33, 100, 333, 1000 microgram/plate Positive control substance(s): With metabolic activation: 2-aminoanthracene (all strains) Without metabolic activation: sodium azide (TA 100, TA 1535), 9aminoacridine (TA 97), 4-nitro-o-phenylenediamine (TA 98) bacterial reverse mutation assay	Test results: positive for gene mutations in Salmonella typhimurium TA97, TA100, TA1535 but not in TA98 ; met. act.: with and without ; cytotoxicity: unknown	2 (reliable with restrictions) key study experimental result **Test material: GMA purity 98%**	Dorothy (1986) OECD (1999)
bacterial reverse mutation assay (e.g. Ames test) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) Test concentrations: With metabolic activation: 32, 100, 320, 1,000 µg/plate Without metabolic activation: 100, 320, 1,000, 2,000 µg/plate Positive control substance(s): With metabolic activation: 2-aminofluorene, 2-aminoanthracene, dimethylbenz(a)anthracene Without metabolic activation: 2-	Test results: positive for gene mutations in S. typhimurium TA 1535, TA 1537 and TA 100 but not in TA98 ; met. act.: with and without ; cytotoxicity: unknown	2 (reliable with restrictions) key study experimental result **Test material: GMA**	Goodyear (1981) OECD (1999)

Method	Results	Remarks	Reference
nitrofluorene, sodium azide, quinacrene mustard Health, Safety and Government Compliance test method, 79-10			
Analysis of the phenotype and the restriction enzyme mapping level of mutations E. coli, other: HB101 (met. act.: not applicable) Test concentrations: 0.1, 0.3 and 1.0 mmol/L	Test results: positive gene mutations in E. coli, other: HB101 ; met. act.: not applicable ; cytotoxicity: unknown	2 (reliable with restrictions) supporting study experimental result **Test material: GMA**	Xie (1990a) OECD (1999)
sister chromatid exchange assay in mammalian cells Chinese hamster lung fibroblasts (V79) (met. act.: without) Test concentrations: 0, 0.02, 0.039, 0.078, 0.16, 0.31 mM	Test results: positive for chromosomal aberrations in Chinese hamster lung fibroblasts (V79) ; met. act.: without ; cytotoxicity: unknown	2 (reliable with restrictions) supporting study experimental result **Test material: GMA purity 97%**	Hude (1991) OECD (1999)
DNA damage and repair assay, unscheduled DNA synthesis in mammalian cells *in vitro* human lymphocytes Test concentrations: no data	Test results: positive for DNA damage in human lymphocytes	2 (reliable with restrictions) supporting study experimental result **Test material: GMA**	Xie (1990b) OECD (1999)
DNA damage and repair assay, unscheduled DNA synthesis in mammalian cells *in vitro* rat lymphocytes Test concentrations: no data	Test results: positive for DNA damage in rat lymphocytes	2 (reliable with restrictions) supporting study experimental result **Test material: GMA**	Xie (1990b) OECD (1999)
DNA replication human lymphocytes Test concentrations: no data	Test results: positive for non-reverse type inhibition of DNA replication in human lymphocytes	2 (reliable with restrictions) supporting study experimental result **Test material: GMA**	Xie (1989) OECD (1999)
DNA replication	Test results: positive for non-reverse type	2 (reliable with restrictions)	Xie (1989)

Method	Results	Remarks	Reference
rat lymphocytes Test concentrations: no data	inhibition of DNA replication in rat lymphocytes	supporting study experimental result **Test material: GMA**	OECD (1999)
in vitro mammalian cell transformation assay Golden Syrian hamster embryo cells (diploid) Test concentrations: 0.9 - 14.2 mg/L	Test results: positive for transformation in Golden Syrian hamster embryo cells (diploid)	2 (reliable with restrictions) supporting study experimental result **Test material: GMA**	Yang (1996) OECD (1999)
in vitro mammalian cell transformation assay Syrian hamster embryonic cells Test concentrations: no data	Test results: positive for transformation in Syrian hamster embryonic cells (diploid)	2 (reliable with restrictions) supporting study experimental result **Test material: GMA**	Xie (1992) OECD (1999)
DNA binding study Calf thymus DNA (met. act.: not applicable) Test concentrations: no data	Test results: Positive for DNA binding to Calf thymus DNA ; met. act.: not applicable	2 (reliable with restrictions) supporting study experimental result **Test material: GMA**	Xie (1990b) OECD (1999)

Bacterial tests

GMA was mutagenic to Salmonella typhimurium TA97, TA100, TA1535 with and without metabolic activation but not to TA98 (Dorothy et al.: 1986, The Goodyear Tire & Rubber Company: 1981 (key study), OuYang et al.: 1988).

GMA was mutagenic to Klebsiella pneumoniae without metabolic activation (Voogd et al.: 1981). In Escherichia coli, this substance induced SOS repair with and without metabolic activation (von der Hude et al.: 1990). This substance was shown to react with the DNA of the gene governing tetracycline resistance in the plasmid pBR322. The modified DNA was transferred to a receptor cell (Escherichia coli HB 101) to screen for mutations based on alterations in phenotypic changes. Results showed the mutations caused by reactions of GMA with the plasmid were stable and heritable (Xie et al.: 1990a).

Non-bacterial tests *in vitro*

Key studies

Ministry of Health and Welfare, Japan (1997)

Structural chromosomal aberrations (including gap) and polyploidy were induced. Structural chromosome aberrations were induced by GMA in CHL/ IU cells with and without metabolic activation (Table 20b and c). However, a trend test showed no dose-dependency for the induction of polyploidy with the 24 hours continuous treatment and the short-term treatment with the metabolic activation system.

Table 20b: Summary of *in vitro* mutagenicity studies with GMA (Ministry of Health and Welfare, Japan, 1997)

Table 1 Chromosome analysis of Chinese hamster cells (CHL/IU) continuously treated with 2, 3- epoxypropyl methacrylate (EPMA)** without S9 mix

Group	Concentration (mg/ml)	Time of exposure (h)	No. of cells analysed	No. of structural aberrations							Others[3]	No. of cells with aberrations		Polyploid[4] (%)	Trend test[5]		Concurrent[6] cytotoxicity (%)
				gap	ctb	cte	csb	cse	mul[2]	total		TAG (%)	TA (%)		SA	NA	
Control[1]			200	0	0	3	5	0	0	8	0	2 (1.0)	2 (1.0)	0.38			——
Solvent[1]	0	24	200	1	1	0	0	0	0	2	0	2 (1.0)	1 (0.5)	0.25			100.0
EPMA	0.0063	24	200	1	2	1	0	0	0	4	0	4 (2.0)	3 (1.5)	0.26[7]			50.0
EPMA	0.013	24	200	1	5	1	0	0	0	7	1	7 *(3.5)	6 (3.0)	1.38 *	+	−	66.1
EPMA	0.025	24	200	11	339	129	3	0	460	942	0	179 *(89.5)	177 (88.5)	0.50			41.9
EPMA	0.050 ***	24	——											——			——
MC	0.00005	24	200	5	35	118	1	0	20	179	0	95 (47.5)	95 (47.5)	0.25			——
Solvent[1]	0	48	200	0	0	0	0	0	0	0	5	0 (0.0)	0 (0.0)	0.00[8]			100.0
EPMA	0.0063	48	200	1	0	1	2	0	0	4	0	4 (2.0)	3 (1.5)	0.38			62.8
EPMA	0.013	48	200	0	2	2	2	0	0	6	0	4 (2.0)	4 (2.0)	0.52[9]	+	+	30.2
EPMA	0.025	48	200	8	56	141	5	0	80	290	0	97 *(48.5)	95 (47.5)	9.40[10] *			32.6
EPMA	0.050 ***	48	——											——			——
MC	0.00005	48	200	4	38	88	1	4	0	135	5	66 (33.0)	64 (32.0)	0.25			——

Abbreviations, gap : chromatid gap and chromosome gap, ctb : chromatid break, cte: chromatid exchange, csb : chromosome break, cse : chromosome exchange (dicentric and ring), mul : multiple aberrations, TAG : total no. of cells with aberrations, TA : total no. of cells with aberrations except gap, SA : structural aberration, NA : numerical aberration, MC : mitomycin C.
1) Dimethylsulfoxide was used as solvent. 2) More than nine aberrations in a cell were scored as 10. 3) Others, such as attenuation and premature chromosome condensation, were excluded from the no. of structural aberrations. 4) Eight hundred cells were analysed in each group. 5) Cochran • Armitage's trend test was done at $p<0.05$. 6) Relative metaphase frequency to the solvent control, representing cytotoxicity, was caluculated. 7) Seven hundred and eighty one cells were analysed. 8) Seven hundred and ninety one cells were analysed. 9) Seven hundred and sixty three cells were analysed. 10) Seven hundred and sixty six cells were analysed.
* : Significantly different from historical solvent control data at $p<0.05$ by Fisher's exact test using a Bonferroni correction for multiple comparisons. ** : Purity of test substance was 99.93 %. 2- Methyl- 3- methoxypropanoic acid 2, 3- epoxypropyl ester (0.07 %) and hydroquinone monomethyl ether (46 ppm) were contained as impurities. *** : Chromosome analysis was not performed because there were small number of metaphase due to cytotoxicity.

Table 20c: Summary of *in vitro* mutagenicity studies with GMA (Ministry of Health and Welfare, Japan,1997)

Table 2 Chromosome analysis of Chinese hamster cells (CHL/IU) treated with 2, 3- epoxypropyl methacrylate (EPMA)** with and without S9 mix

Group	Concentration (mg/ml)	S 9 mix	Time of exposure (h)	No. of cells analysed	No. of structural aberrations gap	ctb	cte	csb	cse	mul[2]	total	Others[3]	No. of cells with aberrations TAG (%)	TA (%)	Polyploid[4] (%)	Trend test[5] SA	NA	Concurrent[6] cytotoxicity (%)
Control[1]				200	1	0	0	0	0	0	1	0	1 (0.5)	0 (0.0)	0.13			——
Solvent	0	−	6 - (18)	200	1	0	0	0	0	0	1	0	1 (0.5)	0 (0.0)	0.25			100.0
EPMA	0.011	−	6 - (18)	200	0	1	1	0	0	0	2	0	2 (1.0)	2 (1.0)	0.13			72.1
EPMA	0.022	−	6 - (18)	200	0	0	4	0	0	0	4	0	4 (2.0)	4 (2.0)	0.75	+	+	56.6
EPMA	0.044	−	6 - (18)	200	6	45	59	0	2	10	122	0	57 *(28.5)	53 (26.5)	0.88 *			24.6
EPMA	0.088 ***	−	6 - (18)	——														——
CPA	0.005	−	6 - (18)	200	0	0	0	0	0	0	0	1	0 (0.0)	0 (0.0)	0.13			——
Solvent[1]	0	+	6 - (18)	200	0	0	1	0	0	0	1	0	1 (0.5)	1 (0.5)	0.13			100.0
EPMA	0.044	+	6 - (18)	200	0	1	0	0	0	0	1	0	1 (0.5)	1 (0.5)	0.38			117.3
EPMA	0.088	+	6 - (18)	200	0	5	4	2	0	0	11	0	7 (3.5)	7 (3.5)	0.88 *	+	−	94.2
EPMA	0.18	+	6 - (18)	200	1	5	24	0	0	0	30	0	19 *(9.5)	18 (9.0)	0.38			80.8
EPMA	0.35 ***	+	6 - (18)	——														——
CPA	0.005	+	6 - (18)	200	17	30	129	0	0	0	176	0	102 (51.0)	98 (49.0)	0.39[7]			——

Abbreviations, gap : chromatid gap and chromosome gap, ctb : chromatid break, cte: chromatid exchange, csb : chromosome break, cse : chromosome exchange (dicentric and ring), mul : multiple aberrations, TAG : total no. of cells with aberrations, TA : total no. of cells with aberrations except gap, SA : structural aberration, NA : numerical aberration, CPA : cyclophosphamide.
1) Dimethylsulfoxide was used as solvent. 2) More than nine aberrations in a cell were scored as 10. 3) Others, such as attenuation and premature chromosome condensation, were excluded from the no. of structural aberrations. 4) Eight hundred cells were analysed in each group. 5) CochranÅEArmitage's trend test was done at $p<0.05$. 6) Relative metaphase frequency to the solvent control, representing cytotoxicity, was caluculated. 7) Seven hundred and seventy nine cells were analysed. * : Significantly different from historical solvent control data at $p<0.05$ by Fisher's exact test using a Bonferroni correction for multiple comparisons. ** : Purity of test substance was 99.93 %. 2- Methyl- 3- methoxypropanoic acid 2, 3- epoxypropyl ester (0.07 %) and hydroquinone monomethyl ether (46 ppm) were contained as impurities. *** : Chromosome analysis was not performed because there were small number of metaphases due to cytotoxicity.

In cell cultures, GMA induced hypoxanthine-guanine-phosphoribosyl transferase forward gene mutation with metabolic activation in Chinese hamster ovary cell (Linscombe and Engle: 1995), very slight increase of unscheduled DNA synthesis in lymphocytes of human and/or rat (Xie et al.: 1990b), non-reverse type inhibition of the DNA replication in lymphocytes of human and/or rat (Xie et al.: 1989), sister-chromatid exchange without metabolic activation in Chinese hamster V79 cells (von der Hude et al.: 1991), transformation of Syrian hamster embryonic cells (SHE) (Xie et al., 1992) and transformation in diploid golden Syrian hamster embryo (SHE) cells (Yang et al.; 1996). This substance was strongly and covalently bound with calf thymus DNA *in vitro* (Xie et al.: 1990b).

4.9.1.2 *In vivo* data

Table 21a: Summary table of relevant *in vivo* mutagenicity studies

Method	Results	Remarks	Reference
Micronucleus assay mouse (BDF1) 5 male + 5 female/group oral: gavage Male: 188, 375 and 750 mg/kg. Female 250, 500 and 1000 mg/kg (nominal in diet) Positive control substance(s): yes, cyclophosphamide (50 mg/kg) OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	Test results: Mutagenicity: positive (male/female) Micronucleus test in mice is positive after 48 h but only at the highest doses.	1 (reliable without restriction) key study experimental result **Test material: GMA purity 99.93%**	Ministry of Health and Welfare, Japan (1997) OECD (1999)
Transgenic animal mutagenicity assay rat (transgenic Big Blue(R) Fischer 344 rats) male inhalation: vapour 6 hours per day, 5 days per week for 2 weeks followed by 4 weeks for fixation. 1, 10, 25 ppm (5.82, 58.2, 145.5 mg/m^3. Calculated daily dose: 0.71, 7.08, 17.70 mg/kg/day) (nominal conc.)	Test results: Mutagenicity: negative (male) in olfactory and respiratory epithelium	2 (reliable with restrictions) key study experimental result **Test material: GMA**	Gollapudi et al. (1999) OECD (1999)
Micronucleus assay mouse male intraperitoneal (2 treatments, 24 h	Test results: Mutagenicity: ambiguous (male) There was an increase in the	2 (reliable with restrictions) supporting study **Test material:**	Ou-Yang (1988) OECD (1999)

Method	Results	Remarks	Reference
apart) 25, 50 and 100 mg/kg bw Positive control substance(s): methyl pterine	number of cells with micronuclei 6h after last treatment, but this change was very slight and inversed dose-response.	**GMA purity 92%**	
Micronucleus assay mouse (CD-1 (ICR) BR); 5 male + 5 female per group intraperitoneal (single) 75, 150 and 300 mg/kg Positive control substance(s): Cyclophosphamide (120 mg/kg) At 24, 48 and 72 hours after exposure. 40 CFR 798.5395 (The Dow Chemical Company)	Test results: Mutagenicity: negative (male/female)	1 (reliable without restriction) supporting study experimental result **Test material: GMA purity 99.5%**	Lick (1995) OECD (1999)
Micronucleus assay Mouse intraperitoneal 42.2, 133, 422, 464 mg/kg bw	Test results: Mutagenicity: negative	4 (not assignable) supporting study experimental result **Test material: GMA**	INBIFO (1979) OECD (1999)
Unscheduled DNA synthesis 5 Kummimg hybrid male mice/group	Test results: Genotoxicity: ambiguous (male) Unscheduled DNA synthesis was increased in the germ cells, but this change was very slight and not dose related.	2 (reliable with restrictions) supporting study experimental result **Test material: GMA**	Xie (1990b) OECD (1999)

In vivo tests

In a micronucleus assay, mice were administered by gavage with GMA at a single dose of 188, 375 and 750 mg/kg in males and 250, 500 and 1000 mg/kg in females. The frequency of micronucleated polychromatic erythrocytes in both sexes (Table 21c and 21d) was significantly increased only at the highest doses 48 hour after administration. (Ministry of Health and Welfare, Japan: 1997).

Table 21b: Summary of *in vivo* mutagenicity study with GMA (Ministry of Health and Welfare, Japan, 1997)

Table 7. Results of micronucleus test in BDF1 male mice after single administration of 2,3-epoxypropyl methacrylate by gavage

Group	Animal No.	a MNPCE / PCE	b PCE / ERY
Solvent control Olive oil 10 ml/kg	1	5 / 2000	599 / 1000
	2	6 / 2000	542 / 1000
	3	4 / 2000	569 / 1000
	4	4 / 2000	548 / 1000
	5	1 / 2000	609 / 1000
	Total	20 / 10000	2867 / 5000
	%(Mean±S.D.)	(0.20 ± 0.09)	(57.3 ± 3.0)
EPMA 188 mg/kg	6	3 / 2000	521 / 1000
	7	1 / 2000	585 / 1000
	8	6 / 2000	578 / 1000
	9	6 / 2000	569 / 1000
	10	1 / 2000	555 / 1000
	Total	17 / 10000	2808 / 5000
	%(Mean±S.D.)	(0.17 ± 0.13)	(56.2 ± 2.5)
EPMA 375 mg/kg	11	2 / 2000	568 / 1000
	12	5 / 2000	571 / 1000
	13	1 / 2000	495 / 1000
	14	3 / 2000	527 / 1000
	15	4 / 2000	543 / 1000
	Total	15 / 10000	2704 / 5000
	%(Mean±S.D.)	(0.15 ± 0.08)	(54.1 ± 3.1)
EPMA 750mg/kg	16	27 / 2000	474 / 1000
	17	12 / 2000	523 / 1000
	18	14 / 2000	412 / 1000
	19	7 / 2000	394 / 1000
	20	20 / 2000	414 / 1000
	Total	80 / 10000	2217 / 5000
	%(Mean±S.D.)	(0.80 ± 0.39)***	(44.3 ± 5.4)**
Positive control CPA 50 mg/kg	21	56 / 2000	407 / 1000
	22	40 / 2000	482 / 1000
	23	22 / 2000	365 / 1000
	24	38 / 2000	401 / 1000
	25	62 / 2000	437 / 1000
	Total	218 / 10000	2092 / 5000
	%(Mean±S.D.)	(2.18 ± 0.79)***	(41.8 ± 4.4)***

a: Number of micronucleated polychromatic erythrocytes / total number of polychromatic erythrocytes observed
b: Number of polychromatic erythrocytes / total number of erythrocytes observed
CPA: Cyclophosphamide
EPMA: 2,3-epoxypropyl methacrylate
Purity was 99.93 wt % and 0.07 wt % 2,3-epoxypropyl 2-methyl-3-methoxypropanoate and 46 ppm hydroquinone monomethyl ether were contained as impurities.
**: Data significantly different from the solvent control at 1 % level
***: Data significantly different from the solvent control at 0.1 % level

Table 21c: Summary of *in vivo* mutagenicity study with GMA (Ministry of Health and Welfare, Japan, 1997)

Results of micronucleus test in BDF_1 female mice after single administration of 2,3-epoxypropyl methacrylate by gavage

Group	Animal No.	a MNPCE / PCE	b PCE / ERY
Solvent control Olive oil 10 ml/kg	51	5 / 2000	614 / 1000
	52	1 / 2000	602 / 1000
	53	5 / 2000	639 / 1000
	54	5 / 2000	616 / 1000
	55	3 / 2000	573 / 1000
	Total	19 / 10000	3044 / 5000
	%(Mean±S.D.)	(0.19 ± 0.09)	(60.9 ± 2.4)
EPMA 250 mg/kg	56	6 / 2000	572 / 1000
	57	2 / 2000	648 / 1000
	58	4 / 2000	617 / 1000
	59	5 / 2000	554 / 1000
	60	4 / 2000	581 / 1000
	Total	21 / 10000	2972 / 5000
	%(Mean±S.D.)	(0.21 ± 0.07)	(59.4 ± 3.8)
EPMA 500 mg/kg	61	9 / 2000	621 / 1000
	62	5 / 2000	634 / 1000
	63	10 / 2000	582 / 1000
	64	5 / 2000	572 / 1000
	65	3 / 2000	580 / 1000
	Total	32 / 10000	2989 / 5000
	%(Mean±S.D.)	(0.32 ± 0.15)	(59.8 ± 2.8)
EPMA 1000mg/kg	66	15 / 2000	467 / 1000
	67	9 / 2000	599 / 1000
	68	13 / 2000	503 / 1000
	69	20 / 2000	527 / 1000
	70	6 / 2000	556 / 1000
	Total	63 / 10000	2652 / 5000
	%(Mean±S.D.)	(0.63 ± 0.27)***	(53.0 ± 5.0)*
Positive control CPA 50 mg/kg	71	66 / 2000	547 / 1000
	72	71 / 2000	622 / 1000
	73	55 / 2000	563 / 1000
	74	45 / 2000	548 / 1000
	75	33 / 2000	515 / 1000
	Total	270 / 10000	2795 / 5000
	%(Mean±S.D.)	(2.70 ± 0.77)***	(55.9 ± 3.9)

a: Number of micronucleated polychromatic erythrocytes / total number of polychromatic erythrocytes observed
b: Number of polychromatic erythrocytes / total number of erythrocytes observed
CPA: Cyclophosphamide
EPMA: 2,3-epoxypropyl methacrylate
Purity was 99.93 wt % and 0.07 wt % 2,3-epoxypropyl 2-methyl-3-methoxypropanoate and 46 ppm hydroquinone monomethyl ether were contained as impurities.
*: Data significantly different from the solvent control at 5 % level
***: Data significantly different from the solvent control at 0.1 % level

Three additional mouse bone marrow micronucleus tests by intraperitoneal (ip) administration have been reported. In one study, this substance produced a 2-3 fold increase in micronuclei at all administered doses relative to the control although it showed an inverse dose response (Ou-Yang et al., 1988). On the other hand, this substance did not cause an increase in the number of cells containing micronuclei in two other ip injection studies with GMA doses of 75, 150, and 300 mg/kg (Lick et al., 1995) or doses of 42.2, 133, 422, and 464 mg/kg (INBIFO: 1979). The study by Lick (1995) fulfils the OECD criteria. The highest dose of 300 mg/kg bw was based on a range finding in

which 100% mortality occurred at 500 mg/kg bw and no mortality at 250 mg/kg bw. At the highest dose tested there was no change in the PCE%.

Lick (1995)

Glycidyl Methacrylate (GMA) was evaluated in the mouse bone marrow micronucleus test. The micronucleus test is capable of detecting agents causing chromosomal aberrations and spindle malfunction. The test material was administered to CD-1 mice by single intraperitoneal injection at dose levels of 0 (negative control), 75, 150, and 300 mg/kg body weight (BW). The highest dose level of 300 mg/kgBW was based upon the results of a range-finding test. The concentrations of the test material in the dosing solutions were verified by either gas chromatography with flame ionization detection or high performance liquid chromatography. Groups of animals were sacrificed at 24, 48, or 72 h after treatment. Mice treated orally with 120 mg/kgBW cyclophosphamide and sacrificed at 24 h served as positive controls. There were five animals per sex per dose level per sacrifice time. One thousand polychromatic erythrocytes (PCE) were evaluated from each surviving animal and the frequencies of micronucleated polychromatic erythrocytes (MN-PCE) were recorded. There were no statistically significant increases in the frequencies of MN-PCE in groups treated with the test material as compared to negative controls. The positive control mice showed significant increases in MN-PCE. Hence, under the experimental conditions used, the test material was considered to be negative in the mouse bone marrow micronucleus test.

Xie (1990b)

Sperm abnormality tests and assays of unscheduled DNA synthesis in germ cells of male mice were conducted to study the *in vivo* genotoxicity of GMA. The results revealed that GMA could damage DNA, increase sperm abnormality frequency, and reduce the number of sperm cells.This substance increased unscheduled DNA synthesis in germ cell of male mice but this effect was very slight (~25% above controls for all doses administered) and not dose-related (Xie et al.: 1990b).

Assay of UDS in the Germ Cell of Male Mice Exposed in Vivo to the GMA

Kunmimg hybrid male mice, 10–14 weeks old at the start of the experiment, were used. GMA was dissolved in peanut oil, and the concentration of GMA was adjusted so that a standard 30-g mouse would receive an ip injection of 0.5 ml. The animals were randomly divided into five groups with five mice in each group. The negative control animals were injected ip with peanut oil. Those in the GMA experiments were injected ip with 25, 50, and 100 mg of GMA per kg body wt, respectively. Those in the positive control group were injected ip with 200 mg of cyclophosphamide (CP) per kg body wt. Each mouse was immediately anesthetized with ether after the injections were given. Each of these animals received a dose of 72 μCi of [^{3}H]TdR divided into equal parts and injected separately into testis in 0.05 ml of 2% ethanol. All animals were killed 16 days after the treatments. The caudal epididymides were removed and washed with physiological saline. After filtration and centrifugation, the sperm cells were recovered for hematocytometer counts. Thereafter the sperm cells were transferred onto glass fiber membrane and dried and a toluene:PPO:POPOP scintillation mixture was added. The radioactivity was measured in a Beckman liquid scintillation counter. Results were expressed as mean values of cpm/10^6 sperm cells.

Table 21d: Unscheduled DNA synthesis induced by GMA in germ cells of male mice (Xie et al. 1990b)

UDS Induced by GMA in Germ Cells of Male Mice

Group	Dose (mg/kg)	No. of animals	cpm/10^6 Sperms ($\bar{X} \pm$ SD)
Control	0	6	3.77 ± 0.09
GMA	25	5	5.07 ± 0.34[a]
	50	5	4.98 ± 0.15[a]
	100	5	4.99 ± 0.19[a]
CP[b]	200	5	5.30 ± 0.34[a]

[a] Control vs GMA group, $P < 0.01$.
[b] Cyclophosphamide.

GMA was evaluated in an *in vivo* assay for the induction of gene mutations at the lacI locus of transgenic Big Blue Fischer 344 (F-344) rats. The rats (15 males/group) were exposed to GMA vapours by inhalation at targeted concentrations of 0 (negative control), 1, 10, and 25 ppm for 2 weeks, 6 hrs/day, 5 days/week followed by a 4 week fixation period. There were no statistically significant increases in the frequencies of lacI mutants in either the olfactory or respiratory epithelium of rats exposed to 25 ppm (145.5 mg/cm^3) (17.7 mg/kg/day) when compared to the corresponding negative control values. Based upon these results, the authors concluded that GMA was not mutagenic to the nasal epithelium of rats under the conditions of the study (Gollapudi et al., 1999).

4.9.2 Human information

No relevant information available

4.9.3 Other relevant information

Table 21e: Additional mutagenicity data on Glycidol for read-across analysis

Method	**Results**	**Remarks**	**Reference**
micronucleus assay (chromosome aberration) mouse (B6C3F1) male intraperitoneal 2 times at 24h intervals Positive control substance(s): Mitomycin C (1 mg/kg) OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	Evaluation of results: positive Test results: Genotoxicity: positive (male); toxicity: no effects ; vehicle controls valid: yes; negative controls valid: yes; positive controls valid: yes	1 (reliable without restrictions) supporting study experimental result **Test material: 2,3-epoxypropan-1-ol (glycidol) purity 94%** Form: liquid	Irwin et al. (1990)

Metabolism of GMA to glycidol

In view of the fact that GMA is metabolised to glycidol, information is being provided below on the genotoxicity of glycidol, as supporting data for an evaluation of the genotoxic potential of GMA.

Glycidol was evaluated for the potential to cause micronuclei in mouse bone marrow *in vivo* (NTP, 1990). Male mice were given two intraperitoneal injections of 37.5, 75 and 150 mg/kg, 24 hours apart, with the glycidol dissolved in phosphate-buffered saline; the total dose volume was 0.4 ml. Solvent control animals were injected with 0.4 ml phosphate-buffered saline only. The positive control mice received injections of mitomycin C. Twenty-four hours after the second injection, the mice were killed by cervical dislocation, and smears were prepared of the bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained; 2,000 PCEs were scored for the number of micronucleated cells in each of five animals per dose group. The results were tabulated as the mean of the pooled results from all animals within a dose group +/- standard error of the mean. Preliminary range-finding studies were performed to determine appropriate doses for the *in vivo* micronucleus test. Dose selection in this study was based on animal lethality; no decrease in the percentage of polychromatic erythrocytes (PCEs) in the bone marrow was observed in any of the dose groups. In the first trial, the incidence of micronuclei was increased statistically above concurrent phosphate-buffered saline controls. Micronuclei incidence was increased at doses of 75 and 150 mg/kg. In a second trial, incidences of micronuclei were statistically increased above concurrent control values at 37.5 and 150 mg/kg but not at 75 mg/kg. (It is noted that the control for the second trial was lower that the control in the first trial.)

Table 21f: Summary of micronucleus study with Glycidol (NTP, 1990)

TABLE H7. INCIDENCE OF MICRONUCLEI IN BONE MARROW POLYCHROMATIC ERYTHROCYTES OF MICE ADMINISTERED GLYCIDOL (a)

	Dose (mg/kg)	Micronucleated Cells/1,000 Cells (b) Trial 1	Trial 2
Vehicle controls (c)	0	1.5 ± 0.4	0.6 ± 0.2
Glycidol	37.5	1.5 ± 0.3	1.3 ± 0.3
	75	2.4 ± 0.4	0.7 ± 0.3
	150	4.4 ± 0.8	1.9 ± 0.6
		P<0.001	0.01<P<0.05
Mitomycin C (d)	1	37.7 ± 4.6	30.2 ± 2.7

(a) Study performed at Environmental Health Research and Testing, Inc. Glycidol, dissolved in phosphate-buffered saline, was administered by intraperitoneal injection to male $B6C3F_1$ mice two times, at 24-hour intervals; bone marrow smears were prepared 24 hours after the second injection. For each trial, 2,000 polychromatic erythrocytes were scored for the number of micronuclei in each of five animals per dose group.
(b) Mean ± standard error of the mean
(c) Vehicle control animals received injections of 0.4 ml phosphate-buffered saline.
(d) Positive control material was dissolved in phosphate-buffered saline and administered by intraperitoneal injection.

Glycidol has a harmonised classification as a germ cell mutagen category 2 under CLP.

4.9.4 Summary and discussion of mutagenicity

Summary

Genotoxicity studies on GMA *in vitro* showed positive results. In micronucleus tests *in vivo*, oral administration of GMA increased the frequency of micronucleated polychromatic erythrocytes at the highest dose only, while mostly negative results were shown in other *in vivo* genotoxicity studies including micronucleus tests by intraperitoneal administration and including the gene mutation study using transgenic Big Blue Fischer 344 rats. The negative i.p. micronucleus test (Lick, 1995) was performed with lower exposure levels compared to the positive oral study. This could be explained by a higher mortality after i.p. exposure of the unprotected peritoneal compared

to the stomach after oral exposure. The dose exposure levels applied in the i.p. studies were also negative in the oral study. Also no decrease in PCE% was observed in the i.p study at the highest dose level which was observed at the highest dose level in the oral study. Further, *in vitro* and *in vivo* studies indicate carboxylesterase mediated hydrolysis of GMA to glycidol. Glycidol, a metabolite of GMA, is classified as a Category 2 germ cell mutagen under CLP. Based on the available studies for GMA itself and read across data for glycidol, GMA is considered to be a substance mutagenic towards somatic cells. There is only one in vivo study on germ cells which showed a significant increase in UDS in sperm cells. However, the increase was small and not dose related.

4.9.5 Comparison with criteria

Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans.
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.

Note: Substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

This proposal is based on a positive somatic cell mutagenicity test in vivo in mammals (micronuclei tests, Ministry of Health and Welfare, 1997)) although a comparable test was negative. This could be explained by the lower dose level in the negative test. However, the positive tests are supported by positive tests in vitro and by the formation of the metabolite glycidol, which is already classified as a category 2 germ cell mutagen.

There is only very limited information regarding germ cell mutagenicity in which only a slight and not dose-related increase in unscheduled DNA synthesis was observed in the germ cells. This is not sufficient for category 1B.

Category 1A is not applicable as there are no human data.

4.9.6 Conclusions on classification and labelling

According to the available studies GMA must be classified according to CLP for Germ cell Mutagenicity Category 2 (H341).

RAC evaluation of germ cell mutagenicity
Summary of the Dossier Submitter's proposal
Genotoxicity studies on GMA *in vitro* showed positive results. In a micronucleus tests *in vivo*, oral administration of GMA increased the frequency of micronucleated polychromatic erythrocytes at the highest dose only. Other *in vivo* genotoxicity studies were mostly negative, including micronucleus tests with intraperitoneal (IP) administration and a gene mutation study with transgenic Big Blue Fischer 344 rats. The negative IP micronucleus test by Lick *et al.* (1995) was performed at lower exposure levels compared to the positive oral

micronucleus study. According to the DS the choice of a lower dose in the IP studies could be explained by a higher mortality after IP exposure to the unprotected peritoneal compartment as compared to the stomach after oral exposure. Similar dose levels as applied in the IP studies did not induce positive responses either in the oral study. Also no decrease in PCE% was observed in the IP study at the highest dose level in contrast to what was observed at the highest dose level in the oral study.

Furthermore, *in vitro* and *in vivo* studies indicated carboxylesterase-mediated hydrolysis of GMA to glycidol. Glycidol, a metabolite of GMA, is already classified for Muta. 2 under CLP. Based on the available studies with GMA itself and on evidence on glycidol, GMA was considered by the DS to be mutagenic in somatic cells. There was only one *in vivo* study on germ cells of mice which showed a significant increase in unscheduled DNA synthesis (UDS) in male germ cells. However, the increase was small and not dose related. The DS concluded that GMA should be classified as Muta. 2; H341.

Comments received during public consultation

Classification as Muta. 2; H341 was supported by one MS. Another MS suggested Muta. 1B; H340 based on the sperm abnormalities observed in one study.

Additional key elements

GMA induced *in vitro* gene mutations in bacteria and mammalian cells, chromosomal aberrations and genotoxic damages detected by SOS-chromotest, UDS and inhibition of DNA-replication. GMA was also positive in transformation assays and showed DNA binding. Although some studies had limitations such as low or unknown purity, induction of structural aberrations was demonstrated in a study of good quality (MHWJ, 1997) using GMA of 99.93% purity. Effects were observed with and without metabolic activation. In this study polyploidy was also observed but the absence of dose-dependency was noted in some parts of the assay.

In vivo genotoxicity studies are summarised in the tables below. Micronuclei were induced in erythrocytes 48 hours after administration of GMA by gavage at the highest dose of 750 mg/kg bw in male mice and at 1000 mg/kg bw in female mice in a guideline-compliant study. Via the inhalation route, no gene mutations were observed in the nasal epithelium of transgenic Big Blue ® rats at doses that were expected to produce some local toxicity (very slight necrosis at 58 mg/m^3 under similar exposure conditions in Landry *et al.* (1991)). Negative or equivocal results were obtained by the intraperitoneal route in micronucleus assays in the bone marrow, including a guideline-compliant study (Lick *et al.*, 1995) reporting a clear negative result.

Summary of *in vivo* studies - somatic cells

Type of test	Species	Method	Concentrations	Remarks	Results	Reference
Micronucleus assay	Mice	Single gavage	Males: 188, 375 or 750 mg/kg bw Females: 250, 500 or 1000 mg/kg bw	Purity: 99.93% OECD TG 474	Positive	MHWJ, 1997
Transgenic animal gene mutation assay	Male Big Blue ® Fisher 344 rats	Vapour inhalation 6h/d, 5d/wk for 2 wk	5.82, 58.2 or 145.5 mg/m^3	Purity not known.	Negative (olfactory and respiratory epithelium)	Gollapudi *et al.,* 1999

Bone marrow micronucleus test	Male mice	Two IP injections 24h apart	25, 50 or 100 mg/kg bw	Purity: 92%	Equivocal (2-3 fold increase at all doses but inverse dose-response)	Ou-Yang *et al.,* 1988
Bone marrow micronucleus test	CD-1 mice	Single IP injection	75, 150 or 300 mg/kg bw	Purity 99.5% OECD guideline No change in the PCE%	Negative	Lick *et al.,* 1995
Bone marrow micronucleus test	Mice	IP injection	42.2, 133, 422 or 464 mg/kg bw	Purity not known. Limited information	Negative	INBIFO, 1979

One study investigated germ cell mutagenicity and is summarised below.

In vivo study - germ cells

Type of test	**Species**	**Method**	**Concentrations**	**Remarks**	**Results**	**Reference**
UDS (Uncheduled DNA synthesis) (Cpm)	Kumming hybrid male mice	5 daily IP injections	25, 50 or 100 mg/kg bw	Purity not known	Equivocal Group / Dose (mg/kg) / Cpm /10^6 sperm cells Control / 0 / 3.77±0.09 GMA / 25 / 5.07±0.34* GMA / 50 / 4.98±0.15* GMA / 100 / 4.99±0.19* Cyclophos-phamide / 200 / 5.30±0.34* Slight increase. No dose-response.	Xie *et al.,* 1990b

Toxicokinetic data shows that GMA is metabolised into glycidol (and methacrylic acid). Glycidol is classified as Muta. 2; H341 and an *in vivo* study on glycidol is presented in the CLH report and is summarised below.

In vivo study - glycidol

Type of test	**Species**	**Method**	**Concentrations**	**Remarks**	**Results**	**Reference**
Bone marrow micronucleus test	Mice	Two IP injection 24h apart	37.5, 75 or 150 mg/kg	Purity 94% OECD guideline No change in the PCE% - selection of doses based on mortality.	Positive (significant increase in the two trials – incidence of micronuclei 3 times higher in high dose group vs controls)	NTP, 1990

Assessment and comparison with the classification criteria

According to the CLP criteria:

> "*The classification in Category 1A is based on positive evidence from human epidemiological studies*" and targets "*substances to be regarded as if they induce heritable mutations in the germ cells of humans*".

No human data are were available and classifications as Muta. 1A is not warranted.

"The classification in Category 1B is based on:
– positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or
– positive result(s) 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 metabolite(s) 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".

One study (Xie *et al.*, 1990b) provided some evidence of genotoxicity in germ cells *in vivo*. The observed unscheduled DNA synthesis (UDS) was slight (24-25% above controls) but the effect induced by the positive control was also slight (+29%). The reliability of this study was, however, uncertain and the result was obtained via the IP route. Infertility observed in males at 100 mg/kg bw/day via the oral route (MHWJ, 1997) may also indicate that GMA is able to reach the germ cells and to induce mutagenity. However, the mode of action has not been investigated and there is no direct evidence that GMA is bioavailable to germ cells and can induce mutagenicity in germ cells via a physiological route of exposure. The data are therefore considered insufficient to warrant classification as Muta. 1B.

"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."

GMA induced micronuclei in mouse erythrocytes in an OECD TG study (MHWJ, 1997) providing evidence of somatic cell mutagenicity *in vivo* meeting the classification criteria for Muta. 2. In contrast, negative results were obtained in an IP study of good quality (Lick *et al.*, 1995). However, it is noted that positive results were obtained at the high dose (750/1000 mg/kg bw) via the oral route whereas the highest dose tested by the IP route was only 300 mg/kg bw. Comparison of the bioavailablility of the test substance/metabolite(s) in the target cells after dosing via two different routes of exposure is usually not directly possible in the absence of information on the relative absorption via the two routes. However, it is noted that the LD_{50} values obtained via the IP route (290-350 mg/kg bw) do not differ very substantially from LD_{50} values obtained via the oral route (390-1050 mg/kg bw). The discrepancy between the results on mutagenicity obtained via the oral and IP routes remains overall unclear, but RAC concludes that the negative result via the IP route cannot invalidate the clear mutagenic response observed via oral route at the high dose. The mutagenic potential of GMA is also supported by a consistent induction of genotoxic effects in the numerous *in vitro* studies that are available.

In addition, GMA is metabolised into glycidol (and methacrylic acid). Glycidol induces micronuclei and has an existing entry in Annex VI to the CLP Regulation as Muta. 2; H341. Glycidol also induces tumours in multiple tissues, which is consistent with a mutagenic mode of action. Glycidol data are considered as supportive evidence of GMA mutagenicity.

Considering these data, RAC concludes that a classification of GMA as **Muta. 2; H341** is warranted.

4.10 Carcinogenicity

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

In a very limited one-year study (Hadidian et al., 1968), rats (3 males and 3 females) were dosed 5 days/week by gavage at 0.1 mg/kg. Groups of 15 male and 15 female rats were also dosed at 0.3 mg/kg. The authors concluded that GMA gave a tumor incidence pattern similar to that of controls. However the doses applied are considered to be too low and the number of animals was very small.

Table 22: Summary of carcinogenicity study (Hadidian et al. 1968)

Compound	(Number)	Dose† (mg)	Number and sex	Average‡		Average body weight (g)			Liver (g)	Neoplastic lesions, No. of rats with lesions	Non-neoplastic lesions
				Number Rx	Survival (days)	Initial	Week 18	Final			
Glycidyl methacrylate.....	(25)	0.3	3 M	260	557	65	302	447	17(16-17)		
			3 F	260	451	57	182	305	12(10-14)	Ad-Lu 1; FAd-Ma 1	
		0.1	15 M	257	509	54	275	416	17(10-22)	Ic-Ts 4	Hep-Tox 1
			15 F	260	526	54	176	288	11 (8-15)	Ly 1; Ad-Br 1	Pol-Ut 1
		0.001 -0.03	12 M	260	541	62	287	430	18(14-21)	Ic-Ts 5	Hep-Tox 1
			12 F	260	564	48	175	306	12 (8-16)	FAd-Ma 2	Pol-Ut 2

*For abbreviations *see* table 3. †Dose refers to mg per day per animal. ‡Number Rx = number of individual doses; Survival = survival period for group.

Organs

Adr	= Adrenal	Ma	= Mammary		
Br	= Bronchus	Mo	= Mouth		
Bl	= Bladder	Pan	= Pancreas		
Ce	= Cecum	Ph	= Pharynx		
Co	= Colon	Pi	= Pituitary		
Duo	= Duodenum	Pr	= Prostate		
Ea	= Ear	Seb	= Sebaceous gland		
En	= Endometrium				
Es	= Esophagus	Sk	= Skin		
Hep	= Liver	Sp	= Spleen		
Il	= Ileum	St	= Stomach		
Int	= Intestine	Th	= Thyroid		
Ki	= Kidney	Tn	= Tongue		
La	= Larynx	Tr	= Trachea		
Li	= Lip	Ts	= Testes		
Lu	= Lung	Ut	= Uterus		

Lesions

AdCa	= Adenocarcinoma	He	= Hepatoma		
Ad	= Adenoma	Hyp	= Hypertrophy		
At	= Atrophy	Hypl	= Hyperplasia		
Ba	= Basal cell tumor	Ic	= Interstitial cell tumor		
Ca	= Carcinoma	Lei	= Leiomyoma		
Ci	= Cirrhosis	Lp	= Lipoma		
Ch	= Cholangioma	Ly	= Lymphoma		
Cy	= Cyst	Me	= Mesothelioma		
FAd	= Fibroadenoma	Met	= Metastasis		
Fi	= Fibroma	Pa	= Papilloma		
FSa	= Fibrosarcoma	Pol	= Polyp		
Gr	= Granulosa cell tumor	Sa	= Sarcoma		
		Sq	= Squamous cell		
Gran	= Granuloma	Tox	= Toxic		

4.10.1.2 Carcinogenicity: inhalation

In a 26-weeks inhalation toxicity study at concentrations of 15.3 and 206 mg/m^3 in rats and rabbits (Ouyang Guoshun et al., 1990), a wide range of toxic effects were observed in both species at both concentrations. A wide range of chronic toxic effects, such as changes of liver and spleen weight, and enzyme (transaminase) levels in blood or tissue, and lesion in central nervous system, cardiovascular system, liver and kidney, were observed. At 206 mg/m^3, all the changes were more pronounced and the pathological lesions only got worsened after the exposure was ceased. On the other hand, the changes at 15.3 mg/m^3 were sparse and slight, and almost all vanished one month after the exposure had ended. Because of the higher vapor pressure and lower purity, the author suggested that the test material used in this study contained components other than GMA, which may have contributed to the toxicity observed. Therefore, the systemic toxicity observed in the studies are questionable.

4.10.1.3 Carcinogenicity: dermal

No relevant information available

4.10.2 Human information

No relevant information available

4.10.3 Other relevant information

There are few reports on chronic exposure studies with GMA, but each one has significant methodological deficiencies (low doses, few animals and low GMA purity) which make their results questionable. Consequently, a read-across for GMA was used with the rationale that although the kinetics of carboxylesterase-mediated hydrolysis of GMA appear to be species dependent, the primary metabolite of GMA found in humans, rats and rabbits is glycidol. Chronic bioassay data were located for glycidol in rats and mice.

Table 23a: Summary table of relevance for carcinogenic assessment of GMA

Method	**Results**	**Remarks**	**Reference**
rat (Fischer 344) male/female: 0, 37.5 and 75 mg/kg/d (nominal) Mouse (B6C3F1) male/female: 0, 25 or 50 mg/kg bw/day (nominal) 50 animals/sex/group oral: gavage Vehicle: water Exposure: 103 weeks (5 days per week)	Positive. An increase in tumors at multiple sites in both sexes of both species.	1 (reliable without restrictions) read-across from supporting substance (structural analogue or surrogate) **Test material: 2,3-epoxypropan-1-ol (glycidol) purity 94%** See endpoint summary for justification of read-across Form: liquid	Irwin et al. (1990) IARC (2000)

The results of studies on carcinogenicity after oral administration of glycidol are summarized as follows:

Groups of 50 male and 50 female Fischer 344 rats, eight weeks of age, were administered 0, 37.5 or 75 mg/kg bw of glycidol (purity, 94%) in distilled water by gavage on five days per week for 103 weeks. Survival of rats was significantly lower in the treated groups in both males and females than in the control groups, with the mean survival being 92, 82 and 66 weeks for the control, mid- and high-dose males, respectively, and 97, 85 and 78 weeks for the female dose groups. As shown in Table 3 of the IARC monograph, respectively in the main table in the abstract of the NTP report (Irwin, 1990), there was a statistically significant increase in the incidence of mesotheliomas of the tunica vaginalis/peritoneum in males at both 37.5 and 75 mg/kg bw. There was a statistically significant increase in the incidence of fibroadenoma and adenocarcinoma of the mammary gland in female rats, and of mammary fibroadenoma in male rats at both doses in each

case. The incidences in the forestomach of squamous-cell papilloma and of squamous papilloma or carcinoma combined were significantly increased in female rats at both doses and in male rats at the high dose. Gliomas of the brain were significantly increased in both sexes at the high dose. Other tumour types with increased incidence included squamous-cell papillomas or carcinomas of the mouth or tongue, adenomas or carcinomas of the clitoral gland and leukaemia in female rats; and Zymbal gland carcinomas, thyroid follicular-cell adenomas or carcinomas, skin tumours and adenomatous polyps or adenocarcinomas of the intestine combined in male rats. According to the National Toxicology Program Review Panel, there was clear evidence of carcinogenicity in male and female Fischer 344 rats. (Irwin, 1990; IARC, 2000).

Table 23b: Tumor incidence at each dose level of Glycidol (Irwin, 1990)

NEOPLASMS ASSOCIATED WITH THE TWO-YEAR GAVAGE ADMINISTRATION OF GLYCIDOL (a)

Site/Neoplasm	Male			Female		
RATS	**Veh. Control**	**37.5 mg/kg**	**75 mg/kg**	**Veh. Control**	**37.5 mg/kg**	**75 mg/kg**
Tunica vaginalis/peritoneum						
Mesothelioma	3/49	34/50	39/47			
Mammary gland						
Fibroadenoma	3/45	8/39	7/17	14/49	32/46	29/44
Adenocarcinoma				1/50	11/48	16/48
Brain						
Glioma	0/46	5/50	6/30	0/49	4/46	4/46
Oral mucosa						
Papilloma or carcinoma				1/46	3/37	7/26
Forestomach						
Papilloma or carcinoma	1/46	2/50	6/32	0/47	4/38	11/30
Intestine						
Adenomatous polyp or adenocarcinoma	0/47	1/50	4/37			
Skin						
Sebaceous gland adenoma, basal cell tumor, or sebaceous gland adenocarcinoma	0/45	5/41	4/18			
Zymbal gland						
Carcinoma	1/49	3/50	6/48			
Clitoral gland						
Adenoma, adenocarcinoma, or carcinoma				5/49	9/47	12/45
Thyroid gland						
Follicular cell adenoma or carcinoma	1/46	4/42	6/19	0/49	1/38	3/35
Hematopoietic system						
Leukemia				13/49	14/44	20/41
MICE	**Veh. Control**	**25 mg/kg**	**50 mg/kg**	**Veh. Control**	**25 mg/kg**	**50 mg/kg**
Harderian gland (b)						
Adenoma or adenocarcinoma	8/46	12/41	22/44	4/46	11/43	17/43
Mammary gland						
Adenoma, fibroadenoma, or adenocarcinoma				2/50	6/50	15/50
Forestomach						
Squamous cell papilloma or carcinoma	1/50	2/50	10/50			
Uterus						
Carcinoma or adenocarcinoma				0/50	3/50	3/50
Subcutaneous tissue						
Sarcoma or fibrosarcoma				0/50	3/50	9/50
Skin						
Squamous cell papilloma or carcinoma	0/50	0/50	4/50	0/50	0/50	2/50
Liver						
Adenoma or carcinoma	24/50	31/50	35/50			
Lung						
Alveolar/bronchiolar adenoma or carcinoma	13/50	11/50	21/50			

(a) A blank space indicates that the tumor incidence at that site and in that sex was not increased by chemical exposure. Tumor incidence is expressed as the number of tumor-bearing animals divided by the number of animals alive in each group at the time the first tumor was observed in any of the three groups.
(b) The denominators for the incidence of harderian gland tumors are the actual number of harderian glands available for microscopic examination.

Groups of 50 male and 50 female B6C3F1 mice, nine weeks of age, were administered 0, 25 or 50 mg/kg bw of glycidol (94% purity, with the primary impurity, as determined by gas chromatography, being diglycidyl ether at a concentration of 2.8%, and 3-methoxy-1,2-propanediol (1.2%), 2,6-dimethanol-1,4-dioxane (1.1%), 3-chloro-1,2-propanediol (0.4%) and methanol (0.1%) as lesser impurities) in distilled water by gavage on five days per week for 103 weeks. The survival of female mice at the high dose was significantly lower after week 101 than in the controls. As shown in Table 2 of the IARC monograph, there was a significantly increased incidence of Harderian gland adenomas and adenocarcinomas combined in the high-dose males and in the high- and mid-dose females, and of Harderian gland adenocarcinomas in the high-dose males. In male mice only, the incidences of adenomas and carcinomas of the liver, squamous-cell papillomas of the forestomach and skin and alveolar/bronchiolar adenomas of the lung were significantly increased at the high dose; in females only, the incidences of mammary gland adenocarcinomas and of subcutaneous sarcomas and fibrosarcomas combined were significantly increased at the high dose. There was also a slight increase in uterine glandular carcinomas in female mice (Irwin, 1990; IARC, 2000).

Glycidol has a harmonised classification as Carc 1B (entry 603-063-00-8).

4.10.4 Summary and discussion of carcinogenicity

There are reports on chronic exposure studies with GMA, but each one has significant methodological deficiencies such that the conclusion is that there are no acceptable chronic studies with GMA. Consequently a Read Across for GMA was used. Rationale: although the kinetics of carboxylesterase-mediated hydrolysis of GMA appear to be species dependent, the primary metabolite of GMA found in humans, rats and rabbits is glycidol. Therefore, glycidol is the breakdown product of GMA as required for read-across according to REACH Annex XI paragraph 1.5 option (2). Read-across is also supported by the comparable effects on fertility of both substances. Chronic bioassay data were located for glycidol in rats and mice. These were clearly positive and have resulted in a harmonised classification of glycidol as Carc. 1B. Therefore, the comparable induction of tumors is also expected for GMA.

4.10.5 Comparison with criteria

No data in humans exist and limited animal studies, but the study results from a read-across to glycidol which is the main (if not the only) metabolite of GMA clearly support a category 1B classification for which the requirements are (quote from the CLP Regulation):

3.6.2.2. *'A substance that has not been tested for carcinogenicity may in certain instances be classified in Category 1A, Category 1B or Category 2 based on tumour data from a structural analogue together with substantial support from consideration of other important factors such as formation of common significant metabolites, e.g. for benzidine congener dyes.'*

This classification is further supported by the *in vivo* mutagenicity to somatic cells as shown in chapter 4.9.

4.10.6 Conclusions on classification and labelling

According to the available studies on glycidol, GMA must be classified according to CLP as Carcinogenic Category 1B (H350).

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

There were reports on chronic exposure studies with GMA, but each one had significant methodological deficiencies; thus the DS concluded that there were no acceptable chronic studies with GMA.

In a very limited one-year study (Hadidian *et al.*, 1968), rats (3 males and 3 females) were dosed 5 days/week by gavage at 0.1 mg/kg bw/day. Groups of 15 male and 15 female rats were also dosed at 0.3 mg/kg bw/day. The authors concluded that no tissue effects related to the treatment were found. However the doses applied were considered too low. There was also a 26-week inhalation toxicity study at concentrations of 15.3 and 206 mg/m^3 in rats and rabbits (Ouyang Guoshun *et al.*, 1990). A wide range of toxic effects were observed in both species at both concentrations. However, because of the low purity of the material used (92%), the authors of the study suggested that the effects may have been caused by the impurities present. Therefore the DS considered it questionable whether the systemic toxicity was caused by GMA.

Consequently, a read across approach was used by the DS for GMA: although the kinetics of carboxylesterase-mediated hydrolysis of GMA appeared to be species-dependent, the primary metabolite of GMA in humans, rats and rabbits was glycidol. Chronic bioassay data were located for glycidol in rats and mice, which were clearly positive and glycidol is classified as Carc. 1B according to Annex VI, CLP. Thus, the read-across approach was based on the formation of a metabolite of GMA which is a known carcinogen (Carc. 1B).

Based on the available studies on glycidol, the DS proposed to classify GMA as Carc.1B; H350.

Comments received during public consultation

Two MS were in support of the proposed classification as Carc. 1B.

Additional key elements

The main study discussed for carcinogenicity of GMA is that performed by NTP on the GMA metabolite glycidol (Irwin *et al.*, 1990). Mice and rats (50/sex/dose) were exposed to glycidol at 37.5 or 50 mg/kg bw/day by gavage for 103 weeks. Increases in tumour incidences that were statistically significant or above historical controls are reported in the table below. The NTP has concluded that the study provided clear evidence of carcinogenicity in male and female rats and mice.

Summary of tumour incidences in rats and mice treated with glycidol (Irwin *et al.,* 1990)

	Males			**Females**		
Doses	0	37.5 mg/kg	75 mg/kg	0	37.5 mg/kg	75 mg/kg
RATS						
Tunica vaginalis /peritoneal mesoth.	6%	68%*	83%*			
Mammary gland - fibroadenoma - adenocarc.	7%	21%	41%*	29% 2%	70%* 23%*	66%* 33%*
Brain glioma	0%	10%*	20%*	0%	9%	9%
Forestomach - sq. cell papill. - sq. cell carc. - combined	 0% 2% 2%	 2% 2% 4%	 16%* 4% 19%*	 0% 0% 0%	 11%* 0% 11%*	 27%* 17%* 37%*
Oral cavity - sq. cell papill. - sq. cell carc. - combined				 2% 0% 2%	 8%* 0% 8%*	 23%* 2% 27%*

Intestine (combined): adenomat. polyp or adenocarc.	0%	2%	11%			
Skin (combined): sebaceous gland adeno./ adenocarc. or basal cell carc.	0%	12%*	22%*			
Zymbal gland carc.	2%	6%	13%			
Thyroid follic. cell - adenoma - carc. - combined	 0% 2% 2%	 4% 5% 10%	 4% 26%* 32%*	 0% 0% 0%	 3% 0% 3%	 0% 9% 9%
Clitoral gland - adenoma - adenocarc. - carc. - combined				 6% 0% 4% 10%	 18% 3% 3% 19%	 23%* 0% 17% 27%*
Leukaemia				27%	32%	49%*
MICE						
Harderian gland - adenoma - adenocarc. - combined	 15% 2% 17%	 24% 5% 29%*	 36%* 16%* 50%*	 9% 0% 9%	 23% 2% 26%*	 37%* 2% 40%*
Forestomach - sq. cell papill. - sq. cell carc. - combined	 0% 2% 2%	 4% 0% 4%	 18%* 2% 20%*			
Skin – sq. cell papill. - sq. cell carc. - combined	 0%	 0%	 8%*	 0% 0% 0%	 0% 0% 0%	 2% 2% 4%
Liver - adenoma - carcinoma - combined	 36% 20% 48%	 32% 34% 62%	 60%* 16% 70%*			
Lung - alv./bronch. aden. - alv./bronch. carc. - combined	 12% 14% 26%	 12% 10% 22%	 16% 28%* 42%*			
Mammary gland - adenoma - fibroadenoma - adenocarc. - combined				 0% 2% 2% 4%	 2% 0% 10% 12%	 0% 0% 30%* 30%*
Uterus/cervix - carcinoma - adenocarcinoma - combined				 0% 0% 0%	 0% 6% 6%	 2% 4% 6%
Subcutaneous tissue - sarcoma - fibrosarcoma - combined				 0% 0% 0%	 2% 4% 6%	 12%* 6% 18%*

*p<0.05 ; mesoth.=mesothelioma; papill.=papilloma; carc.=carcinoma; sq.=squamous ; adeno.=adenoma ; adenomat.=adenomatous; alv./bronch.=alveolar/bronchiolar

Assessment and comparison with the classification criteria

RAC concludes that the chronic data available on GMA present methodological limitations (low number of animals, limited purity) and were not performed at sufficiently high doses or for sufficient duration to provide a reliable information on the carcinogenic potential of GMA. The assessment of the carcinogenic potential of GMA is therefore based on data available for the metabolite glycidol.

Glycidol induced benign and malignant tumours in multiple organs in rats and mice of both sexes (Irwin *et al.*, 1990). It is noted that the increase in several tumours was observed from the lowest dose of 37.5 mg/kg bw/day. Glycidol has an existing entry in Annex VI to the CLP

Regulation with a classification as Carc. 1B; H350.

Toxicokinetic data show that GMA is extensively metabolised into glycidol (and methacrylic acid) which is further supported by the consistency between the systemic toxicity profile of GMA and glycidol. RAC notes that although a more comprehensive analysis of other substances metabolised into glycidol (or to similar metabolites) would have provided additional support, GMA is expected to produce similar systemic effects as glycidol, on the basis of the available data. Local toxicity is different between GMA and glycidol and the corrosivity of GMA may prevent a high exposure to glycidol arising from metabolism of GMA. However, the data on male fertility supports the conclusion that the most sensitive systemic effects of glycidol can be observed after oral exposure to GMA in the absence of severe local toxicity. In addition, the induction of tumours at multiple sites in both males and females in two rodent species is consistent with the identification of glycidol as a genotoxic, non-threshold carcinogen and GMA is also identified as genotoxic *in vivo*.

Altogether, RAC concludes that classification as **Carc. 1B; H350** is warranted for GMA.

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Table 24a: Summary table of relevant reproductive toxicity studies (fertility)

Method	Results	Remarks	Reference
rat (Crj: CD(SD)) 12 male/12 female oral: gavage 10, 30, 100 mg/kg/day (nominal in diet) Vehicle: corn oil Exposure: 40-47 days (daily) OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test)	NOAEL (P): 30 mg/kg bw/day (nominal) (male/female) (At 100 mg/k the fertility index (number of delivered animals/ number of mated animals) dropped to 16.7 %, compared to 81.8 %, 100 % and 91.7 % at 0, 10 and 30 mg/kg, respectively. There were no effects on the oestrous cycle, copulation index, or gestation length. No significant changes in the numbers of corpora lutea, implants, pups born and live pups as well as the implantation and delivery indices were observed. There were no significant differences in the gestation index, live birth index or viability index on day 4. Histopathological analysis of the gonads showed no significant effect. No change in the number of gonocyte per	1 (reliable without restriction) key study experimental result **Test material: GMA purity 99.93%**	Ministry of Health and Welfare, Japan (1997) OECD (1999)

Method	Results	Remarks	Reference
	Sertoli cell was observed in epithelium of seminiferous tubules (stage VIII) of all survival males at 100 mg/kg.) NOEL (F1): 100 mg/kg bw/day (nominal) (male/female) (Toxicity to offspring: No abnormalities were noted in the body weights of live pups or on necropsy of pups of any treated group).		
5 mouse male (CD-1)/group sperm abnormality test intraperitoneal 0, 1, 5, 25 and 100 mg/kg/day Exposure: Daily for 5 consecutive days Necropsy was performed on Day 36	NOEL (P): 5 mg/kg bw/day (nominal) (male) (At 100 mg/kg mice had decreased caudal epididymal weights, slightly lower testicular weights, decreased sperm counts and increased abnormal sperm. Mice given 25 mg/kg/day showed decreased sperm counts and increased abnormal sperm. The NOAEL for spermatotoxicity was 5 mg/kg/day).	2 (reliable with restrictions) supporting study experimental result **Test material: GMA**	Vedula (1994) OECD (1999)
5 male mice (Kunming hybrid)/group Sperm abnormality test Intraperitoneal 0, 25, 50, 100 mg/kg Exposure: Daily for 5 consecutive days Necropsy was performed on Day 36	LOAEL (P): 25 mg/kg (male) (At 25 mg/kg mice, the number of sperm cells decreased and there was an increase in sperm abnormalities) There was no NOAEL in this study.	2 (reliable with restrictions) supporting study experimental result **Test material: GMA**	Xie (1990b)

Oral toxicity study was performed in SD (Crj: CD) rats by an OECD combined repeated dose and reproductive/developmental toxicity screening test (OECD TG 422). Administration was conducted by gavage at doses of 10, 30 and 100 mg/kg/day from 14 days before mating to 14 days after mating in males and from 14 days before mating to day 3 of lactation in females (Ministry of Health and Welfare, Japan: 1997).

Maternal toxicity was mainly limited to effects on the stomach at all dose levels due to the local irritating/corrosive properties of GMA (see 4.7). Many animals were infertile apparent in the 100 mg/kg group, however, morphological abnormalities were not apparent in the epididymis, seminal vesicles, prostate, uterus, or pituitary gland. Moreover, counts of Stage VIII seminiferous tubules in the testes of the 100 mg/kg group did not reveal any effects attributed to GMA exposure. The fertility index decreased significantly in the 100 mg/kg group, presumably due to the low sperm mobility. No sperm analysis was performed in the original investigation as this is not required according to OECD 422. Secondary investigations showed reduced motility in sperm but no further details were provided in the report. There were no effects on the estrous cycle, copulation index, gestation length or parturition. Slight decreases in the numbers of corpora lutea, implants, pups born and live pups as well as the implantation and delivery indices were observed in the 100 mg/kg group. However, clear effects attributable to the administration of GMA could not be concluded due to the few cases. There were no significant differences in gestation index, liver birth index or viability index on day 4. No abnormalities attributable to GMA were noted in body weights of live pups or on necropsy of pups in any GMA-treated group. Therefore, NOAELs for reproductive performance of parents (males and females) and pup development were considered to be 30 mg/kg/day and 100 mg/kg/day, respectively (Ministry of Health and Welfare, Japan: 1997).

Table 24b: Copulation and fertility results in rats (Ministry of Health and Welfare, 1997)

Dose level (mg/kg)	0	10	30	100
No. of pairs mated	11	12	12	12
No. of pairs copulated	11	12	12	12
No. of pregnant females	9	12	11	2
Copulation index (%) 1)	100.0	100.0	100.0	100.0
Fertility index (%) 2)	81.8	100.0	91.7	16.7**
Estrus cycle (days) (Mean±S.D.)	4.5± 0.5 (12)	4.5± 0.6 (12)	4.4± 0.5 (12)	4.6± 0.7 (12)

1) (No. of animals with successful copulation / no. of animals mated) x 100
2) (No. of pregnant animals / no. of animals with successful copulation) x 100
Values in parentheses are expressed no. of animals observed
Significant difference from control group; **: p ≦ 0.01

Table 24c: Reproductive and foetal findings in rats in rats (Ministry of Health and Welfare, 1997)

Dose level (mg/kg)	0	10	30	100
No. of dams observed	10	12	11	2
No. of dams delivered live pups	10	12	11	2
Duration of gestation (Mean ±S.D.)	22.6 ± 0.5	22.8 ± 0.4	22.7 ± 0.6	23.0 ± 0.0
No. of total corpora lutea (Mean ±S.D.)	195(19.5± 3.6)	233(19.4± 2.4)	212(19.3± 2.2)	31(15.5± 0.7)
No. of total implants (Mean ±S.D.)	166(16.6± 2.7)	200(16.7± 1.9)	184(16.7± 1.6)	24(12.0± 2.8)
No. of total pups born (Mean ±S.D.)	152(15.2± 3.3)	176(14.7± 2.6)	160(14.5± 1.6)	18(9.0± 1.4)
No. of total live pups born (Mean ±S.D.)	151(15.1± 3.3)	174(14.5± 2.9)	160(14.5± 1.6)	18(9.0± 1.4)
Male	66(6.6± 2.5) a)	85(7.1± 3.0)	79(7.2± 2.5) a)	9(4.5± 0.7)
Female	85(8.5± 2.7) a)	89(7.4± 2.5) a)	81(7.4± 2.5)	9(4.5± 2.1)
Sex ratio (Mean ±S.D.)	0.88± 0.46	1.10± 0.57	1.15± 0.65	1.17± 0.71
No. of total live pups on day 4 (Mean ±S.D.)				
Male	61(6.1± 2.6)	82(6.8± 2.7)	76(6.9± 2.5)	9(4.5± 0.7)
Female	81(8.1± 2.4)	83(6.9± 2.8)	77(7.0± 2.8)	8(4.0± 1.4)
No. of total dead pups born (Mean ±S.D.)	1(0.1±0.3)	2(0.2±0.6)	0(0.0±0.0)	0(0.0±0.0)
stillbirth	0(0.0±0.0)	0(0.0±0.0)	0(0.0±0.0)	0(0.0±0.0)
cannibalism	1(0.1±0.3)	2(0.2±0.6)	0(0.0±0.0)	0(0.0±0.0)
Gestation index (%) 1)	100.0	100.0	100.0	100.0
Implantation index (%,Mean±S.D.) 2)	86.0± 12.1	86.5± 10.2	87.5± 10.0	77.9± 21.8
Delivery index (%,Mean±S.D.) 3)	91.0± 7.8	88.0± 11.3	87.0± 5.0	75.7± 6.1
Live birth index (%,Mean±S.D.) 4)	99.3± 2.1	98.5± 5.2	100.0± 0.0	100.0± 0.0
Viability index on day 4 (%,Mean±S.D.) 5)				
Male	90.2± 17.8	97.6± 4.5	96.3± 6.4	100.0± 0.0
Female	96.1± 5.1	92.3± 13.7	93.5± 12.9	91.7± 11.8

1) (No. of females with live pups / no. of pregnant females) x 100
2) (No. of implants / no. of corpora lutea) x 100
3) (No. of pups born / no. of implants) x 100
4) (No. of live pups born / no. of pups born) x 100
5) (No. of live pups on day 4 after birth / no. of live pups born) x 100
a) Includes live pups died before observations

Male mice injected i.p. with 5 consecutive daily doses of 0, 25, 50 or 100 mg/kg/day showed an increase in the percentage of abnormal sperm and decrease in the number of sperm (Table 24d) (Xie *et al*.1990b).

Induction of Sperm Abnormalities in Mice by GMA

Male mice of Kunming hybrid, 11–15 weeks old, were used. GMA was dissolved in maize oil and administered to the animals by five consecutive daily ip injections of 25, 50, and 100 mg/kg body wt. Groups of five mice were used in each dose level of the chemical. The negative control group received only maize oil, while the positive control group received ip injections of cyclophosphamide (40 mg/kg body wt) daily for 5 days. The mice were killed by cervical dislocation 35 days after the first injection. The caudal epididymides were removed. Sperm suspensions, smears, and slides were made and sperm abnormalities were evaluated according to Wyrobek and Bruce (1975). The morphological abnormalities included sperm with a banana-like form, without the usual hook, swollen head, or amorphous.

Table 24d: Sperm abnormalities of male mice induced by GMA (Xie et al. 1990b)

Sperm Abnormality of Male Mice Induced by GMA

Group	Dose (mg/kg)	Sperm abnormality (%)	No. of sperm × 10^6 ($\bar{X}$ ± SD)
Control	0	6.15	1.48 ± 0.11
GMA	25	16[a]	1.08 ± 0.15[a]
	50	18[a]	0.66 ± 0.08[a]
	100	17[a]	0.66 ± 0.08[a]
CP	200	25[a]	0.72 ± 0.08[a]

[a] Control vs GMA and CP groups, $P < 0.01$.

These results were confirmed in a subsequent study where mice were dosed i.p. with 0, 1, 5, 25 or 100 mg/kg for five consecutive days and on day 36 complete necropsy was performed (Vedula *et al.*, 1994). At 100 mg/kg, mice had decreased caudal epididymal weights and slightly lower testicular weights (Table 24e), decreased sperm counts (Table 24g) and increased abnormal sperm (Table 24h). Mice given 25 mg/kg/day showed decreased sperm counts and increased abnormal sperm.

Table 24e: Summary of male reproductive organ weights (Vedula et al. 1994)

ORGAN AND ORGAN/BODY WEIGHTS - MALES

DOSE MG/KG/DAY		FINAL BODY WT. (G)	EPIDIDYMIS (G)	EPIDIDYMIS (G/100)	TESTES (G)	TESTES (G/100)	CAUDA (G)	CAUDA (G/100)
0	MEAN	38.6	0.111	0.286	0.258	0.668	0.034	0.087
	S.D.	1.4	0.010	0.021	0.022	0.059	0.003	0.007
	N=	5	5	5	5	5	5	5
1	MEAN	36.4	0.109	0.300	0.254	0.701	0.034	0.092
	S.D.	0.8	0.014	0.035	0.039	0.116	0.005	0.012
	N=	4	4	4	4	4	4	4
5	MEAN	37.5	0.098	0.262	0.243	0.648	0.030	0.080
	S.D.	1.3	0.011	0.026	0.011	0.042	0.006	0.015
	N=	5	5	5	5	5	5	5
25	MEAN	37.8	0.103	0.273	0.243	0.644	0.030	0.079
	S.D.	1.3	0.009	0.022	0.030	0.073	0.006	0.017
	N=	5	5	5	5	5	5	5
100	MEAN	38.6	0.111	0.292	0.212	0.550	0.026$	0.068$
	S.D.	1.9	0.030	0.095	0.035	0.097	0.004	0.009
	N=	5	5	5	5	5	5	5
40 (CP)	MEAN	36.5	0.095	0.259	0.228	0.626	0.027$	0.075
	S.D.	2.3	0.009	0.008	0.035	0.091	0.002	0.007
	N=	5	5	5	5	5	5	5

$ STATISTICALLY DIFFERENT FROM CONTROL MEAN BY WILCOXON'S TEST, ALPHA = 0.05.

Table 24f: Summary of gross pathologic observations in male reproductive organs (Vedula et al. 1994)

GROSS PATHOLOGIC OBSERVATIONS@

SEX	MALES					
DOSE IN MG/KG/DAY	0	1	5	25	100	40$
NUMBER OF MICE EXAMINED	5	4	5	5	5	5
TESTES						
WITHIN NORMAL LIMITS.	4	4	4	5	4	4
CYST, UNILATERAL:	0	0	1	0	0	0
DECREASED SIZE, UNILATERAL:	1	0	1	0	1	1
FOCUS - ELEVATED, UNILATERAL:	0	0	0	0	0	1

Table 24g: Summary of sperm count effects induced by GMA (Vedula et al. 1994)

CAUDAL EPIDIDYMAL SPERM COUNT

DOSE MG/KG/DAY		TOTAL SPERM ($\times 10^6$)	SPERM COUNT/G CAUDA EPIDIDYMIS ($\times 10^9$)
0	MEAN	54.2	1.63
	S.D.	12.4	0.44
	N=	5	5
1	MEAN	65.4	1.98
	S.D.	6.2	0.29
	N=	4	4
5	MEAN	48.9	1.59
	S.D.	19.7	0.52
	N=	5	5
25	MEAN	39.7	1.37
	S.D.	6.5	0.32
	N=	5	5
100	MEAN	29.8$	1.11
	S.D.	8.4	0.22
	N=	5	5
40 (CP)	MEAN	33.8	1.23
	S.D.	11.5	0.37
	N=	5	5

$ STATISTICALLY DIFFERENT FROM CONTROL MEAN BY WILCOXON'S TEST, ALPHA = 0.05.

Table 24h: Summary of abnormal sperm effects induced by GMA (Vedula et al. 1994)

CAUDAL EPIDIDYMAL SPERM MORPHOLOGY

DOSE MG/KG/DAY		% ABNORMAL
0	MEAN	4.6
	S.D.	1.9
	N=	5
1	MEAN	4.3
	S.D.	1.7
	N=	4
5	MEAN	5.4
	S.D.	1.1
	N=	5
25	MEAN	7.0
	S.D.	1.5
	N=	5
100	MEAN	9.6*
	S.D.	3.7
	N=	5
40	MEAN	14.0*
	S.D.	4.4
	N=	5

* STATISTICALLY DIFFERENT FROM CONTROL MEAN BY DUNNETT'S TEST, ALPHA = 0.05.

These results might support the decreased fertility index of rat study at 100 mg/kg/day.

4.11.1.2 Human information

No relevant information available

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Table 24i: Summary table of relevant reproductive toxicity studies (developmental toxicity)

Method	Results	Remarks	Reference
rat (Wistar) oral: gavage 5.38, 10.76, 21.52 and 108.0 mg/kg/day Exposure: Day 5 to day 15 of gestation (Daily)	NOAEL (maternal toxicity): 21.52 mg/kg bw/day (nominal). NOAEL (teratogenicity): 108 mg/kg bw/day (nominal). Pregnancy/litter data: At 108.0 mg/kg, a statistically significant increase in the fetal resorption rate (12.7 %,	2 (reliable with restrictions) key study experimental result **Test material: GMA purity 92%**	Ou-Yang (1988) OECD (1999)

Method	Results	Remarks	Reference
no data	compared to 5.18 % of control group) Foetal data: No external, skeletal or organ abnormalities. No significant difference in body weight from the control. Developmental toxicity in rats by oral administration is not observed at the highest dose, 108 mg/kg/day which induces maternal toxicity.		
rabbit (New Zealand White) inhalation: vapour 0.5, 2, and 10 ppm (2.91, 11.6 and 58.2 mg/m^3. Calculated daily dose: 0.31, 1.22 and 6.11 mg/kg/day (nominal conc.) Exposure: Day 7 to 19 of gestation (7 hours/day, daily) yes	NOAEL (maternal toxicity): 2.91 mg/m³ air (nominal) (The principal indication of maternal toxicity was inflammation of the nasal olfactory and respiratory epithelium at the 11.6 and 58.2 mg/m^3.) NOAEL (teratogenicity): 58.2 mg/m³ air (nominal) (There was no teratogenic effect)	2 (reliable with restrictions) key study experimental result **Test material: GMA purity 99.5%**	Vedula (1996) OECD (1999)
rat (Crj: CD(SD)) male/female oral: gavage 10, 30, 100 mg/kg/day (nominal in diet) Vehicle: corn oil Exposure: 40-47 days (daily) OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test)	NOEL (F1): 100 mg/kg bw/day (nominal) (male/female) (Toxicity to offspring. No abnormalities were noted in the body weights of live pups or on necropsy of pups of any treated group).	1 (reliable without restriction) key study experimental result **Test material: GMA purity 99.93%**	Ministry of Health and Welfare, Japan (1997) OECD (1999)
rabbit (New Zealand White) inhalation: vapour 5, 10 and 50 ppm (29.1, 58.2 and 291 mg/m^3). Calculated daily dose 2.62, 5.24 and 26.2 mg/kg/day) (nominal conc.) Exposure: From day 7 through day 19 of gestation (6 hours/day, daily)	LOAEL (maternal toxicity): 29.1 mg/m³ air (nominal) (At 29.1 mg/m^3 histopathologic alterations of the nasal respiratory and olfactory epithelium (hyperplasia, necrosis, etc.) in all animals. At 58.2 mg/m^3 reddened eyes, swollen eyes and mucus discharge from eyes, and wet	2 (reliable with restrictions) supporting study experimental result **Test material: GMA**	Vedula (1995) OECD (1999)

Method	Results	Remarks	Reference
	muzzle and sneezing after exposure. Histopathologic alterations of the nasal respiratory and olfactory epithelium (hyperplasia, necrosis, etc.) in all animals At 291 mg/m^3 decrease in feed consumption and faecal output during the exposure period in all animals (group removed from study after third exposure). Laboured breathing, reddened eyes and nares, swollen eyelids, squinting, decreased activity, nasal congestion, lacrimation, dorsal extension of the head, wet muzzle, excessive sneezing after exposure and coloured nasal discharge Histopathologic alterations of the nasal respiratory and olfactory epithelium (hyperplasia, degeneration, etc.) in all animals. Pregnancy/litter data: No adverse effect on any reproductive parameters at 29.1 and 58.2 mg/m^3) NOAEL (teratogenicity): 58.2 mg/m³ air (nominal) (Foetal data: No adverse effect on any embryo/foetal parameters at 29.1 and 58.2 mg/m^3)		

GMA was administered by oral gavage to rats during day 5 to day 15 of gestation at doses of 5.38, 10.76, 21.52 and 108.0 mg/kg/day. The animals were sacrificed on day 19 of pregnancy. (OuYang et al.: 1988). As maternal toxicity, there was significant decrease in body weight gain at 108.0 mg/kg. There was a statistically significant increase in the fetal resorption rate at the 108.0 mg/kg. The percentage of pups stillborn was somewhat higher than control at all dose levels (0 % for control, and 1.35 %, 7.58 %, 1.26 % and 6.03 % for treated group at 5.38, 10.76, 21.52 and 108.0 mg/kg/day, respectively). However, because this change was not dose-dependent and statistically significant change only at 10.76 mg/kg, this was not considered to be chemical-related change. Neither birth defects nor fetal abnormalities were noted in rats treated with GMA. There was also no significant difference in fetal body weight from the control. Therefore, NOAELs were considered to be 21.52 mg/kg/day for maternal toxicity and 108.0 mg/kg/day for teratogenicity.

Table 24j: Fetal resorption data as provided by OuYang et al. (1988)

表5 GMA大鼠致畸试验结果

组别	剂量 (mg/kg)	孕鼠数 (只)	平均体重增长值 (g)	平均活胎数 (只)	平均窝重 (g)	死胎率 (%)	吸收胎率 (%)
阴性对照		14	76.78	9.14	32.63	0	5.18
GMA1/100LD$_{50}$	5.38	17	66.94	8.70	35.40	1.35	2.60
GMA1/50LD$_{50}$	10.76	15	65.40	8.80	37.36	7.58**	3.40
GMA1/20LD$_{50}$	21.52	18	68.10	8.80	33.38	1.26	1.23
GMA1/5LD$_{50}$	108.0	14	47.14 *	8.30	36.93	6.03	12.70*
致枯双	1.0	15	35.80 *	2.40**	16.50**	0	73.10**

In an oral toxicity study performed in SD (Crj: CD) rats by an OECD combined repeated dose and reproductive/developmental toxicity screening test (OECD TG 422), administration was conducted by gavage at doses of 10, 30 and 100 mg/kg/day from 14 days before mating to 14 days after mating in males and from 14 days before mating to day 3 of lactation in females. No abnormalities attributable to the administration of GMA were noted in the body weights of live pups or on necropsy of pups in any treated group. Therefore, in this study there were no teratogenic effects induced by GMA in rats with a NOAEL of 100 mg/kg/day (Ministry of Health and Welfare, Japan, 1997).

There were two inhalation tests on developmental toxicity. In the first, rabbits were exposed to GMA at concentrations of 29.1, 58.2 and 291 mg/m^3 (daily intake is calculated as 2.62, 5.24 and 26.2 mg/kg/day, respectively), 6 hours/day, daily during day 7 to day 19 of gestation (Vedula et al.: 1995). Respiratory distress and decrease in feed consumption was observed at 291 mg/m^3. Less severe signs of ocular and respiratory irritation consisting of reddened eyes, wet muzzle and sneezing after exposure were observed at 58.2 mg/m^3. Treatment-related histopathologic alterations of the nasal tissues (hyperplasia, necrosis, etc.) were present in all animals treated with GMA. Because of respiratory distress, animals at 291 mg/m3 were removed early from study after the third exposure. Therefore, evaluation of reproductive and embryonal/fetal parameter was precluded. There was no adverse effect on any reproductive and embryo/fetal parameter at 29.1 and 58.2 mg/m^3. LOAEL for maternal toxicity was 29.1 mg/m^3 (2.62 mg/kg/day) and NOAEL for teratogenicity was 58.2 mg/m^3 (5.24 mg/kg/day).

In the second, rabbits were exposed to GMA at concentrations of 2.91, 11.6 and 58.2 mg/m^3 (daily intake 0.31, 1.22, 6.11 mg/kg/day, respectively) 7 hours/day, daily during day 7 to day 19 of gestation (Vedula et al.: 1995). The principal indication of maternal toxicity was inflammation of the nasal olfactory and respiratory epithelium at the 11.6 and 58.2 mg/m^3. There was no adverse effect on any reproductive and embryo/fetal parameter at any doses. Therefore, NOAEL for maternal toxicity was 2.91 mg/m^3 (0.31 mg/kg/day) and NOAEL for teratogenicity was 58.2 mg/m^3 (6.11 mg/kg/day).

4.11.2.2 Human information

No relevant information available

4.11.3 Other relevant information

As shown in chapter 4.1, GMA is metabolised into glycidol. Glycidol has a harmonised classification for reproductive toxicity in category 1B with H360F indicating an effect on fertility.

Summaries of the main studies showing effects of **glycidol** on fertility are provided below and were copied from the C&L proposal of glycidol (ECBI-92/95-add.3). In addition, a summary of the 13-week study by the NTP in rats and mice is also summarized below.

Thirteen-Week Studies with glycidol: Doses for groups of 10 rats ranged from 25 to 400 mg/kg, and doses for groups of 10 mice ranged from 19 to 300 mg/kg; vehicle controls received distilled water. All rats that received 400 mg/kg died by week 2; three males and one female that received 200 mg/kg died during weeks 11-12. Final mean body weights of male rats that received 50, 100, or 200 mg/kg were 96%- 85% that of vehicle controls; final mean body weights of female rats receiving the same doses were 94%-89% that of vehicle controls. **Sperm count and sperm motility were reduced in male rats that received 100 or 200 mg/kg**. Necrosis of the cerebellum, demyelination in the medulla of the brain, tubular degeneration and/or necrosis of the kidney, lymphoid necrosis of the thymus, and testicular atrophy and or degeneration occurred in rats that received 400 mg/kg (NTP, 1990).

All mice that received 300 mg/kg died by week 2; deaths of mice that received 150 mg/kg occurred during weeks 4-8 for males and weeks 1-5 for females. Mean body weights of chemically exposed mice surviving to the end of the studies were generally 90%-94% those of vehicle controls. Sperm count and sperm motility were reduced in dosed male mice. Compound-related histopathologic lesions included demyelination of the brain in males and females that received 150 or 300 mg/kg, **testicular atrophy in males at all doses**, and renal tubular cell degeneration in male mice that received 300 mg/kg (NTP, 1990).

Table 24k: Summary table of relevant reproductive toxicity studies (fertility) with Glycidol

Species	Route	Dose	Exposure time	Observations and remarks
Rat (Sprague-Dawley)	Oral	15mg/kg	12 days	In a single generation study, fertile male rats were given glycidol and at different times during treatment 2 pre-oestrous females were placed in the cage of each male. After 7 days in each case, fertility was evaluated on the basis of the number of uterine implantations. Within one week, the males became infertile, although this effect was reversible within one week. Neither libido nor ejaculation ability was adversely affected by treatment. Histological examination of testes, epididymides, prostate and seminal vesicles on day 12 of treatment showed no difference from controls. The study was only briefly reported and no comments on systemic toxicity were recorded (Hahn, 1970). **The study provides evidence for impaired fertility in male rats.**
Rat (Wistar)	Oral	20, 40, 100, 200mg/kg	5 days	In a single generation study, male rats were given glycidol and mated with females of proven fertility. At 20mg/kg there were no treatment related effects. When mating was commenced on day 3 after the first dose of 40mg/kg, preimplantation losses were 40% in that week, rising to 95% in the second week. At 100mg/kg, sterility was observed in the first 2 weeks with no effect on sperm motility or mating activity. At 200mg/kg, glycidol induced epididymal spermatoceles. This study was briefly reported and made no specific reference to systemic toxicity (Jackson *et al*, 1970). **The data demonstrate impaired fertility in male rats.**

Rat (Wistar)	Oral	200mg/kg	1-5 days	Male rats were examined histologically between 1 and 12 weeks after dosing. Single oral doses of glycidol had no adverse effect on male rat fertility or on histology of the testis and its ducts. After five daily doses, males were sterile for 3 weeks, although histology was normal. In one animal, a large spermatocele occupied the cauda epididymidis, and in another, a small spermatocele was seen after 5 weeks in one ductulus efferens near its origin. Normal spermatogenic activity was evident in the testes at both 5 and 8 weeks and the epididymides were filled with spermatozoa. More prolonged treatment failed to produce sustained adverse effects on fertility and histology was normal. The methodological details in this paper were only briefly described (Cooper *et al*, 1974). **The study indicates that glycidol can cause impaired fertility in male rats.**
Mouse ($B6C3F_1$)	Oral	0, 19, 75, 150mg/kg	5 days per week for 13 weeks	Group size: 10 male mice and 10 male rats. Treatment-related deaths occurred: 4/10 mice given 150 mg/kg and 3/10 rats receiving 200 mg/kg died before study completion. Subsequently, sperm analysis was conducted on 5 animals from each group.
Rat (F344/N)		0, 25, 100, 200mg/kg		In mice a reduction in sperm count was observed in treated animals, achieving statistical significance at 75 and 150mg/kg. Sperm motility, recorded on a scale of 0-4, in mice given 0, 19, 75, 150mg/kg, was respectively, 3.6, 3.2, 2.8, 1.6. A treatment related lesion of the testis (atrophy and or degeneration) was observed but failed to achieve statistical significance. Rats showed a reduction in sperm counts which reached statistical significance in all treatment groups. On a scale of 0-4 sperm motility at doses of 0, 25, 100 and 200mg/kg was respectively, 3.4, 3.0, 2.0, 0.2. Testicular atrophy showed a significantly greater incidence in rats given 200mg/kg (NTP, 1990). **The data show that glycidol reduces sperm count and sperm motility in mice and rats.**

The effects observed with glycidol resemble the effects observed in the combined repeated dose toxicity and reproductive screening study in that effects were observed on the fertility without clear effects on the reproductive organs.

4.11.4 Summary and discussion of reproductive toxicity

In the OECD combined study (TG 422), the NOAEL for reproductive toxicity was considered to be 30 mg/kg/day, based on a decrease in the fertility index (number of delivered animals/ number of mated animals) at 100 mg/kg. No effects on the reproductive organs were observed. Comparable effects on the fertility were observed for glycidol, a metabolite of GMA, supporting the relevance of the effect in the screening study. The effects on fertility were observed in the presence of maternal toxicity which was limited to local irritation of the forestomach.

As three reliable developmental studies by two different routes, oral and inhalation, and the screening study indicated no teratogenicity even at the highest doses which showed maternal toxicity. The only concern is the significant increase in fetal resorption rate at the highest dose tested. This dose also induced maternal toxicity in the form of reduced body weight gain.

4.11.5 Comparison with criteria

No data in humans exist (a Category 1A requirement) but the study results support a category 1B classification for which the requirements are (quote from the CLP Regulation):

Presumed human reproductive toxicant:
The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

These criteria are fulfilled because a clear and statistically significant reduction in the number of pregnant rats was observed at 100 mg/kg bw/day. At this dose level also other toxic effects were observed in the parental rats. However, these effects were limited to changes in absolute and relative kidney and adrenal weights without histopathological changes or increases in total protein and albumin in the blood and not considered adverse. Local effects in the stomach were observed due to the irritating properties of GMA. However, these effects were limited to local effects as no general effect on body weight was observed. Therefore, the reduced pregnancy rate is not considered secondary to the mainly local paternal effects.

The mechanism by which GMA induces the reduced pregnancy is not clear as no effects were observed on the reproductive organs in the male and female rats. The oral developmental study in rats shows an increase in fetal absorption at 108 mg/kg bw/day, indicating that the reduced pregnancy may be due to a developmental effect. However, an ip study showed in mice that 25 and 100 mg/kg bw/day for 5 days resulted in an effect on testes weight and sperm counts and abnormal sperm. This would indicate an effect on the sexual function. However, such effects were determined in the rat oral screening study but not observed. GMA is metabolised into glycidol which has a harmonised classification including Repr. 1B H360F. This was based on studies in which males exposed to glycidol became infertile within one week without clear effects on libido, ejaculation or male reproductive organ histology (C&L proposal for glycidol U033, 1995). The reduction in fertility was observed in studies in which only males were exposed confirming that this was an effect on fertility. The observed effects with glycidol confirm the effects on fertility without effects on the reproductive organs for GMA and justify classification for effects on fertility in category 1B.

The significant increase in fetal resorption was considered for classification for developmental toxicity. However, as this effect was not observed in the comparable OECD 422 study (same route and dose levels), only observed in the presence of maternal toxicity and the main metabolite glycidol has no classification for development, no classification is proposed.

4.11.6 Conclusions on classification and labelling

Based on the available studies classification under CLP of GMA for developmental toxicity is not required.

However based on the same studies the substance must be classified under this Regulation as Toxic to Reproduction Category 1B (H360F).

RAC evaluation of reproductive toxicity
Summary of the Dossier Submitter's proposal
An oral toxicity study was performed on GMA in CD (Crj: CD) rats according to OECD TG 422 (a combined repeated dose and reproductive/developmental toxicity screening test).

Administration was conducted by gavage at doses of 10, 30 and 100 mg/kg bw/day from 14 days before mating to 14 days after mating in males and from 14 days before mating to day 3 of lactation in females. The fertility index (number of pregnant animals/ number of sucessfully mated animals) decreased significantly at 100 mg/kg bw/day.

Male mice injected IP with 5 consecutive daily doses of 0, 25, 50 or 100 mg/kg bw/day of GMA showed an increase in the percentage of abnormal sperm and decrease in the number of sperm. These results were confirmed in a subsequent study where mice were dosed IP with 0, 5, 25 or 100 mg/kg bw/day for five consecutive days. At 100 mg/kg bw/day, mice had decreased caudal epididymal weights, slightly lower testicular weights, decreased sperm counts and increased abnormal sperm. Mice given 25 mg/kg bw/day showed decreased sperm counts and increased abnormal sperm. These results might support the decreased fertility index of the rat study at 100 mg/kg bw/day, according to the DS.

In addition, the DS noted that glycidol, a metabolite of GMA, has an existing entry in Annex VI to the CLP Regulation with a classification as reproductive toxicant category 1B for adverse effects on sexual function and fertility (Repr. 1B; H360F).

Three reliable developmental studies via two different routes of exposure, oral and inhalation, indicated no teratogenicity even at the highest doses which showed maternal toxicity. The significant increase in foetal resorptions was considered as a basis for classification for developmental toxicity. However, as this effect was not observed in the comparable OECD TG 422 study (same route and dose levels), was only observed in the presence of maternal toxicity and since the main metabolite glycidol is not classified for development (Annex VI, CLP), no classification was proposed by the DS.

Based on the available studies on GMA and on data for glycidol, the DS proposed to classify GMA in category 1B for adverse effects on sexual function and fertility (Repr. 1B; H360F).

Comments received during public consultation

Two MS were in support of the proposed classification Repr. 1B for adverse effects on sexual function and fertility.

Additional key elements

Fertility

A combined repeated dose and reproductive toxicity screening study (OECD TG 422) (MHWJ, 1997) is available. Rats were exposed to 10, 30 or 100 mg/kg bw/day GMA by gavage. The main reproductive finding consisted of a dramatic decrease in fertility at 100 mg/kg bw/day as summarised in the table below. No sperm analysis was performed as part of the original investigation. Secondary investigations were reported to have shown reduced mobility in sperm but these were not further described. The mean number of corpora lutea (and subsequently the number of implantations and number of pups born) was lower in the high dose group compared to other groups (15.5±0.7 vs 19.5±3.6). The effect was not statistically significant. It was obtained only in 2 pregnant dams at this dose and the overall significance of this finding is considered equivocal by RAC. No other significant effects on reproductive or developmental parameters were noted. General toxicity in this study consisted mainly of local effects (see STOT RE section). At 100 mg/kg bw/day, an increase in kidney and adrenal weights, salivation and squamous hyperplasia and cell infiltration in the forestomach were observed in males and cell infiltration was observed in the forestomach of females.

Fertility index (number of pregnant animals/number of sucessfully mated animals) in MHWJ (1997).

Dose (mg/kg bw/day)	0	10	30	100
Fertility index	81.8%	100%	91.7%	16.7%*

* $p < 0.05$

Two studies administering GMA by IP injections investigated effects of GMA on sperm parameters.

In Xie *et al.* (1990b), male Kunming mice (5/dose) were exposed by IP injection to 0, 25, 50 or 100 mg/kg bw/day GMA for 5 consecutive days and sacrificed 35 days after the 1st injection. Sperm abnormalities were investigated and the results are summarised in the table below.

Findings in sperm parameters in Xie *et al.* (1990b)

Dose (mg/kg bw/day)	0	25	50	100
Sperm abnormality (%)	6.15	16*	18*	17*
No of sperm x 10^6	1.48±0.11	1.08±0.15*	0.66±0.08*	0.66±0.08*

* $p < 0.01$

In Vedula *et al.* (1994), male mice (5/dose) were exposed by IP injection to 0, 1, 5, 25 or 100 mg/kg bw/day GMA for 5 consecutive days and sacrificed 35 days after the 1st injection. Sperm parameters and reproductive organ weights were investigated and the results are summarised in the table below.

Findings in sperm parameters in Vedula *et al.* (1994)

Dose (mg/kg bw/day)	0	1	5	25	100
Relative caudal epididymal weight (g/100g)	0.087±0.007	0.092±0.012	0.080±0.0.015	0.079±0.017	0.068±0.009*
Sperm abnormality (%)	4.6±1.9	4.3±1.7	5.4±1.1	7.0±1.5	9.6±3.7*
No of sperm x 10^6	54.2±12.4	65.4±6.2	48.9±19.7	39.7±6.5	29.8±8.4*

* $p < 0.05$

The toxicokinetic data shows that GMA is metabolised into glycidol (and methacrylic acid). In a 90-day study (Irwin *et al.*, 1990) with glycidol exposure, a decrease in sperm count and motility was observed in rats from 100 mg/kg bw/day and in mice from 19 mg/kg bw/day. Testicular atrophy was reported in rats at 400 mg/kg bw/day of glycidol and in mice at all doses (from 19 mg/kg bw/day). Additional studies in male rats exposed via oral route demonstrated reversible infertility after 7 days of exposure to 15 mg/kg bw/day of glycidol (Hahn, 1970), massive preimplantation losses after 5 days of exposure at 40 mg/kg bw/day, sterility from 100 mg/kg bw/day (Jackson *et al.*, 1970) and reversible sterility after 5 days at 200 mg/kg bw/day (Cooper *et al.*, 1974). When investigated, sperm parameters and reproductive organ histology were not affected. Glycidol is classified as Repr. 1B; H360F in Annex VI of CLP.

Developmental toxicity

In addition to the OECD TG 422 reproductive screening study described above, prenatal toxicity studies were performed in the rat via oral route and in the rabbit via inhalation route.

In rats (Ou Yang *et al.*, 1988), oral administration (gavage) of GMA at 5.38, 10.76, 21.52 or 108 mg/kg bw/day from gestation day 5 to 15 induced significant increases in fetal resorptions and in the number of stillborn pups as summarised in the table below. The latter effect was not dose-related and its relationship to the treatment is equivocal uncertain.

Developmental effects in Ou Yang *et al.* (1988).

Dose (mg/kg bw/day)	0	5.38	10.76	21.52	108
Fetal resorptions (%)	5.18	2.60	3.40	1.23	12.70*
Stillborn pups (%)	0	1.35	7.58**	1.26	6.03

Rabbits were exposed via inhalation to 29.1, 58.2 or 291 mg/m^3 6h/day during gestation days 7-19 (Vedula, 1995). No adverse effects on any reproductive or developmental parameters were observed despite local toxicity at all doses that was severe at the highest dose.

In a subsequent study, rabbits were exposed to GMA via inhalation to 2.91, 11.6 or 58.2 mg/m^3 6h/day during gestation days 7-19 (Vedula, 1995). No adverse effects on any reproductive or developmental parameters were observed despite local toxicity (inflammation) from 11.6 mg/m^3.

Assessment and comparison with the classification criteria

Fertility

No human data are available and classification Repr. 1A is therefore not appropriate.

Clear evidence of an impaired fertility is available in rats via oral route from a guideline study (MHWJ, 1999). The effect occurred at the highest dose of 100 mg/kg bw/day. At this dose, effects on adrenal and kidney weight without histopathological changes as well as local toxicity in the gastrointestinal tract (squamous cell hyperplasia and cell infiltration in the forestomach) were observed. However, RAC considers that the clear effect on fertility cannot be a secondary non-specific consequence of the other toxic effects, that were mainly local effects.

Additional studies via the IP route provide evidence that the fertility in males is affected. Investigations of sperm parameters and reproductive organs have identified an effect on sperm count as well as on sperm morphology. RAC notes that genotoxicity was also reported in male germ cells after IP injections (Xie *et al.*, 1990b). An effect on sperm motility without further details, was also reported in infertile rats exposed to GMA via the oral route (MHWJ, 1999).

Toxicokinetic data shows that GMA is extensively metabolised into glycidol (and methacrylic acid). RAC notes that although a more comprehensive analysis of other substances metabolised into glycidol (or to similar metabolites), would have provided additional support, GMA is expected to produce similar systemic effects as glycidol, on the basis of the available data. Studies investigating fertility showed that glycidol induced male infertility. Although effects on sperm parameters were not affected in several studies, a decrease in sperm count and motility was identified in rats and mice in one study with glycidol (Irwin *et al.*, 1990). These data are consistent with the results of studies with GMA. It supports the conclusion that GMA can induce male infertility and that the effect is not secondary to the local toxicity of GMA, in particular since local toxicity is not observed with glycidol.

Although data point toward a direct action on sperm cells, there is overall no clear understanding on the mode of action of the effects of glycidol and GMA on male fertility and none of the available information raises questions concerning the relevance of the effects to humans.

In conclusion, RAC agrees with the DS that GMA meets the classification criteria as **Repr. 1B; H360F**

Developmental toxicity

Developmental toxicity was not observed in two inhalation rabbit studies or in one oral (gavage) OECD TG 422 rat study (MHWJ, 1997) A significant increase in fetal resorptions at the highest dose of 108 mg/kg bw/day was observed in a second oral (gavage) rat study (Ou Yang *et al.*, 1988). Such an effect was not observed in the OECD TG 422 guideline rat study at the same dose (MHWJ, 1997). However, only two dams were pregnant at this dose in the MHWJ (1997) study. Thus, based solely on this study, it is not possible to draw a sound conclusion on the possibility of GMS to induce fetal resorptions at 100 mg/kg bw/day. No

effects were observed at lower doses in any of the studies available.

RAC notes that the study by Ou Yang *et al.* (1988) was performed on GMA of low purity and it cannot be excluded that some impurities may have played a role in the induction of fetal resorptions at the highest dose. Thus, the reliability of this result is uncertain.

RAC concludes that no classification is justified for developmental effects based on the lack of reliable data, in particular the data from the highest doses via the oral route.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Table 25: Studies on neurotoxicity

Method	Results	Remarks	Reference
rat (Fischer 344) (inhalation: vapour) 0.5, 2 and 15 ppm Exposure: 13 weeks (6 hours per day, 5 days per week) 13-wk inhalation neurotoxicity study in Fisher 344 rats	NOAEL : > 15 ppm (nominal) (13-week inhalation neurotoxicity study was performed in Fischer 344 rats. At week 4, there was a low incidence of nasal discharge and enlarged nostrils at 3.9 and 12 mg/m^3. There were no treatment-related effects in any of the other measures. There was no evidence of neurotoxic effects at any exposure level.	2 (reliable with restrictions) key study experimental result **Test material: GMA**	Mattsson (1996) OECD (1999)

4.12.1.2 Immunotoxicity

No relevant information available

4.12.1.3 Specific investigations: other studies

No relevant information available

4.12.1.4 Human information

No relevant information available

4.12.2 Summary and discussion

Neurotoxicity

Fischer 344 rats were exposed by inhalation to GMA at approximately 0.5, 2 or 15 ppm (2.9, 12, 87 mg/m^3), 6 hours/day, 5 days/ week for 13 weeks (calculated daily dose: 0.35, 1.46, 10.59 mg/kg/day). At week 4, there was a low incidence of rat with nasal discharge and enlarged nostrils at 2 and 15 ppm. There were no other treatment-related effects. A functional observation battery (FOB) and motor activity (MA) were conducted pre-exposure and at the end of each month of exposure. In addition, the post-exposure neurotoxicity evaluation focused on evoked potential testing of the visual (FEP), auditory (ABR), somatosensory system (SEP), and caudal nerves (CNAP), and a comprehensive neuropathological examination.

There was no evidence of neurotoxic effects at any exposure level (Mattsson et al.: 1996).

4.12.3 Comparison with criteria

Based on the available studies and based on the other classifications already presented, no further classification of GMA for STOT RE is required under CLP.

4.12.4 Conclusions on classification and labelling

Based on the available studies classification under CLP of GMA is not required.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not assessed.

6 OTHER INFORMATION

No further information available

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8 ANNEXES

None