Committee for Risk Assessment
RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

m-bis(2,3-epoxypropoxy)benzene; resorcinol diglycidyl ether

EC Number: 202-987-5
CAS Number: 101-90-6

CLH-O-0000001412-86-250/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

30 November 2018

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

1,3-bis(2,3-epoxypropoxy)benzene; resorcinol diglycidyl ether

EC Number: 202-987-5

CAS Number: 101-90-6

Index Number: 603-065-00-9

Contact details for dossier submitter:

Bureau REACH
National Institute for Public Health and the Environment (RIVM)
The Netherlands
bureau-reach@rivm.nl

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Note on confidential information

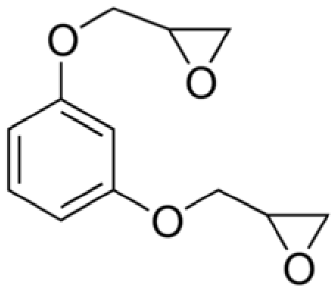
Please be aware that this report is intended to be made publicly available. Therefore it should not contain any confidential information. Such information should be provided in a separate confidential Annex to this report, clearly marked as such.

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	1,3-bis(2,3-epoxypropoxy)benzene
Other names (usual name, trade name, abbreviation)	1,3-diglycidyloxybenzene; m-bis(2,3-epoxypropoxy)benzene; 2,2’-(1,3-phenylenebis(oxymethylene))bisoxirane; resorcinol diglycidyl ether; DGRE
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	202-987-5
EC name (if available and appropriate)	Resorcinol diglycidyl ether
CAS number (if available)	101-90-6
Other identity code (if available)	-
Molecular formula	$C_{12}H_{14}O_4$
Structural formula	
SMILES notation (if available)	-
Molecular weight or molecular weight range	222.2
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	Not applicable

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Resorcinol diglycidyl ether	Not applicable	Acute Tox. 4* (H302) Acute Tox. 4* (H312) Skin Irrit. 2 (H315) Eye Irrit. 2 (H319) Skin Sens. 1 (H317) Muta. 2 (H341) Carc. 2 (H351) Aquatic Chronic 3 (H412)	Acute Tox. 4 (H302) Acute Tox. 4 (H312) Skin Irrit. 2 (H315) Eye Irrit. 2 (H319) Skin Sens. 1 (H317) Muta. 2 (H341) Carc. 2 (H351) Aquatic Chronic 3 (H412)

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
No information				

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

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
No information					

Table 5: Test substances (non-confidential information)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
Resorcinol diglycidyl ether	81%	No information		NTP 1986; Krishna-Murthy et al., 1990
Resorcinol diglycidyl ether	Not specified	No information		Hine et al., 1958; Westrick and Gross (1960), as cited in Gardiner et al., 1992; Van Duuren et al., 1965;

Identification of test substance	**Purity**	**Impurities and additives (identity, %, classification if available)**	**Other information**	**The study(ies) in which the test substance is used**
				Kotin and Falk, 1963; McCammon et al., 1957;
Resorcinol diglycidyl ether	Not specified	No information	Xylene (Acute Tox. 4* (H312); Acute Tox. 4* (H332)) is used as vehicle for dermal and inhalation testing	Westrick and Gross (1960), as cited in Gardiner et al., 1992

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors, ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	603-065-00-9	m-bis(2,3-epoxypropoxy)benzene; resorcinol diglycidyl ether	202-987-5	101-90-6	Acute Tox. 4* Acute Tox. 4* Skin Irrit. 2 Eye Irrit. 2 Skin Sens. 1 Muta. 2 Carc. 2 Aquatic Chronic 3	H302 H312 H315 H319 H317 H341 H351 H412	GHS08 GHS07 Wng	H302 H312 H315 H319 H317 H341 H351 H412			
Dossier submitters proposal	603-065-00-9	m-bis(2,3-epoxypropoxy)benzene; resorcinol diglycidyl ether	202-987-5	101-90-6	**Modify** Acute Tox. 4 Acute Tox. 3 Carc. 1B	**Modify** H302 H311 H350	**Add** GHS06 Dgr **Remove** GHS07 Wng	**Modify** H302 H311 H350		ATE-oral: 980 mg/kg bw ATE-dermal: 744 mg/kg bw	
Resulting Annex VI entry if agreed by RAC and	603-065-00-9	m-bis(2,3-epoxypropoxy)benzene; resorcinol diglycidyl ether	202-987-5	101-90-6	**Acute Tox. 4** **Acute Tox. 3** Skin Irrit. 2 Eye Irrit. 2	**H302** **H311** H315 H319	GHS08 GHS06 Dgr	**H302** **H311** H315 H319		ATE-oral: 980 mg/kg bw ATE-dermal: 744 mg/kg bw	

COM					Skin Sens. 1 Muta. 2 **Carc. 1B** Aquatic Chronic 3	H317 H341 **H350** H412		H317 H341 **H350** H412			

In **bold:** the classifications that are proposed to be changed

Table 7: Reason for not proposing harmonised classification and status under public consultation

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

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Resorcinol diglycidyl ether has previously been assessed for harmonised classification by TC C&L. Resorcinol diglycidyl ether has an Annex VI entry as Acute Tox. 4* (H302), Acute Tox. 4* (H312), Skin Irrit. 2 (H315), Eye Irrit. 2 (H319), Skin Sens. 1 (H317), Muta. 2 (H341), Carc. 2 (H351), Aquatic Chronic 3 (H412).

Resorcinol diglycidyl ether is not registered under REACH (February 2018).

In 1985 and 1999, IARC concluded that there are no data on the carcinogenicity of resorcinol diglycidyl ether to humans, but that there is sufficient evidence for the carcinogenicity of a technical grade of resorcinol diglycidyl ether in experimental animals (IARC, 1985+1999). IARC classified resorcinol diglycidyl ether (technical grade) as possibly carcinogenic to humans (Group 2B).

RAC general comment
Resorcinol diglycidyl ether (RDGE) has been assessed for harmonised classification by the Technical Committee for Classification and Labelling (TC C&L) under the Dangerous Substance Directive (DSD) 67/548/EEC in 1997. The current CLP classification arises from translation of the harmonised classification under DSD. The current CLH proposal is limited to two hazard classes, acute toxicity and carcinogenicity. The current existing classification for acute toxicity (oral and dermal) is considered as a minimum classification and was re-evaluated by the Dossier Submitter (DS) in the CLH proposal. The DS re-evaluated also the carcinogenicity classification and proposed an update after a comparison with the CLP criteria for carcinogenicity that are slightly different than the classification criteria under DSD. The CLH report is based on a recent report of the Health Council of the Netherlands (2016) using the monograph of the International Agency for Research on Cancer (IARC) as the starting point. IARC (1985, 1999) concluded diglycidyl resorcinol ether (technical grade) is possibly carcinogenic to humans (Group 2B).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[B.] Justification that action is needed at Community level is required.

Reason for a need for action at Community level:

Change in existing entry due to changes in the criteria

Further detail on need of action at Community level

The Health Council of the Netherlands published an evaluation of this substance in 1995 and concluded that resorcinol diglycidyl ether should be regarded as a genotoxic carcinogen (Health Council of the Netherlands, 1995). In 1999, it further concluded that the carcinogenicity studies were inappropriate for a quantitative extrapolation for an inhalation based occupational cancer risk value (Health Council of the Netherlands, 1999).

In 2016, the Health Council performed a re-evaluation of the mutagenic and carcinogenic properties of resorcinol diglycidyl ether. In this re-evaluation, the Health Council concluded that resorcinol diglycidyl ether is suspected to be carcinogenic to man and recommended to classify this substance in category 1B. Furthermore, they recommended classifying resorcinol diglycidyl ether as germ cell mutagen in category

2. They considered that the substance acts by a stochastic genotoxic mechanism (Health Council of the Netherlands, 2016).
This 2016 re-evaluation by the Health Council forms the basis for the current proposal for an update of the harmonised classification of resorcinol diglycidyl ether from Cat. 2 to Cat. 1B (H350) for carcinogenicity, taking into account that the criteria under CLP for carcinogenicity are slightly different than under DSD.

Further, the current CLP classification is based on a translation of the harmonised classification under DSD. As a result, the current classification for acute toxicity (oral and dermal) considers a minimum classification.

This CLH proposal is therefore limited to these hazard classes.

Sub-categorisation was considered for Skin Sens. 1. However, the available data did not allow differentiation between category 1A and 1B.

5 IDENTIFIED USES

Resorcinol diglycidyl ether is used as an epoxy resin and as a reactive diluent in the production of other epoxy resins. It is also used as a curing agent in the production of polysulfide rubber. In recent years, it has been primarily used in the aerospace industry.

6 DATA SOURCES

This CLH report is based on a recent report of the Health Council of the Netherlands (2016), "Resorcinol diglycidyl ether. Evaluation of the carcinogenicity and genotoxicity", No. 2016/03, The Hague, February 29, 2016. Starting point of their report was the monograph of the International Agency for Research on Cancer (IARC). Other sources as cited in the text and tables are mentioned in the reference list.

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Physical state at 20°C and 101,3 kPa	Straw yellow liquid		
Melting point	32-33 °C	DFG 1992	
Boiling point	172 °C	IARC 1985	
Relative density	1.21	ICSC 1991	
Vapour pressure	Low, $4x10^{-5}$ mm Hg at 25 °C	NTP 2011	
Surface tension	-		
Water solubility	Insoluble in water	Chemiekaarten 2017	
Partition coefficient n-octanol/water	No experimental data (1.23 calculated)		
Flash point	177 °C (open cup)	ICSC 1991	
Flammability			
Explosive properties	Reacts with strong oxidants; presumed to perform explosive	ICSC 1991	

Property	Value	Reference	Comment (e.g. measured or estimated)
	peroxides		
Self-ignition temperature	-		
Oxidising properties	-		
Granulometry	-		
Stability in organic solvents and identity of relevant degradation products	-		
Dissociation constant	-		
Viscosity	-		

8 EVALUATION OF PHYSICAL HAZARDS

This hazard class has not been evaluated.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

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

Only limited information on the toxicokinetics of resorcinol diglycidyl ether is available. One study was retrieved.

In a study by Seiler (1984a), male and female ICR-mice were treated orally (single dose) with ^{14}C labelled resorcinol diglycidyl ether and urine (collected for 1-4 hr after dosing) was analysed for metabolic products (the number of replicates was not reported). Four per cent of the metabolites detected in the urine was the phenol-diol metabolite, 64% was the bis-diol metabolite and 21% of the metabolites could not be identified. No bis-epoxide or diol-epoxide was excreted The total amount of radioactivity recovered from urine collected up to 4 hours after a single oral dose of 1,000 mg/kg body weight was nearly 50% of the applied dose. In addition, Seiler incubated epoxidase hydrolase containing liver homogenates (S9) with resorcinol diglycidyl ether and measured remaining alkylating activity. Resorcinol diglycidyl ether showed apparent first-order kinetics and a half-life of about 6 minutes. This study indicates that resorcinol diglycidyl ether is rapidly converted via the diol-epoxide to the inactive bis-diol substance.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Table 9: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
Non-guideline, non-GLP	Rat, Long-Evans, male Number of animals/group not specified	Resorcinol diglycidyl ether; propylene glycol was used as vehicle where necessary for ease of administration Intragastric administration	Single exposure, (except for highest dose*); Dose levels not specified; 10-day postexposure observation period	2570 mg/kg bw	Hine et al., 1958 Klimisch score: 2
Non-guideline, non-GLP	Mouse, Webster, male Number of animals/group not specified	Resorcinol diglycidyl ether; propylene glycol was used as vehicle where necessary for ease of administration Intragastric administration	Single exposure; Dose levels not specified; 10-day postexposure observation period	980 mg/kg bw	Hine et al., 1958 Klimisch score: 2
Non-guideline, non-GLP	Rabbit, albino, male Number of animals/group not specified	Resorcinol diglycidyl ether; propylene glycol was used as vehicle where necessary for ease of administration Intragastric administration	Single exposure; Dose levels not specified; 10-day postexposure observation period	1240 mg/kg bw	Hine et al., 1958 Klimisch score: 2

** because of the large volume of the highest intragastric dose for rats, the suspension was given in two aliquots, three hours apart, to fasted animals*

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

An acute toxicity study via the oral route was conducted in three species, i.e. Long-Evans rat, Webster mouse, and Albino rabbit (Hine et al., 1958). Resorcinol diglycidyl ether was administered via intragastric application. The resultant LD_{50} values were 2570, 980, 1240 mg/kg bw, respectively. In addition to lethality, there was moderate depression, slight dyspnea, and in surviving animals loss of weight and diarrhea observed.

10.1.2 Comparison with the CLP criteria

The LD_{50} value of the rat study (i.e. 2570 mg/kg bw) is outside the border for Acute oral Category 4 of 300-2000 mg/kg bw. The LD_{50} values of the mouse and rabbit studies (i.e. 980 and 1240 mg/kg bw, respectively) fall within the range for Acute oral Category 4 of 300-2000 mg/kg bw. This warrants classification as Acute Tox. 4.

The lowest LD_{50} of 980 mg/kg bw is suggested as ATE for acute oral toxicity.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Classification of resorcinol diglycidyl ether for acute toxicity via the oral route as Acute Tox. 4 (H302: Harmful if swallowed) is required.

It is proposed to assign an ATE of 980 mg/kg bw for acute oral toxicity.

10.2 Acute toxicity - dermal route

Table 10: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
Non-guideline, non-GLP	Rabbit, strain and sex not specified Number of animals/group not specified	Resorcinol diglycidyl ether	The test substance was applied as a 60% solution in xylene; Non-occlusion conditions; 7 hour exposure period	2420 mg/kg bw (2.0 ml/kg bw) Number of deaths and clinical signs not detailed. Further, it is noted that the contribution of xylene to the observed effects cannot be excluded.	Westrick and Gross (1960), as cited in Gardiner et al., 1992 Klimisch score: 3 (limited details available (secondary literature); co-exposure with xylene which might interfere with the outcome)
Non-guideline, non-GLP	Rabbit, strain and sex not specified Number of animals/group not specified	Resorcinol diglycidyl ether	Dose levels not specified; Continuous exposure, not further specified	744 mg/kg bw (0.64 ml/kg bw) No details provided.	Westrick and Gross (1960), as cited in Gardiner et al., 1992 Klimisch score: 4 (limited details available (secondary literature))

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

An acute toxicity study via the dermal route, only available as secondary source, was available in which rabbits were exposed for 7 hours (under non-occluded conditions) to resorcinol diglycidyl ether as a 60% solution in xylene (Westrick and Gross (1960), as cited in Gardiner et al., 1992). The LD_{50} was reported to be 2420 mg/kg bw. The original study, where this LD_{50} was based on was not available to the Dossier Submitter. Further, it is noted that xylene has a harmonized classification for acute dermal toxicity as Acute Tox. 4* (H312: Harmful in contact with skin). It is not clear what the contribution of xylene to the observed deaths in this study was. Therefore, no conclusion with respect to the acute dermal toxicity of resorcinol diglycidyl ether can be drawn based on this study.

In a second study, only available as secondary source, rabbits were continuously exposed (total exposure period not specified) to resorcinol diglycidyl ether (Westrick and Gross (1960), as cited in Gardiner et al., 1992). The LD_{50} was reported to be 744 mg/kg bw. No further details were provided on the number of animals and decedents and clinical signs. The original study, where this LD_{50} was based on, was not available to the Dossier Submitter. However, the data of this study were previously used by TC C&L to conclude that classification may be appropriate and resorcinol diglycidyl ether was subsequently classified at that time with R21 (The original CLH proposal from 1997 is provided in chapter 15 of this CLH-report.). Therefore, these data will also be used for current classification proposal.

10.2.2 Comparison with the CLP criteria

The reported dermal rabbit LD_{50} of 744 mg/kg bw fall within the range for Acute dermal Category 3 of 200-1000 mg/kg bw. This warrants classification as Acute Tox. 3.

An LD_{50} value of 744 mg/kg bw is suggested as ATE for acute dermal toxicity.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Classification of resorcinol diglycidyl ether for acute toxicity via the dermal route as Acute Tox. 3 (H311: Toxic in contact with skin) is required.

It is proposed to assign an ATE of 744 mg/kg bw for acute dermal toxicity.

10.3 Acute toxicity - inhalation route

Table 11: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, , form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Non-guideline, non-GLP	Rat, Long-Evans, male Number of animals/group not specified	Resorcinol diglycidyl ether	Single 8 hour exposure; Saturated air concentration; Concentration level not specified; 10-day postexposure observation period	No deaths observed; LC_{50} was greater than the highest vapour concentration attained	Hine et al., 1958 Klimisch score: 2
Non-guideline, non-GLP	Mouse, Webster, male Number of animals/group not specified	Resorcinol diglycidyl ether	Single 8 hour exposure; Saturated air concentration,; Concentration level not specified; 10-day postexposure observation	No deaths observed; LC_{50} was greater than the highest vapour concentration attained	Hine et al., 1958 Klimisch score: 2

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, , form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
			period		
Non-guideline, non-GLP	Rat, strain and sex not specified Number of animals/group not specified	Resorcinol diglycidyl ether	Single 4 hour exposure; 44.8 mg resorcinol diglycidyl ether (60% in xylene) per liter of air; aerosol.	All rats died within 5 days postexposure. However, it is noted that the contribution of xylene to the observed effects cannot be excluded.	Westrick and Gross (1960), as cited in Gardiner et al., 1992 Klimisch score: 3 (limited details available (secondary literature); co-exposure with xylene which might interfere with the outcome)

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

An acute toxicity study via the inhalation route was conducted in two species, i.e. Long-Evans rat and Webster mouse (Hine et al., 1958). Animals were exposed during 8 hours to resorcinol diglycidyl ether (as a saturated test atmosphere). No deaths were observed.

In a second study, only available as secondary source, rats were exposed for 4 hours to 44.8 mg resorcinol diglycidyl ether (60% in xylene) per liter of air (Westrick and Gross (1960), as cited in Gardiner et al., 1992). All animals died within 5 days postexposure. It is noted that xylene has a harmonized classification for acute inhalation toxicity as Acute Tox. 4* (H332: Harmful if inhaled). It is not clear what the contribution of xylene to the observed deaths in this study was. Therefore, no conclusion with respect to the acute inhalation toxicity of resorcinol diglycidyl ether can be drawn based on this study.

10.3.2 Comparison with the CLP criteria

As no deaths were observed in an acute inhalation study in rats and mice (Hine et al., 1958) up to the saturated vapour pressure, this does not warrant classification. However, seen the low saturated vapour pressure, it cannot be excluded that testing the mist would result in a requirement for classification but such data is not available. Although deaths were observed in a second inhalation study (Westrick and Gross (1960), as cited in Gardiner et al., 1992), the findings might be confounded by the presence of xylene in the test atmosphere. Therefore, this study cannot be used for classification purposes of resorcinol diglycidyl ether.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Classification of resorcinol diglycidyl ether for acute toxicity via the inhalation route is not required.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity: oral route

RDGE was tested for acute oral toxicity by intragastric application in male Long-Evans rats, male Webster mice and male Albino rabbits with a 10-day post-exposure observation period. Only a summary report of these studies (Hine et al., 1958) was available to the DS without information on the dose levels and number of animals/group. The reported LD_{50} values were 2570 mg/kg bw, 980 mg/kg bw, and 1240 mg/kg bw for rats, mice, and rabbits, respectively. The DS proposed a classification as acute toxicity category 4 (H302: Harmful if swallowed) with the lowest LD_{50} value of 980 mg/kg as the ATE value for acute oral toxicity.

Acute toxicity: dermal route

RDGE was tested for acute dermal toxicity in two studies in rabbits. The studies were available only as secondary sources (cited as Westrick and Gross (1960) in Gardiner et al., 1992) to the DS. Strain, sex, and number of animals/group were not specified.

In the first study an LD_{50} value of 744 mg/kg bw after a continuous exposure to RDGE was reported, but the dose levels, duration of exposure, clinical signs and the number of deaths were not reported. In the second study RDGE was applied as a 60% solution in xylene via a non-occlusive method for a 7-hour exposure period and an LD_{50} value of 2420 mg/kg bw was reported. The DS considered this study inadequate for classification purposes due to co-exposure to xylene, which could have interfered with the outcome of the study.

Based on the LD_{50} value of 744 mg/kg bw, the DS proposed a classification for acute toxicity category 3 (H311: Toxic in contact with skin) and suggested an ATE value of 744 mg/kg bw for dermal toxicity.

Acute toxicity: inhalation route

RDGE was tested for acute inhalation toxicity in two studies.

In the first study (Hine et al., 1958), a saturated air concentration of RDGE was tested in male Long Evans rats and male Webster mice via a single 8-hour exposure followed by a 10-day post-exposure observation period. The study was available to the DS only as a summary report without information on the tested concentrations or number of animals/group. No deaths were observed and the LC_{50} values were determined to be greater than the highest vapour concentrations attained. In the second study, 44.8 mg RDGE (60% in xylene) per litre of air was tested in rats via a single 4-hour exposure. The study was available only as a secondary source (cited as Westrick and Gross (1960) in Gardiner et al., 1992) to the DS. The strain, sex, and number of animals/group were not specified. All animals died within 5 days post-exposure. The DS considered this study inadequate for classification purposes due to co-exposure to xylene which could have interfered with the outcome of the study.

The DS proposed to not classify RDGE for acute toxicity via the inhalation route.

Comments received during public consultation

One Member State Competent Authority (MSCA) agreed to the proposed classification for acute toxicity and ATE values in the absence of more reliable data and limited information available on the study protocols. The DS responded that the present data had previously been used by the TC C&L to conclude on the classification for acute toxicity of RDGE and should therefore be included also in the current evaluation for classification, although acknowledged that the data might not entirely fulfil the current standards.

Assessment and comparison with the classification criteria

RDGE has been tested for acute oral, dermal and inhalation toxicity. The studies are available only as summary reports (Hine et al., 1958) or as secondary sources (cited as Westrick and Gross (1960) in Gardiner et al 1992) with limited information available. The same information has been previously considered by the TC C&L as documented in Annex I to the CLH report presenting the original classification proposal for RDGE from 1997.

In the absence of more reliable information, RAC agrees on the classification proposal of the DS. RAC agrees with the DS that the co-exposure to xylene in the dermal and inhalation tests invalidates the tests to be considered for the classification of RDGE due to the harmonised classification of xylene as Acute tox 4* (H312 and H332).

For acute oral toxicity, the reported LD_{50} values were 2570 mg/kg bw, 980 mg/kg bw, and 1240 mg/kg bw for rats, mice, and rabbits, respectively. The LD_{50} values of 980 mg/kg bw and 1240 mg/kg bw are within the borders of classification for acute toxicity 4 ($300 < ATE \leq 2000$). RAC takes also into consideration a micronucleus assay presented in the CLH report, in which a single oral dose of 300 or 600 mg/kg bw (98% RDGE in PEG-400) was administered and 1 out of 4 animals (ICR mice) died at 600 mg/kg bw within 48 hours (Seiler, 1984b). RAC therefore concludes that the **classification of RDGE as Acute Tox 4; H302 is warranted.**

To ensure a consistent classification of mixtures containing RDGE, RAC concludes also on a harmonised ATE value. The lowest LD_{50} value of 980 mg/kg bw was obtained in male mice. However, the reporting of the studies was very poor and no additional details are available to RAC. In the absence of more details on the available studies, RAC concludes to set the converted **acute toxicity point estimate of 500 mg/kg bw (Annex I CLP, table 3.1.2) as the ATE value for acute oral toxicity** .

For the dermal route, an LD_{50} value of 744 mg/kg bw after continuous exposure to rabbits is reported, which is within the borders of classification for acute dermal toxicity 3 (200 mg/kg bw < ATE ≤ 1000 mg/kg bw). RAC notes that in rabbits the dermal LD_{50} value is lower than the oral LD_{50} value. There is no information on the length of dermal exposure, clinical signs, number of animals and decedents. The death might be related to local skin effects (severe irritation), however due to the limited information available, no reliable analysis is possible for RAC. RAC agrees with the DS that classification of RGDE as **Acute Tox 3; H311 is warranted**. The DS proposed an ATE value of 744 mg/kg bw, but in the absence of more details on the available study, RAC concludes to set the converted **acute toxicity point estimate of 300 mg/kg bw (Annex I CLP, table 3.1.2) as the ATE value for acute dermal toxicity**.

In the acute inhalation toxicity study, no deaths were observed and the LC_{50} value was greater than the highest attained vapour concentration. No data on inhalation toxicity of RDGE mist is available. RAC concludes that a **classification for inhalation toxicity is not warranted**.

10.4 Skin corrosion/irritation

This hazard class has not been evaluated. However, in support of the evaluation of the endpoint carcinogencity, a summary table and a short summary of the skin irritation/corrosion studies are presented below. The individual studies can be found in Annex I.

Table 12: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
Draize method Non-guideline, non-GLP	Rabbit, Albino (sex not specified) Number of animals not specified	Resorcinol diglycidyl ether	Concentration not specified 24 h exposure, occlusive, readings at 24 and 72 h	Moderately irritating, with Draize score of 5 out of possible 8	Hine et al., 1958 Klimisch score: 2
Non-guideline, non-GLP	Rabbit, Albino (sex not specified) Four animals	Resorcinol diglycidyl ether	Concentration not specified; 7 daily applications of 7 h exposure; Readings at 24 h intervals (prior to subsequent application)	Severe irritation reported, score 8 out of possible 8 for erythema and edema (individual daily scores not reported). Three animals died by day 8.	Hine et al., 1958 Klimisch score: 2
Non-guideline, non-GLP	Rabbit, Five animals	Resorcinol diglycidyl ether	0.01 ml of a 10% solution in acetone; Details on the exposure period and time points of evaluation provided	Scar tissue formation was observed in one animal, and a definite erythema and edema were observed in the other four rabbits	Westrick and Gross, 1960 Klimisch score: 4 (limited details available (secondary literature))
Non-guideline, non-GLP	Rabbit (strain en sex not specified) Number of animals not	Resorcinol diglycidyl ether	0.5 ml of a 60% solution in xylene; 24 h exposure	Severe irritation which progressed to necrosis. Further, it is noted that the contribution of xylene to the observed effects cannot be excluded.	Westrick and Gross, 1960 Klimisch score: 3 (limited details

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results -Observations and time point of onset -Mean scores/animal -Reversibility**	**Reference**
	specified				available (secondary literature); co-exposure with xylene which might interfere with the outcome)

Summary on skin irritation/corrosion:

In a skin irritation study in which resorcinol diglycidyl ether was applied to rabbit skin for 24h, resorcinol diglycidyl ether was a moderate skin irritant (Draize score 5/8) (Hine et al., 1958). In a second study in which 4 rabbits were treated with 7 daily applications of 7 h each, severe irritation was reported (Draize score 8/8) (Hine et al., 1958). Within this study, 3 deaths, which occurred by treatment-day 8, were attributed to severe irritation. Finally, two studies were described (via secondary source) that reported irritation upon single exposure to resorcinol diglycidyl ether (Westrick and Gross, 1960).

Resorcinol has a current harmonized classification for skin irritation as Skin Irrit. 2 (H315). The original CLH proposal from 1997 is provided in chapter 15 of this CLH-report.

10.5 Serious eye damage/eye irritation

This hazard class has not been evaluated.

10.6 Respiratory sensitisation

This hazard class has not been evaluated.

10.7 Skin sensitisation

This hazard class has not been evaluated.

10.8 Germ cell mutagenicity

This hazard class has not been evaluated.

However, in support of the evaluation of the endpoint carcinogencity, a summary table and a short summary of the *in vitro* and *in vivo* mutagenicity studies are presented below. The individual studies can be found in Annex I.

Table 13: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	**Test substance, concentration levels, controls, etc**	**Observations**	**Reference**
Micro-organisms			
Reverse Mutation *Salmonella typhimurium* *Strains*: TA98, TA100, TA1535, TA1537	*Purity*: 87.9% (analyzed; method not reported) *Method*: 5 doses in DMSO using triplicate plates, retest at least one week later *Concentrations (µg/plate)* *Initial study*: 0-333(-S9 mix), 0-2,000(+S9 mix) *Retest*: 0-100(-S9 mix), 0-1,000/1,500(+S9 mix) *Metabolic system*: Liver S9 mix from Aroclor 1,254-induced male Sprague-Dawley rats and Syrian hamsters *Control*: Negative: vehicle; Positive: -S9 mix: sodium azide (TA100, TA1535), 9-aminoacridine (TA1537), 4-nitro-o-phenylenediamine (TA98); +S9 mix 2-aminoanthracene (all strains) *Statistical analysis*: not used	*Outcome*: TA98: negative TA1537: negative TA1535: positive with and without metabolic activation TA100: positive without metabolic activation and with rat S9 mix; equivocal with hamster S9 mix *Cytotoxicity*: Slight clearing of background lawn in the highest and sometimes second to highest dose tested	Canter et al., 1986; NTP, 1986 Klimisch score 2
Reverse Mutation Salmonella typhimurium *Strains*: TA100	*Purity*: >98% (HPLC) *Concentrations*: 0, 50, 100, 200, 500, 1,000 µg/plate *Metabolic system*: not used *Control*: Negative control: not specified, Positive control: not used *Statistical analysis*: not used	*Outcome*: positive Revertant colonies: 116, 438, 609, 772, 117, toxic, for 0, 50, 100, 200, 500, 1,000 µg/plate, for control and lowest through highest concentration, resp. *Cytotoxicity*: In 500 and 1000 µg/plate test	Seiler, 1984b Klimisch score: 3 (only one strain; no metabolic activation; no information on potential solvent used; no positive control; not specified negative control; number of replicates unknown)
Mammalian cells			
Gene mutation Mouse lymphoma L5178Y cells, *tk* locus	*Method*: Test performed in duplicate at *tk* *Concentrations*: 0, 0.25, 0.5, 1, 2, 4 µg/ml *Metabolic activation*: not used *Controls*: Negative: dimethylsulfoxide; Positive: ethyl methanesulfonate	*Outcome*: Mutant frequency (no. of mutant clones/million viable clones) *Tk*: Positive (5.3 fold increase mutant fraction) respectively: 60, 339, 783 761, lethal, lethal (1st test), 35, 182, 369, 689, 982, lethal (2nd test) *Cytotoxicity*: Relative total growth	McGregor et al., 1988 Klimisch score 2

Method, guideline, deviations if any	**Test substance, concentration levels, controls, etc**	**Observations**	**Reference**
	Purity: unknown *Solvent*: unknown *Statistical analysis*: dose-trend test and variance analysis		
Gene mutation Mouse lymphoma L5178Y cells, *tk* locus, *hprt* locus	*Method*: Test performed in duplicate at *tk* and *hprt* locus *Concentrations*: 0, 0.1, 0.4, 0.7 µg/ml (first exp.), 0, 0.1, 0.2, 0.4 µg/ml (second exp.) *Metabolic activation*: not used *Controls*: Negative: used, but not specified; Positive: ethyl methanesulfonate *Purity*: unknown *Solvent*: unknown *Statistical analysis*: not used	*Outcome*: Mutant frequency (no. of mutant clones/million viable clones) *Tk*: Positive, respectively: 14, 45, 157, 238 (1st test), 21, 48, 99, 173 (2nd test): *Hprt*: negative, 4, -, 8, 22 (first test), 12, 7, 4, 16 (2nd test) *Cytotoxicity*: Relative total growth	McGregor et al., 1996 Kimisch score 3 (no metabolic activation, no information on potential solvent used, purity unknown, negative control not specified)
Chromosome Aberration Chinese Hamster Ovary cells	*Method*: Positive results were repeated *Concentrations* (µg/ml): 0, 0.5, 1.6, 5, 16 (-S9); 0, 5, 16, (25 only in 2nd test), 50 (+S9) *Metabolic activation*: Liver S9 mix from Aroclor 1254-induced male Sprague-Dawley rats *Controls*: Negative: vehicle; Positive: mitomycin C (-S9), cyclophosphamide (+S9) *Purity*: >87.9% (analyzed; method not reported) *Solvent*: DMSO *Statistical analysis*: conducted on slopes of the dose-response curves and on individual dose points	*Outcome*: Positive with and without metabolic activation; % cells with aberrations (* indicates statistical significance): 3, 1, 4, 14*, 61* (-S9, 1st test); 0, 5*, 6*, 40*, 69* (-S9, 2nd test); 3, 3,10, 58* (+S9, 1st test); 3, 5, 8, 6, 27* (+S9, 2nd test) *Cytotoxicity*: No information reported	Gulati et al., 1989 Klimisch score 2
Chromosome Aberration Chinese Hamster Ovary cells	*Method*: 6 and 24 hours exposure *Solvent*: DMSO *Concentrations*: 2.5, 8, 25 µg/ml *Metabolic system*: not used *Control*: Negative control: not specified, Positive control: not used *Purity*: >98% (HPLC) *Statistical analysis*: estimated with the aid of the tables of Kastenbaum and Bowman (1970)	*Outcome*: Positive; % aberrant metaphases (number of metaphases scored): 2 (100), 8 (100), 24 (33), 44 (25) for 6 hr exposure, 2 (100), 9 (100), 48 (50), 93 (15) for 24 hr exposure for control and lowest through highest concentration, resp.	Seiler 1984b Klimisch score 3 (no information on check cell line absence of mycoplasma, number of chromosomes); no metabolic activation; no information on potential solvent used,

Method, guideline, deviations if any	**Test substance, concentration levels, controls, etc**	**Observations**	**Reference**
		Cytotoxicity: high at 8 and 25 µg/ml	negative control not specified; no positive controls; number of replicates unknown; no standard deviations reported; low numbers of metaphases scored at cytotoxic concentrations)
Other studies			
Sister chromatid Exchange Chinese Hamster Ovary cells	*Method*: Positive results were repeated *Concentrations* (µg/ml): 0, 0.05, 0.16, 0.5, (1.6 only in 1st test) (-S9); 0, 0.5, 1.6, 5, 16 (+S9) *Metabolic activation*: Liver S9 mix from Aroclor 1254-induced male Sprague-Dawley rats *Controls*: Negative: vehicle; Positive: mitomycin C (-S9), cyclophosphamide (+S9) *Purity*: >87.9% (analyzed; method not reported) *Solvent*: DMSO *Statistical analysis*: conducted on slopes of the dose-response curves and on individual dose points	*Outcome*: Positive with and without metabolic activation Number of SCE/cell (* indicates statistical significance): 7.7, 9.7*, 10*, 30*, 71* (-S9, 1st test); 9.1, 8.4, 21*, 49* (-S9, 2nd test); 9.6, 9.8, 10, 13*, 51* (+S9, 1st test); 9.4, 8.5, 9.9, 14*, 39* (+S9, 2nd test) *Cytotoxicity*: No information reported	Gulati et al., 1989 Klimisch score 2
Alkylating potency using the 4-(4-nitrobenzyl) pyridine assay Epoxyhydrolase containing rat and mice liver homogenates	*Method*: According to Friedman and Boger (1961) *Concentrations*: 12.5, 25, 50, 100 µg *Control*: Negative control: not specified, Positive control: not used *Purity*: >98% (HPLC) *Solvent*: unknown *Statistical analysis*: no descriptive or comparative statistics reported	*Outcome*: positive; Optical density at 450 nm (measured against negative control): 0.23, 0.55, 1.17, 2.18, respectively	Seiler 1984b

Table 14: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	**Test substance, concentration levels, controls, etc**	**Observations**	**Reference**
Somatic cell mutagenicity			
Micronucleus	*Method*: 5 animals per dose, test	*Outcome*: Overall result:	Shelby et al., 1993

Method, guideline, deviations if any	Test substance, concentration levels, controls, etc	Observations	Reference
Male B6C3F1 mice, bone marrow	performed in triplicate, intraperitoneal injection on three consecutive days, bone marrow cells sampled 24 hr after last treatment *Concentrations*: 15.2, 30.4, 60.8 mg/kg (first and second test), 30.4, 60.8, 91.2 mg/kg (third test) *Controls*: Negative: vehicle; Positive: dimethylbenzanthracene *Purity*: unknown *Solvent*: corn oil *Statistical analysis*: %PCEb: ANOVA; micronucleated PCE: unadjusted one-tailed Pearson chi-square test (pairwise comparison with solvent control group) and one-tailed trend test	negative; first test was positive: dose-related increase in micronuclei (highest dose: p=0.0442, trend: p=0.038), the other two tests were negative *Toxicity*: All animals survived, no cytotoxicity to PCE observed	Kllimisch score 2
Micronucleus (follow up previous test with higher concentrations) Male B6C3F1 mice; bone marrow cells	*Method*: 5 animals per dose, single intraperitoneal injection, sampled 24 hr after treatment *Concentrations*: 90, 180, 270 mg/kg *Controls*: Negative: vehicle; Positive: dimethylbenzanthracene *Purity*: unknown *Solvent*: corn oil *Statistical analysis*: unadjusted one-tailed Pearson chi-square test (pairwise comparison with solvent control group) and one-tailed trend test	*Outcome*: Positive: dose-related increase in micronuclei (highest dose: p=0.0008, trend: p=0.001) *Toxicity*: no information on survival/clinical signs of toxicity and toxicity to bone marrow	Shelby et al., 1993 Klimisch score: 2
Micronucleus ICR mice (male and female)	*Method*: single oral dose, 4 animals per dose *Doses*: 300 mg/kg with 24h fixation time; 600 mg/kg with 24, 48 and 72h fixation time *Control*: Negative control: not specified, Positive control: not used *Purity*: >98% (HPLC) *Solvent*: polyethylene-glycol (PEG 400) *Statistical analysis*: not used	*Outcome* Negative: inactive with respect to micronuclei formation *Toxicity*: 1 out of 4 animals died within 48 h in the experiments with 48h and 72h fixation time	Seiler, 1984b Klimisch score 3 (negative control not specified; no positive controls; no information on toxicity to bone marrow, low number of animals)
Other test systems			
Sex-linked recessive lethal induction *Drosophila melanogaster*	*Exposure*: 3 days to 50,000 ppm in feeding solution *Controls*: Negative: solvent; Positive: nitrosodimethylamine and β-propiolactone *Purity*: 87.9% *Solvent*: 9% ethanol, 1% Tween-80;	*Outcome*: Mutagenic: 0.19 and 1.31% lethals for control and exposed groups, resp. *Toxicity*: no mortality or sterility	Valencia et al., 1985; Woodruff et al., 1984 Klimisch score 3 (Classification based on studies in mammalians; no OECD guideline anymore)

Method, guideline, deviations if any	**Test substance, concentration levels, controls, etc**	**Observations**	**Reference**
	initial solution was diluted with aqueous 5% sucrose for feeding *Statistical analysis*: Poisson distribution to correct for spontaneous mutations. Normal test as suggested by Margolin et al. (1983)		
Reciprocal translocations induction *Drosophila melanogaster*	*Exposure*: three days to 50,000 ppm in feeding solution *Controls*: No concurrent negative controls (results were compared to combined historical control for three laboratories which was very low, namely 0.001%); Positive: N-nitrosodimethylamine and β-propiolactone *Purity*: 87.9% *Solvent*: 9% ethanol, 1% Tween-80; initial solution was diluted with aqueous 5% sucrose for feeding *Statistical analysis*: Conditional binomial test	*Outcome*: Mutagenic: total reciprocal translocations: 11 in 4,661 tests (0.24%)	Valencia et al., 1985; Woodruff et al., 1984 Klimisch score 3 (Classification based on studies in mammalians; no OECD guideline anymore)

<u>Germ cell genotoxicity</u>

Genotoxicity studies of resorcinol diglycidyl ether in germ cells, which can be considered relevant for humans, are not available.

<u>Somatic genotoxicity</u>

Resorcinol diglycidyl ether was investigated in genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, structural and numerical chromosome aberrations.

In vitro, resorcinol diglycidyl ether induced gene mutations in bacteria (TA100 and TA1535 strains, with and without metabolic activation) and in mammalian cells (mouse lymphoma study, tk locus). Exposure to resorcinol diglycidyl ether did also result in an increase in cells with chromosome aberrations with and without metabolic activation. The supporting genotoxicity tests confirmed the positive findings in *in vitro* tests.

In vivo, positive results were found in micronucleus tests at triplicate intraperitoneal doses of 60.8 mg /kg bw and at single intraperitoneal doses of 270 mg/kg bw.

The available data are in line with the existing harmonised classification as Muta. 2. The original CLH proposal from 1997 is provided in chapter 15 of this CLH-report.

10.9 Carcinogenicity

Table 15: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
F344/N rats 50 rats per dose/sex	Resorcinol diglycidyl ether; purity: 81% Gavage, 5 times/week, vehicle corn oil, 0, 25, 50 mg/kg bw/d Exposure period: 103 weeks Observation period: 104-105 weeks Statistical analysis tumour incidences: Fisher's exact test for pairwise comparison, Cochran-Armitage linear trend test for dose response trends. Two methods adjusting for intercurrent mortality using combining contingency tables by Mantel and Haenszel (1959) (life table test & incidental tumour test).	*Survival:* At end of study (week 104-105): males: 84, 10, 0%; females: 74, 32, 2% for control, low, and high-dose respectively. *Adverse effects*: Wheezing and respiratory distress. Body weights: High dose: lower than control after week 30; Low dose: lower than control after week 80 Increased incidence of hyperkeratosis and basal cell hyperplasia in forestomach in both dose groups and both sexes *Tumours*: For control, low, and high-dose respectively Forestomach: squamous cell papillomas: males: 0, 34, 12% (Adjusted for intercurrent mortality: 0, 40.9, 33.5%); females: 0, 14, 2% (Adjusted: 0, 24.2, 14.3%) Forestomach: squamous cell carcinoma: males: 0, 76, 8% (adjusted: 0, 100, 100%); females: 0, 68, 6% (adjusted: 0, 97, 100%)	NTP 1986; Krishna-Murthy et al., 1990 Klimisch score: 2
Supplemental to previous study F344/N rats 50 rats per dose/sex	Resorcinol diglycidyl ether; purity: 81% Gavage, 5 times/week, vehicle corn oil, 0, 12 mg/kg bw/d exposure period: 103 weeks observation period: 104-105 weeks Statistical analysis tumour incidences: Fisher's exact test for pairwise comparison, Two methods adjusting for intercurrent mortality using combining contingency tables by Mantel and Haenszel (1959) (life table test & incidental tumour test).	*Survival*: At end of study (week 104-105): males: 78, 46%; females: 78, 70% for control and treated respectively *Adverse effects*: Increased incidence of hyperkeratosis and basal cell hyperplasia in forestomach in both sexes *Tumours*: For control and treated respectively. Forestomach: squamous cell papillomas: males: 0, 32% (Adjusted for intercurrent mortality: 0, 51.7%); females: 0, 38% (Adjusted: 0, 48.4%) Forestomach: squamous cell carcinoma: males: 0, 78% (adjusted: 0, 92.8%); females: 0, 54% (adjusted: 0, 64%)	NTP 1986; Krishna-Murthy et al., 1990 Klimisch score: 2
B6C3F1 mice	Resorcinol diglycidyl ether; purity: 81%	*Survival*: At end of study (week 104-105): males: 60, 52, 68%; females: 40, 26, 20% for control, low, and high-dose respectively	NTP 1986; Krishna-Murthy et al.,

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
50 mice per dose/sex	Gavage, 5 times/week, vehicle corn oil, 0, 50, 100 mg/kg bw/d exposure period: 103 weeks observation period: 104-105 weeks Statistical analysis tumour incidences: Fisher's exact test for pairwise comparison, Cochran-Armitage linear trend test for dose response trends. Two methods adjusting for intercurrent mortality using combining contingency tables by Mantel and Haenszel (1959) (llfe table test & incidental tumour test).	*Adverse effects*: Body weights: High dose female mice: lower than control after week 20; Other groups were comparable to control. Increased incidence of hyperkeratosis and epithelial cell hyperplasia in forestomach in both dose groups and both sexes *Tumours*: For control, low, and high-dose respectively Forestomach: squamous cell papillomas or papillomatosis: males: 0, 8, 20% (Adjusted for intercurrent mortality: 0, 14, 29.4%); females: 0, 10, 20% (Adjusted: 0, 33.4, 73.1%) Forestomach: squamous cell carcinoma: males: 0, 29, 50% (adjusted: 0, 40.7, 55.5%); females: 0, 24, 47% (adjusted: 0, 53.3, 70.5%) Liver: hepatocellular carcinoma: females: 0, 2, 6% (adjusted 0, 6.3, 25%) Liver: hepatocellular carcinoma and adenoma combined: females: 6, 2, 14% (adjusted 16, 6, 43%)	1990 Klimisch score: 2
Swiss-Millerton female mice 30 treated; 60 untreated controls; 60 vehicle controls	Resorcinol diglycidyl ether Purity: not specified Dermal application (to clipped dorsal skin) 1% in benzene, three times per week, about 100 mg of solution per application Exposure + observation period: life-span The study was continued until there were no survivors	*Survival*: Median survival time: 441, 408 and 491 days for untreated control, vehicle control and treated mice, resp *Tumours*: No tumours observed in any group	Van Duuren et al., 1965 Klimisch score: 3
C57/B1 mice 20 treated	Resorcinol diglycidyl ether Total concentration 0.75 mM Exposure route, frequency and duration, vehicle, purity test material, observation period, method of tumour detection: not specified	*Survival*: 14/20 after 8 months *Tumours*: One skin tumour observed (after 8 months)	Kotin and Falk, 1963 Klimisch score: 3 (not adequate for carcinogenicity asssessment)
C57/B1 mice	Resorcinol diglycidyl ether Purity: not specified Intrascapular painting three times a week	Authors state that substance was carcinogenic; organs not mentioned	McCammon et al., 1957 Klimisch score: 4
Long-Evans	Resorcinol diglycidyl ether	Authors state that substance was carcinogenic; organs not	McCammon et

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
rats	Purity: not specified Subcutaneous injection	mentioned	al., 1957 Klimisch score: 4

The table above summarizes the carcinogenicity studies in experimental animals. In these studies resorcinol diglycidyl ether was administered orally (gavage), dermally or by subcutaneous injection. No inhalation carcinogenicity studies were available.

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

No data on the carcinogenicity of resorcinol diglycidyl ether in humans are available.

The animal studies published in 1957-1965 have substantial shortcomings in design and reporting and are not adequate for assessment of carcinogenicity. The studies of the NTP were well performed and reported and, therefore, considered suitable for assessing the carcinogenic potential of resorcinol diglycidyl ether.

Long-term oral carcinogencity studies were performed with rats and mice (NTP 1986; Krishna-Murhty et al., 1990). In the 2-year oral gavage study in rats (0, 25, 50 mg/kg bw/d, 5 d/wk, 2 year), observed effects included reduced body weight gain and a dose-related reduced survival in both sexes. At the end of the 2-year study, 42/50, 5/50 and 0/50 male and 37/50, 16/50 and 1/50 female rats of the 0, 25 and 50 mg/kg bw/d dose groups, respectively, had survived. Histopathological examination revealed lesions in forestomach. These included non-neoplastic lesions such as basal cell hyperplasia and hyperkeratosis, and statistically increased increased incidences of benign and malignant neoplastic lesion of the epithelium such as squamous cell papilloma (males: 0/50, 17/50 (34%) and 6/49 (12%), females: 0/49, 7/50 (14%) and 1/50 (2%)) and squamous cell carcinoma (males: 0/50, 38/50 (76%) and 4/49 (8%), females: 0/49, 34/50 (68%) and 3/50 (6%)). It is noted that in the high dose animals, the effects were not as striking, which may be explained by the markedly increased number of deaths (Table 16). Adjustment of these numbers for intercurrent mortality, the incidences of squamous papillomas were 0, 40.9 and 33.5% (0, 25 and 50 mg/kg bw/d) for male rats, and 0, 24.2, 14.3% for female rats. Adjusted incidences for squamous carcinomas were 0, 100 and 100% (0, 25 and 50 mg/kg bw/d) for male rats and 0, 97, 100% for female rats. In addition to the increased incidences of forestomach tumours, some tumour types were observed with reduced incidences (i.e. adrenal pheochromocytoma, leukemia, pituitary adenoma, and thyroid C-cell tumors in males and females; lung adenoma, pancreatic islet cell tumors, and interstitial cell tumors of the testes in males; and mammary glandfibroadenomas and uterine tumors in females). However, none of these decreases were statistically significant when life table analyses were used, and they appeared to be related to the reduced survival observed in the dosed groups relative to those in the controls (NTP, 1986; Krishna-Murthy et al., 1990).

Table 16. Incidences of neoplasms of the stomach in male and female rats administered resorcinol diglycidyl ether in corn oil by gavage for two years (NTP, 1986; Krishna-Murthy, 1990). A: main study, B: supplemental study

A

	Dose resorcinol diglycidyl ether (mg/kg bw/d)					
	0		**25**		**50**	
	m	f	m	f	m	f
Squamous cell papilloma						
Overall incidence	0/50 (0%)	0/49 (0%)	17/50 (34%)	7/50 (14%)	6/49 (12%)	1/50 (2%)
Adjusted incidence[(a)]	0.0%	0.0%	40.9%	24.2%	33.5 %	14.3%
Terminal incidence	0/42 (0%)	0/36 (0%)	0/5 (0%)	1/16 (6%)	0/0 (0%)	0/1 (0%)
Life table test	P<0.001	P<0.001	P<0.001	P=0.002	P<0.001	P=0.125
Cochran-Armitage trend test	P=0.058	P=0.421				
Fischer Exact test			P<0.001	P=0.007	P=0.012	P=0.505
Squamous cell carcinoma						
Overall incidence	0/50 (0%)	0/49 (0%)	38/50 (76%)	34/50 (68%)	4/49 (8%)	3/50 (6%)
Adjusted incidence[(a)]	0.0%	0.0 %	100%	97.0%	100%	100.0%
Terminal incidence	0/42 (0%)	0/36 (0%)	5/5 (100%)	15/16 (94%)	0/0 (0%)	1/1 (100%)
Life table test	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Cochran-Armitage trend test	P=0.199	P=0.300				
Fischer Exact test			P<0.001	P=0.001	P=0.056	P=0.125

(a) Kaplan-Meier estimated tumor incidence at the end of the study after adjusting for intercurrent mortality

B

	Dose resorcinol diglycidyl ether (mg/kg bw/d)			
	0		**12**	
	m	f	m	f
Squamous cell papilloma				
Overall incidence	0/50 (0%)	0/50 (0%)	16/50 (32%)	19/50 (38%)
Adjusted incidence[(a)]	0.0%	0.0%	51.7%	48.4%
Terminal incidence	0/39 (0%)	0/39 (0%)	10/23 (43%)	15/35 (43%)
Life table test			P<0.001	P<0.001
Incidental tumour test			P<0.001	P<0.001
Fischer Exact test			P<0.001	P<0.001
Squamous cell carcinoma				
Overall incidence	0/50 (0%)	0/50 (0%)	39/50 (78%)	27/50 (54%)
Adjusted incidence[(a)]	0.0%	0.0 %	92.8%	64.0%
Terminal incidence	0/39 (0%)	0/39 (0%)	20/23 (87%)	20/35 (57%)
Life table test			P<0.001	P<0.001
Incidental tumour test			P<0.001	P<0.001
Fischer Exact test			P<0.001	P<0.001

(a) Kaplan-Meier estimated tumor incidence at the end of the study after adjusting for intercurrent mortality

Due to the excessive mortality at the high dosed rats, a supplemental rat study was performed using resorcinol diglycidyl ether at dose levels of 0 and 12 mg/kg bw/d (5 d/wk, 2 yr). Survival of male but not female rats was significantly reduced compared to the controls. The reduced survival in males was probably due to the increase in squamous cell carcinomas as the incidence of intercurrent mortality without such tumours was comparable between the treated group ((27-19)/50) and the controls (11/50). The body weights were not affected. Similar lesions of the forestomach were observed upon histopathological examination. Markedly increased incidences of hyperkeratosis and basal cell hyperplasia, and squamous cell papilloma (males: 0/50 (0%), 16/50 (32%); females: 0/50 (0%), 19/50 (38%)) and carcinoma (males: 0/50 (0%), 39/50 (78%); females: 0/50 (0%), 27/50 (54%)) were noticed in both sexes. In addition to the increased incidences of forestomach tumours, some tumour types were observed with reduced incidences (i.e. C-cell tumors of the thyroid in males, and pheochromocytomas of the adrenal medulla in females) (NTP, 1986; Krishna-Murthy et al., 1990).

In the 2-year oral gavage study in mice (0, 50 and 100 mg/kg bw/d, 5 d/wk, 2 year), observed effects included reduced body weight in high dose females. Survival was not significantly affected but survival was low in all female mice groups. Histopathological evaluation revealed treatment-related lesions which were primarily observed in the forestomach of low and high dose mice of both sexes. The incidence of hyperkeratosis and epithelial cell hyperplasia in the forestomach was markedly increased in low- and high-dose mice of both sexes, and squamous cell papillomas and papillomatosis (males: 0/47 (0%), 4/49 (8%), 10/50 (20%); females: 0/47 (0%), 5/49 (10%), 10/49 (20%)) and carcinomas (males: 0/47 (0%), 14/49 (29%), 25/50 (50%); females: 0/47 (0%), 12/49 (24%), 23/49 (47%)) of the forestomach occurred in male and female mice with statistically significant positive trends and the incidences in the high dose groups were significantly higher than those in the controls. Further, a positive trend was observed for hepatocellular carcinoma in female mice and the incidences of combined adenoma and carcinoma in liver were statistically significantly increased. NTP (1986) considered these as not related to treatment. In comparison with historical control data, the incidence in females dosed with the high dose resorcinol diglycidyl ether (6% for carcinoma, 14% for combined adenoma/carcinoma) was lower than that in historical controls at the same laboratory (upper level 8% for carcinoma and 14% for combined adenoma/carcinoma). Historical control data should preferably be taken from the same laboratory and the same strain, using a time period that is close to the time period at which the study under consideration is conducted. The NTP-study were conducted at Mason lab, which is also included in the historical control data as presented by NTP. It is noted that that the exact time period has not been specified for the individual historical studies. No further details are available on the historical control data from NTP. In addition to the increased incidences of forestomach tumours, some tumour types were observed with reduced incidences (i.e. all types of malignant lymphomas in females, and fibroma, fibrosarcoma, or -carcoma in male mice) (NTP 1986; Krishna-Murthy et al., 1990).

Potential mechanism and human relevance of the forestomach tumours.

The precise underlying mechanism of action for any forestomach carcinogen is at present not fully known. The tumorigenic lesions may be the result of a direct, genotoxic action of the compound on the epithelium, an indirect action (a prolonged proliferation stimulus) or a combination of both (RIVM, 2003).

A working group of IARC concluded that carcinogens that are DNA reactive and cause forestomach tumours in rodents – even if they only caused tumours at this site – should be evaluated as if they presented a carcinogenic hazard to humans (IARC, 2003). This conclusion is based on the fact that although humans do not have a forestomach, they do have comparable squamous epithelial tissues in the oral cavity and the upper two-thirds of the oesophagus. Also, the target tissues for carcinogens may differ between experimental animals and humans and a forestomach carcinogen in rodents may target a different tissue in humans.

Proctor et al. (2007) reviewed the relevance of rodent forestomach tumours in cancer risk assessment. Substances that cause forestomach tumour through nongenotox mechanisms they consider not to be relevant for human carcinogenicity because the mode-of-action is specific to the forestomach. Substances that are DNA reactive and cause tumours at multiple sites, in addition to the forestomach, are likely relevant human carcinogens (Proctor et al., 2007).

Further, the CLP-guidance (section 3.6.2.3.2a) states the following with respect to forestomach tumours (i.e. tumours occurring in tissues with no human equivalent): "*Forestomach tumours in rodents following administration by gavage of irritating or corrosive, non mutagenic substances. In rodents, the stomach is divided into two parts by the muco-epidermoid junction separating squamous from glandular epithelium. The proximal part, or forestomach, is non-glandular, forms a continuum with the oesophagus, and is lined by keratinized, stratified squamous epithelium. While humans do not have a forestomach, they do have comparable squamous epithelial tissues in the oral cavity and the upper two-thirds of the oesophagus. See also this Section (k), IARC (2003), and RIVM (2003).*

Tumours occurring in such tissues indicate that the substance has the potential to induce carcinogenic effects in the species tested. It cannot automatically be ruled out that the substance could cause similar tumours of comparable cell/tissue origin (e.g. squamous cell tumours at other epithelial tissues) in humans. Careful consideration and expert judgement of these tumours in the context of the complete tumour response (i.e. if there are also tumours at other sites) and the assumed mode of action is required to decide if these findings would support a classification. However, tumours observed only in these tissues, with no other observed tumours are unlikely to lead to classification. However, such determinations must be evaluated carefully in justifying the carcinogenic potential for humans; any occurrence of other tumours at distant sites must also be considered."

Based on the available *in vitro* and *in vivo* mutagenicity studies, resorcinol diglycidyl ether can be considered a mutagenic subtance. *In vitro* studies showed that resorcinol diglycidyl ether induced gene mutations in bacteria, and mouse lymphoma cells (tk locus) and structural chromosomal aberrations in cultured mammalian cells with and without metabolic activation, which suggests a stochastic genotoxic mechanism. The *in vivo* mouse micronucleus study of Seiler (1984b), applying exposure to resorcinol diglycidyl ether via the oral route, did not find a positive response. The *in vivo* mouse micronucleus of Shelby et al. (1993), applying exposure to resorcinol diglycidyl ether via the intraperitoneal route (which is considered physiologically less relevant to humans), revealed statistically significant increases in cells with micronuclei upon single high exposure only. It is noted that upon a triple-exposure with somewhat lower dose levels, a negative outcome was considered. Shelby et al. (1993) considered that due to the toxicity characteristics of resorcinol diglycidyl ether, a triple-exposure protocol does not permit use of a sufficiently high dose levels to induce observable genetic toxicity.

The data of the toxicokinetic study performed by Seiler (1984a) indicates that oral absorption occurs, which is likely to be followed by systemic distribution (of the parent compound and/or its metabolites). Seiler (1984a) observed that the total amount of radioactivity recovered from urine collected up to 4 hours after a single oral dose of 1000 mg/kg body weight was nearly 50% of the applied dose. Nevertheless, resorcinol diglycidyl ether-induced micronucleus could not be revealed upon oral exposure *in vivo* (Seiler, 1984b), though upon intraperitoneal administration of high doses positive findings were noticed (Shelby et al., 1997). The CLP-guidance states that "*A positive result for somatic or germinal mutagenicity in a test using intraperitoneal administration only shows that the tested substance has an intrinsic mutagenic property, and the fact that negative results are exhibited by other routes of dosage may be related to factors influencing the distribution/ metabolism of the substance which may be characteristic to the tested animal species. It cannot be ruled out that a positive test result in intraperitoneal studies in rodents may be relevant to humans.*" Resorcinol diglycidyl ether-induced tumours were only observed in the forestomach, i.e. the site of contact. Tumours in other, systemic, tissues were not observed (NTP 1986; Krishna-Murthy et al., 1990). The toxicokinetic study performed by Seiler (1984a) also showed that resorcinol diglycidyl ether is rapidly inactivated within the body, which might explain why *in vitro* studies showed clear genotoxic effects whereas not all *in vivo* mutagenicity results were conclusive. This rapid metabolization to genetically inactive substance and the *in vitro* results indicating that this substance does not require metabolic activation might also explain why resorcinol diglycidyl ether-induced tumours were observed only at the site of contact (the forestomach) in the oral (gavage) carcinogenicity studies performed by the NTP, because the active substance is not distributed to other tissues in significant amounts.

The available skin irritation data, the repeated dose studies as well as the carcinogenicity studies point towards an irritative effect at the site of contact upon exposure to resorcinol diglycidyl ether. Resorcinol diglycidyl ether was found to be a moderate skin irritation upon 24h application on rabbit skin (Draize score 5/8) (Hine et al., 1958). Multiple applications (7 daily applications of 7 h each) to rabbit skin resulted in severe irritation (Draize score 8/8), which in some animals even resulted in death (Hine et al., 1958). In the 2-week and 13-week repeated dose studies, resorcinol diglycidyl ether (via oral gavage) was found to induce effects primarily in the forestomach of F344/N rats and B6C3F1 mice of both sexes, causing mucosal cell proliferation, hyperkeratosis, hyperplasia and papillary growth, mucosal ulcers of the forestomach (Ghanayem et al., 1986; NTP, 1986; Krishna-Murthy et al., 1990). When comparing the type/severity of stomach effects in the 2-week and 13-week oral gavage studies with respect to dose levels, mainly local effects with limited severity were noticed in these repeated dose studies. Severe type of effects such as ulceration was only observed at high dose levels (i.e. higher than the dose levels as applied in the carcinogenicity studies). The data of the NTP carcinogenicity study also point towards local irritation in the forestomach as hyperkeratosis and hyperplasia of the epithelium were observed (NTP 1986; Krishna-Murthy et al., 1990). Taking into account these data, this might suggest that chronic tissue damage with resultant hyperplasia may have contributed to the carcinogenic response in the forestomachs of rats and mice.

Based on the available carcinogenicity data and taking into account the data on toxicokinetics, skin irritation, repeated dose toxicity and mutagenicity, it is considered that resorcinol diglycidyl clearly induces local effects at the site of contact. Upon oral exposure this results in mucosal cell proliferation, hyperkeratosis, hyperplasia, papillary growth, mucosal ulcers and squamous cell papillomas and carcinomas in the forestomach, and by that, it may be considered that resorcinol diglycidyl ether acts (at least partly) via an indirect mode of action (i.e. a prolonged proliferation stimulus). However, as resorcinol diglycidyl ether was also found to be a mutagenic substance, though probably acting at the site of contact and not via systemic exposure due to inactivation, it may be considered that the resorcinol diglcidyl ether-induced forestomach tumours are induced via a (local) genotoxic mechanism.

Taking into account the considerations of RIVM (2003) and IARC (2003), the forestomach tumours as observed in F344/N rats and B6C3F1 mice of both sexes (NTP 1986; Krishna-Murthy et al., 1990) should be taken forward for classification of resorcinol diglycidyl ether for the endpoint carcinogenicity. A potential irrelevance for humans is not clearly demonstrated for the resorcinol diglycidyl ether-induced forestomach tumours.

Read-across with other related substances

As far as known to the Dossier submitter no other resorcinol glycidyl ethers have been tested for carcinogenicity. Several other glycidylethers have been tested for carcinogenicity and a number have a harmonised classification for carcinogenicity and/or mutagenicity but mostly in category 2. Probably the most comparable substance that was tested for carcinogenicity is phenyl glycidyl ether (CAS 122-60-1) as it contains one instead of two glycidylether side chains. Phenyl glycidyl ether is classified as carcinogenic 1B and mutagenic 2. The original CLH proposal from 1997 of phenyl diglycidyl ether is provided in chapter 15 of this CLH-report The carcinogenicity classification was based on an increase in nasal tumours in a inhalation carcinogenicity study in rats. Therefore, it is considered that the read-across to the most comparable substance tested for carcinogenicity, phenyl glycidyl ether, may support the proposed classification as carcinogen category 1b. It is acknowledged that the basis for this read-across may be considered limited. However, in the view of the Dossier Submitter, no other data are available that can be used for read-across.

Table 17: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Consistent increase in tumours was observed in both rats and mice	Forestomach tumours; the increase in tumours is limited to the site of exposure in rats and mice	The increase in tumours is limited to the site of exposure in rats and mice	The forestomach tumours in both rats and mice progress to malignancy.	As no forestomach tumours were observed in control rats and mice and in treated animals the tumours were observed partly before the terminal sacrific, the latency period was reduced.	Forestomach tumours were observed in both male and female rats and mice	Local toxicity due to the irritating properties may have contributed to the formation of tumours. Local effects included hyperkeratosis and hyperplasia, indicating no excessive toxicity though these local effects might have contributed to the observed tumour response. In addition, an increase in mortality was observed in the rat and mouse (females) studies. In the rat most early non tumour related mortality were attributable to bronchopneumonia. In female mice the major cause of early dead was necrosuppurative lesion of the ovary. The cause of the lethality is not related to the tumour formation.	The oral route is considered a relevant route of exposure for humans	The available information indicates that both local irritation and (local) mutagencity may have contributed to the increase in forestomach tumours. A genotoxic MoA is considered relevant for humans, while the relevancy of a MoA of local irritation might be questionable. However, no data available to conclude with certainty which MoA is responsible for the forestomach tumours

10.9.2 Comparison with the CLP criteria

No information is available regarding carcinogenicity in humans. Therefore category 1A is not applicable.

Classification in category 1B requires "*a causal relationship between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times* *or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites*".

Adequate studies on carcinogenicity in experimental animals were available for the oral route (NTP 1986; Krishna-Murthy et al., 1990). In these studies resorcinol diglycidyl ether was carcinogenic for two species (i.e. rat and mouse) of both sexes, causing benign and malignant neoplasms of the forestomach. This would basically be sufficient for classification in category 1B (i.e. sufficient evidence for carcinogenity).

An increase in mortality and a reduction in body weight was observed in the chronic study in rats and the tested dose levels could be considered to high. However, an additional dose level was tested without a decrease in body weight and no increase in mortality due to general toxicity showing an increase in the same type of tumours. Therefore, the studies are considered adequate atv least at one or more dose levels for determining the carcinogencity.

With respect to the responsible mode of action, it is known that resorcinol diglycidyl ether is a mutagenic substance. Forestomach tumours, caused by substances that act via a genotoxic mechanism, are considered relevant for humans (IARC, 2003; Proctor, 2007; RIVM, 2003). However, the data of the NTP-study also point towards local irritation in the forestomach as hyperkeratosis and hyperplasia of the epithelium were observed (NTP 1986; Krishna-Murthy et al., 1990). This might also suggest that chronic tissue damage with resultant hyperplasia may have contributed to the carcinogenic response. However, there are currently no data which can exlude a genotoxic mode of action. Therefore, it is assumed that the (local) genotoxicity contributed to the observed tumour response. Consequently, tumour formation at the site of first contact is considered relevant for this substance. Subsequently, the forestomach tumours are considered relevant for humans (IARC, 2003; Proctor, 2007; RIVM, 2003).

Based on these data, it can be concluded that there is sufficient evidence of carcinogenicity. According to the CLP criteria, resorcinol diglycidyl ether should, therefore, be classified in category 1B.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Classification of resorcinol diglycidyl ether for carcinogenicity in category 1B (H350: May cause cancer) is warranted.

RAC evaluation of carcinogenicity
Summary of the Dossier Submitter's proposal The carcinogenicity of RDGE was investigated in two oral (gavage) NTP studies in rats (a primary and a supplemental study), and in one oral (gavage) NTP study in mice. In addition, three older studies in mice were reported by the DS. However, these studies were carried out during the years 1957-1965 and were considered not to be suitable for classification due to substantial shortcomings in their design and reporting. In the primary NTP study in rats (NTP 1986, Krishna-Murthy et al., 1990), male and female F344/N rats (50/sex/dose) received RDGE (purity 81%, vehicle corn oil) 5 times/week by gavage for 103 weeks at 0, 25, 50 mg/kg bw/d. Due to excessive mortality at both doses, a supplemental study was performed with dose levels of 0 and 12 mg/kg bw/d. The histopathological examination revealed lesions of the forestomach including non-neoplastic lesions, basal cell hyperplasia and hyperkeratosis, and a statistically significant increase in the incidence of benign and malignant neoplasms (squamous cell papilloma and squamous cell carcinoma) in both studies at 12, 25, and 50

mg/kg bw/d in both sexes. Metastases (in the brain, liver, lung, lymph nodes, pancreas and spleen) were observed in 20 treated rats in the primary study. No tumours were found in the nasopharynx and oesophagus squamous epithelium, although the substance was hyperkeratotic in some rats. NTP Historical Control Data (HCD, of the research programme including the study performing laboratory) of stomach tumours (NTP 1986, data as of 1983) was provided by the DS as a response to comments received during the public consultation. Incidences for both sexes were close to zero in both HCD bases (NTP research programme HCD: 5/1065 (0.5%) (males) and 5/1073 (0.5%) (females); HCD of the performing laboratory: 1/200 (males) and 0/199 (females))

***Table 1:** Forestomach lesions in rats administered RDGE by gavage for 2 years (NTP 1986; Krishna-Murthy et al., 1990). Data are combined for the primary and the supplementary study.*

Exposure level (mg/kg bw/d)	0	0*	12*	25	50
Male rats					
Mortality[1]	8/50	11/50	27/50	45/50	50/50
Body weight / clinical signs[2]			(96.4%)/-	d (84.2%)/i	d/i
Bronchopneumonia[1]	2/50			17/49	26/50
FORESTOMACH:					
Non-neoplastic lesions					
Hyperkeratosis	1/50 (2%)	0/50 (0%)	38/50 (76%)	12/50 (24%)	43/50 (86%)
Basal cell hyperplasia	1/50 (2%)	6/50 (12%)	37/50 (74%)	16/50 (32%)	34/50 (68%)
Neoplastic lesions					
Squamous cell papilloma					
Overall incidence	0/50 (0%)	0/50 (0%)	16/50 (32%)	17/50 (34%)	6/49 (12%)
Adjusted incidence[3]	0.0%	0.0%	51.7%	40.9%	33.5%
Terminal incidence	0/42 (0%)	0/39 (0%)	10/23 (43%)	0/5 (0%)	0/0 (0%)
Life table test	p<0.001		p<0.001	p<0.001	p<0.001
Cochran-Armitage trend test	p=0.058				
Fisher exact			p<0.001	p<0.001	p=0.012
Incidental tumour test			p<0.001		
Squamous cell carcinoma					
Overall incidence	0/50 (0%)	0/50 (0%)	39/50 (78%)	38/50 (76%)	4/49 (8%)
Adjusted incidence[3]	0.0%	0.0%	92.8%	100%	100%
Terminal incidence	0/42 (0%)	0/39 (0%)	20/23 (87%)	5/5 (100%)	0/0 (0%)
Life table test	p<0.001		p<0.001	p<0.001	p<0.001
Cochran-Armitage trend test	p=0.199				
Fisher exact			p<0.001	p<0.001	p=0.056
Incidental tumour test			p<0.001		
Total number of animals with proliferative lesions / number of stomachs examined	1/50	6/50	48/50	49/50	49/49
Female rats					
Mortality[1]	13/50	11/50	15/50	34/50	49/50
Body weight / clinical signs[2]			(98.1%)/-	d (85.5%)/i	d (79.5%)/i
Bronchopneumonia[1]	0/50			10/50	17/50
FORESTOMACH:					
Non-neoplastic lesions					
Hyperkeratosis	1/49 (2%)	0/50 (0%)	46/50 (92%)	12/50 (24%)	48/50 (96%)
Basal cell hyperplasia	2/49 (4%)	3/50 (6%)	45/50 (90%)	12/50 (24%)	33/50 (66%)
Neoplastic lesions					
Squamous cell papilloma					
Overall incidence	0/49 (0%)	0/50 (0%)	19/50 (38%)	7/50 (14%)	1/50 (2%)
Adjusted incidence[3]	0.0%	0.0%	48.8%	24.2%	14.3%
Terminal incidence	0/36 (0%)	0/39 (0%)	15/35 (43%)	1/16(6%)	0/1 (0%)
Life table test	p<0.001		p<0.001	p=0.002	p=0.125
Cochran-Armitage trend test	p=0.421				
Fisher exact			p<0.001	p=0.007	P=0.505
Incidental tumour test			p<0.001		
Squamous cell carcinoma					
Overall incidence	0/49	0/50 (0%)	27/50 (54%)	34/50 (68%)	3/50 (6%)
Adjusted incidence[3]	0.0%	0.0%	64%	97%	100%

Terminal incidence Life table test Cochran-Armitage trend test Fisher exact Incidental tumour test	0/36 (0%) p<0.001 p=0.300	0/39 (0%)	20/35 (57%) p<0.001 p<0.001 p<0.001	15/16 (94%) p<0.001 p=0.001	1/1 (100%) p<0.001 p=0.125
Total number of animals with proliferative lesions / number of stomachs examined	2/49	3/50	48/50	50/50	50/50

[1] Most of the early deaths in the primary study not related to treatment were attributable to bronchopneumonia with the following incidences: males: 2/59, 17/49, 26/50 for 0, 25, 50 mg/kg bw/d; females: 0/50, 10/50, 17/50 for 0, 25, 50 mg/kg bw/d.
[2] d = decreased, i = increased, - = no effects on clinical signs or body weight; Clinical signs: wheezing and respiratory distress; Body weights expressed as percent of concurrent control values at week 103 (for the high dose no males survived until week 104, mean body weights for this group were 89.9% of the mean control at week 80).
[3] Kaplan-Meier estimated tumour incidence at the end of the study after adjusting for intercurrent mortality.
* Supplementary study started 1 year later due to excessive mortality in the primary study.

In the NTP study in mice, B6C3F1 mice (50/sex/dose) received RDGE (purity 81%, vehicle corn oil) 5 times/week by gavage for 103 weeks at dose levels of 0, 50, 100 mg/kg bw/d. The incidences of hyperkeratosis, epithelial cell hyperplasia, squamous cell papilloma, papillomatosis, and carcinomas of the forestomach were increased in both sexes, with a positive trend and statistically significant increase of neoplasia (carcinoma and papilloma) in high dose animals (carcinoma statistically significant at both treatment doses) as compared to the control group. In the high dose females there was a positive trend for hepatocellular carcinoma and a significant increase by life table test (not by fisher exact test) in the combined incidence of liver adenoma and carcinoma. The incidence in females dosed with the test substance was within the HCD range of the NTP research programme.

Table 2: *Forestomach and liver lesions of mice given RDGE by gavage for 2 years (NTP 1986; Krishna-Murthy et al., 1990).*

Exposure level (mg/kg bw/d)	**0**	**50**	**100**
Males			
Mortality	20/50	24/50	16/50
Body weight / clinical signs[1]	/-	(97.4%)/-	(97.4%)/-
Forestomach lesions non-neoplastic and neoplastic			
Hyperkeratosis	3/47	40/49	42/50
Hyperplasia	1/47	30/49	37/50
Squamous cell papilloma or papillomatosis Adjusted incidence	0/47 0%	4/49 (8%) P=0.064 (14%)	10/50 (20%) p=0.001 29.4%
Squamous cell carcinoma Adjusted incidence	0/47 0%	14/49 (29%) p<0.001 40.7%	25/50 (50%) P<0.001 55.5%
Liver neoplastic lesions			
Hepatocellular adenoma	7/48(15%)	7/50 (14%)	5/50 (10%)
Hepatocellular carcinoma	7/48 (15%)	11/50 (22%)	6/50 (12%)
Adenoma and carcinoma combined	14/48 (29%)	18/50 (36%)	11/50 (22%)
Females			
Mortality[2]	*30/50*	*37/50*	*40/50*
Body weight / clinical signs[1]	/-	(95.3%)/-	d (79.1%)/-
Forestomach lesions non-neoplastic and neoplastic			
Hyperkeratosis	11/47	31/49	46/49
Hyperplasia	3/47	25/49	26/49
Squamous cell papilloma or papillomatosis Adjusted incidence	0/47 0%	5/49 (10%) p=0.031 33.4%	10/49 (20%) p=0.001 73.1%
Squamous cell carcinoma	0/47	12/49 (24%)	23/49 (47%)

Adjusted incidence	0%	P<0.001 53.3%	P<0.001 70.5%
Liver neoplastic lesions*			
Hepatocellular adenoma [HCD study laboratory HCD research programme Overall range]	3/48 (6%) [10/198 (5.1%) 47/1126 (4.2%) 0-10%]	0/50 (0%) P=0.114	5/49 (10%) P=0.369
Hepatocellular carcinoma [HCD study laboratory HCD research programme Overall range]	0/48 (0%) [7/198 (3.5%) 33/1126 (2.9%) 0-8%]	1/50 (2%) p=0.510	3/49 (6%) p=0.073
Hepatocellular carcinoma and adenoma combined [HCD study laboratory HCD research programme Overall range]	3/48 (6%) [17/198 (8.6%) 79/1126 (7.0%) 2-14%]	1/50 (2%) p=0.294	7/49 (14%) p=0.167

[1] d = decreased, - = no effects on clinical signs or body weight; Body weights expressed as percent of concurrent control values at week 103.
[2] The major cause of death in female mice was a necrosuppurative lesion of the ovary, which spread to other areas of the abdominal cavity.
Statistical significance assessed by Fischer exact test.
* NTP historical control data for studies of the research programme including data form the study performing laboratory have been made available. Historical data as of March 16, 1983 for studies of at least 104 weeks; the exact time period of the individual historical control data has not been specified by NTP (1986). Data given for mean and the overall range of the NTP studies.

In both NTP studies, in rats and mice, a reduced incidence of other tumour types was observed which was attributed to the reduced survival of animals. In conclusion, the DS considered the increased incidence of forestomach tumours in rats and mice in both sexes to be treatment-related.

RDGE has a harmonised classification as Muta. 2. In order to support the classification proposal for carcinogenicity, the DS presented the available genotoxicity data on RDGE indicating positive *in vitro* and *in vivo* mutagenicity. In addition, repeated dose toxicity studies were included in the CLH report as supporting information. In these studies, RDGE induced effects mainly in the forestomach of F344/N rats and B6C3F1 mice of both sexes. These effects consisted of mucosal cell proliferation, hyperkeratosis, hyperplasia, papillary growth, and ulcers. The DS considered that the available skin irritation data, repeated dose studies, and carcinogenicity studies suggested that RDGE caused irritation at the site of contact, and that chronic tissue damage may have contributed to the carcinogenic response. Since RDGE was mutagenic, the DS considered also a local genotoxic mechanism at the site of contact likely involved in the forestomach tumour formation.

The DS also suggested to use read-across from phenyl glycidyl ether, containing one instead of two diglycidylether side chains, as supporting evidence for the classification of RDGE, as it had a harmonised classification as Muta. 2 and Carc. 1B due to substance-induced nasal tumours in an inhalation carcinogenicity study.

The DS concluded that there was sufficient evidence for RDGE-induced carcinogenicity based on squamous cell tumours in the forestomach at the site of contact at and above 12 mg/kg bw/d in both sexes of rats and 50 mg/kg bw/d in both sexes of mice following a 103-week oral exposure via gavage.

The DS acknowledged that the precise mechanism of any forestomach tumour formation is not fully known at present, and while humans have no forestomach, they do have comparable squamous epithelial tissues in the oral cavity and the upper oesophagus. Considering that the irrelevance of the RDGE-induced forestomach tumours to humans was not clearly demonstrated, the DS proposed to upgrade the current harmonised classification as Carc. 2; H351 to Carc. 1B; H350. In its proposal the dossier submitter presented compilation factors taken into account (table 17 of the CLH report):

- Tumour induction was consistent observed in two species rats and mice,
- Tumours were observed in both sexes of rats and mice,
- Neoplasms were limited to the site of exposure,
- Apparent progression to malignancy,
- A reduction of latency period since no forestomach tumours were observed in control animals and tumours in treated animals were observed before terminal sacrifice,
- The oral route of exposure is considered relevant for humans,
- The mode of action was considered relevant for humans. Both local mutagenicity and irritation may have contributed. A genotoxic mechanism is considered relevant for humans, while irritation might be of questionable relevance. The available data did not allow a firm conclusion which mechanism is responsible,
- As a confounding factor, local toxicity seen as hyperkeratosis and hyperplasia due to irritating properties may have contributed to the tumour development.

Comments received during public consultation

Two MSCAs provided comments on the classification proposal. One of them disagreed with the proposed upgrade in carcinogenicity classification because tumours were only observed in the forestomach and because the substance was both irritating and mutagenic.

The other MSCA requested a thorough discussion on the relevance of the outcome of the animal studies for humans and to weighing all evidence carefully before making a final decision on classification. While formally the criteria for a Carc 1B classification were considered to be met, with tumours in two species and both sexes, several additional factors were considered to complicate the assessment:

- Tumours were only observed in the forestomach, which had no direct counterpart in humans, and the residence time for food and test material in the human oesophagus (which had comparable squamous epithelium tissue as the forestomach) was expected to be rather short. Thus, the relevance of the two potential MoAs (irritation and genotoxicity) for tumour formation in the human oesophagus was questioned,

- The forestomach presented the site of contact after oral gavage dosing, and no non-neoplastic lesions had been found outside the forestomach. The relevance of the studies for other routes of exposure (inhalation or skin contact) was uncertain,

- Genotoxicity was considered a local, non-systemic effect due to rapid inactivation of epoxy groups (at least *in vitro*). The positive evidence for *in vivo* genotoxicity was limited to a single high dose administration via *intraperitoneal* route,

- With reference to the CLP guidance, tumours occurring only at sites of contact and/or only at excessive doses needed to me carefully evaluated, e.g. forestomach tumours after gavage administration of irritating/corrosive, non-mutagenic chemical may be of questionable relevance. Excessive toxicity, such as at doses exceeding the MTD, can affect carcinogenic response. Cell death with associated regenerative hyperplasia could lead to tumour development as a secondary consequence unrelated to the intrinsic potential of a substance to cause tumours at lower less toxic doses.

Assessment and comparison with the classification criteria

Carcinogenicity at the site of contact

Animal data – oral route

In rats, RDGE (technical grade, 81%) caused hyperkeratosis, hyperplasia, benign and malignant lesions of the squamous epithelium of the forestomach in both sexes at concentrations of 12, 25, 50 mg/kg bw/d by oral gavage dosing in the primary and supplemental NTP study.

Forestomach neoplasia was induced at all tested dose levels. The dose-response evaluation is however compromised because the low dose (12 mg/kg bw/d) was tested only later in the supplemental study due to excessive mortality in the primary study at 25 and 50 mg/kg bw/d (45/50 and 50/50 males and 34/50 and 49/50 females died before the scheduled necropsy, respectively). Incidences of benign papilloma and malignant squamous cell carcinoma were very high at 25 mg/kg bw/d (benign: 34% males, 14% females; malignant: 76% males and 68% females) and at 12 mg/kg bw/d (benign: 32% males, 38% females, malignant: 78% males and 54% females). The absence of a positive dose-response in the incidence of papilloma in females is considered not a critical factor as malignant carcinoma may "overwrite" benign tumours in the histopathology evaluation. Further, the marked increase in the number of early deaths at the high dose (onset of survival reduction at week 30) explains the low incidence of neoplasm in this dose group. At 12 mg/kg bw/d the survival of males (46%) was significantly reduced as compared to controls (78%), but such effect was not observed in females at the same dose. The concurrent and historical control incidences of forestomach tumours were low and close to zero.

The terminal body weights at the mid and high dose (primary study) were 15-20% lower as compared to the control group. The body weight gain was reduced by 23% at 25 mg/kg bw/d as compared to the control group. It is unclear whether the reason is related to toxicity or to reduced food consumption, but it seems likely that the test compound-related gastric lesions have contributed to the body weights. The maximum tolerated dose (MTD) is conventionally described by approximately 10% reduction in body weight gain (CLP guidance section 3.6.2.3.2 j.). When considering this convention, the MTD was reached at the mid and high dose (tested in the primary study). Nevertheless, the low dose of 12 mg/kg bw/d of the supplemental study showed a marked and significant increase in benign and malignant forestomach tumours in the absence of excessive toxicity.

Metastasis was reported for the primary study (14 males at 25 mg/kg bw/d, 1 male at 50 mg/kg bw/d, 5 females at 25 mg/kg bw/d) at several distant sites (regional lymph nodes, pancreas, liver, spleen, lungs, brain). The latency for tumour induction was not analysed by the DS. Forestomach squamous cell carcinomas were detected in deceased animals before the scheduled sacrifice. Based on the individual data available in the NTP study report, RAC notes that squamous cell carcinomas were first reported at week 76 at the low dose (12 mg/kg bw/d) of the supplementary study, at week 61 for the mid dose (25 mg/kg bw/d) and as early as week 42 in one high dose female (50 mg/kg bw/d) of the primary study. Control incidences are equal to zero. It can be stated that treatment was related with a high tumour incidence of up to 100 % (adjusted), with malignant neoplasm at terminal sacrifice but also in early deceased animals, at rather low dose levels (i.e. 12 mg/kg bw/d), indicating a high carcinogenic potency. Malignant neoplasms were accompanied by metastasis at several distant sites, which indicates a high grade of malignancy.

RAC concludes that the test material was carcinogenic in rats inducing forestomach tumours at the site-of-contact after oral gavage administration.

In mice, the NTP study with RDGE (technical grade, 81%) identified similar findings as in rats, i.e. hyperkeratosis, hyperplasia, benign and malignant lesions of the squamous epithelium of the forestomach in both sexes, at somewhat higher gavage dosing concentrations of 50 and 100 mg/kg bw/d as compared to the dose range in the rat study. RAC considers that there were no treatment-related effects on body weights, mortality rates or overt toxicity in males. In females at the high dose, the body weights were reduced (79% of the control value) and the mortality rate was increased with 40/50 deaths compared to 30/50 in the control. Hyperplasia and benign and malignant forestomach neoplasia were significantly and dose-dependently increased in both sexes as compared to the controls, with a maximum of 47% and 50% incidences of squamous cell carcinoma in high dose females and males, respectively. Concurrent and historical control incidences of forestomach neoplasia were low and close to zero.

Metastases were observed at both dose levels (males: 4/10; females: 1/9 at low/high dose) at several distant sites (lung, liver, lymph nodes, spleen, adrenal glands, heart, and kidney). The latency for tumour induction had not been analysed by the DS. Forestomach squamous cell carcinomas had been detected in deceased animals before the scheduled sacrifice. Based on the individual data available in the NTP study report, RAC notes that forestomach squamous cell carcinoma had been detected in the earliest deceased male and female at the low dose (50 mg/kg bw/d) at week 39 and 64, respectively. At the high dose (100 mg/kg bw/d), earliest deaths with squamous cell carcinoma occurred at week 75 for males and week 82 for females, respectively.

RAC concludes that the test material was carcinogenic in mice of both sexes inducing forestomach tumours at the site of contact after oral gavage administration in the absence of marked toxicity. Again, high tumour incidences (up to 70%, adjusted), malignant neoplasms at terminal sacrifice but also in early deceased animals, accompanied by metastasis at several distant sites, indicates a high carcinogenic potency and a high grade of malignancy under the conditions of the study.

The test material of the NTP 2-year carcinogenicity studies in rats and mice was a technical grade with 81% purity. This introduces uncertainties as to whether 19% of impurities could have contributed to the development of forestomach tumours. The DS clarified that 30 impurities were detected by gas-liquid chromatography, with a total area of approximately 14% of the major peak area. One of the impurities had an area that was 3.7% of the major peak area, and two groups of unresolved impurities had a combined area of 3.7% and 2.0% of the major peak area. The remaining impurities had a combined area of less than 4% of the major peak area. The identity of the impurities, however, was not determined by the laboratory. Therefore it is considered difficult to draw conclusions regarding their potential influence on the study results. RAC notes that the best way to synthesise RDGE and other glycidyl ethers is by a reaction of epichlorohydrin in basic (i.e. NaOH) medium and by removing the excess of epichlorohydrin by liquid extractions in water. Epichlorohydrin is therefore a contaminant of technical-grade preparations of glycidyl ethers. Epichlorohydrin is a highly reactive electrophilic chloro-organic epoxide compound and has a harmonised classification as Carc. 1B, Skin Corr. 1B, Skin Sens 1, Acute Tox. 3 (H301, H303). The substance is a mutagen based on positive results for *in vitro* bacterial mutation and *in vivo* cytogenicity after oral and *i.p.* administration[1]. IARC concluded that there was sufficient evidence for the carcinogenicity of epichlorohydrin in experimental animals: the substance was a rodent forestomach carcinogen inducing inflammation, hyperplasia, papilloma and carcinoma after oral gavage and drinking water dosing in Wistar rats, and it induced papillomas and carcinomas of the nasal cavity after inhalation exposure (IARC Monographs Volume 71). Since epichlorohydrin levels in the RDGE technical grade test material are unknown, its influence on the study outcome remains uncertain. However, RAC also notes that RDGE carries two epoxide groups and is thus electrophilic and very reactive itself. Finally, RDGE is not registered under the REACH Regulation and there is no information available on impurities in the products currently on the market.

In conclusion, under the conditions of the studies, RGDE (technical grade) administered by oral gavage induced non-neoplastic forestomach lesions hyperkeratosis and hyperplasia as well as neoplastic effects benign papilloma, and malignant metastasising carcinoma of the squamous epithelium of the forestomach in male and female mice and rats.

<u>Animal data – dermal route</u>

RDGE seems toxic to the stratified squamous epithelium in direct contact as reported by the NTP studies. This raises the question whether the substance could also induce skin tumours after prolonged direct contact. Three dermal studies were reported in the CLH dossier. However, the information available to the DS was poor and no assessment of the studies was possible:

In Swiss Millerton mice (Van Duuren, 1965), 100 mg of 1% RDGE solution in benzene was administered three times/week by dermal application to clipped dorsal skin. Treated animals survived 491 days. No tumours were observed in any dose group. No further information is available to RAC and RAC agrees with the DS that the study has limited value for classification purposes.

The study by Kotin and Falk (1963) reported one skin tumour in C57/B1 mice after 8 months of exposure. However, the CLH report includes hardly any information. RAC notes that the study is also briefly described by NIOSH (NIOSH, 1978): 1 of 14 surviving mice (7%) exposed to RDGE at 0.75 mmol developed a skin tumour. RDGE at 0.25 mmol caused no skin tumours in any of the mice. In a written communication (January 1978), the study author noted that the skin tumours produced by the glycidyl ethers in this study were all benign papilloma and that controls receiving only acetone did not develop any papilloma. RAC agrees with the DS that this study is not adequate for carcinogenicity assessment.

McCammon (1957) tested RDGE (purity not specified) in C57/B1 mice by painting on the interscapular skin three times/week. In addition, Long-Evans rats received the compound by subcutaneous injection. As presented in the CLH dossier, the authors reported that

[1] Registration dossier Epichlorohydrin: https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15559/7/7/3/?documentUUID=220840b4-1516-4b6f-a625-0331037030da

RDGE was tumorigenic in both rats and mice, however, organs were not mentioned in the short abstract available to the DS. The study is also briefly described by NIOSH (NIOSH, 1978): RDGE produced sebaceous gland suppression, intense hyperkeratosis, parakeratosis, and epithelial hyperplasia in mice. Tumours produced were benign papilloma. RAC agrees with the DS that the study information is too limited for carcinogenicity evaluation (e.g. tested doses and exposure duration are not stated).

The original NTP study report (NTP, 1986) subject to current assessment of forestomach lesions and the original classification proposal from 1997 under DSD cite another study, a 2-year skin painting study (Holland et al., 1981) with C3Hf/Bd mice, which failed to cause skin neoplasms.

In summary, there is no valid information available to enable RAC to conclude on a potential of RDGE to induce skin and/or other tumours via the dermal route.

<u>Animal data – inhalation route</u>

No inhalation chronic/carcinogenicity studies are available to enable RAC to conclude on a potential of RDGE to cause tumours in the nasal cavity and to induce carcinogenicity via the inhalation route.

Systemic carcinogenicity

In the NTP carcinogenicity study in mice (NTP, 1986), statistically significant positive trends (by the life table and incidental tumour tests) for hepatocellular carcinomas in females (0%, 2%, 6% for control, low and high dose, respectively), and a significant (only by the life table test) increase for combined incidences of hepatocellular adenoma and carcinoma in high dose females (6%, 2%, 14% for control, low and high dose, respectively) were observed. The incidences were not statistically significant by the fisher exact test. As indicated by the HCD for the NTP research programme and the study performing laboratory (NTP 1986), the incidence of liver carcinoma of the concurrent control and that of the mid dose group were lower than those of the historical control groups of the same laboratory. The combined incidence of 14% for adenoma and carcinoma at the high dose was within the range of HCD of the research programme as pointed out by the DS. RAC notes that HCD should preferably be from the same laboratory and thus considers these pooled data of limited value. However, the B6C3F1 mouse is known for high background rates of liver tumours. In addition, no liver tumours were observed in rats. Thus, RAC agrees with the conclusion by the NTP study author and DS that these findings may not be related to the administration of the test material.

In the NTP study in rats, the squamous epithelium of the oesophagus and nasopharynx was hyperkeratotic in some rats, but no tumours were found.

In both NTP studies, a variety of other tumours appeared with a reduced incidence in rats and mice, and RAC concludes that this was likely related to the reduced survival of the animals and not a direct treatment-related effect.

RAC concludes that there is no sufficient evidence for treatment-related systemic tumour induction by RDGE at distant sites.

Mode of action considerations for rodent forestomach lesions

In rodents the proximal part of the stomach, the non-glandular forestomach, forms a continuum with the oesophagus, and is lined with keratinised and stratified squamous epithelium. There is no site concordance, because humans do not have a forestomach. However, humans do have comparable squamous epithelium in the oral cavity and upper two-third of the oesophagus. Forestomach squamous cell tumours are most frequently induced after oral administration of a chemical, either by gavage resulting in a bolus, or via the diet although less frequently. Upon exposure, forestomach neoplasia generally appear to be a continuum, progressing from hyperplasia and dysplasia to benign tumours and eventually to metastasising carcinoma. The precise underlying mechanism of action for any forestomach carcinogen is not fully known at present. Cytotoxicity and regenerative cell proliferation of the epithelium are involved in the induction of forestomach neoplasia by many chemicals administered by oral route. For non-genotoxic chemicals irritation may be essential for the tumour development. However, the majority of forestomach carcinogens are genotoxic and cell regenerative proliferation as provoked by irritants could make an important contribution. The historical NTP data suggest these kind of tumours to be susceptible to a local combination of irritation/wound healing and mutagenicity (IARC, 2003; NTP[2]).

With respect to the mode of action, the DS concluded that "*it is known that RDGE is a mutagenic substance. Forestomach tumours caused by substances that act via a genotoxic mechanism are considered relevant for humans. However, the data of the NTP study also point towards local irritation in the forestomach as hyperkeratosis and hyperplasia of the epithelium were observed. This might also suggest that chronic tissue damage with resultant hyperplasia may have contributed to the observed tumour response. However there are currently no data which can exclude a genotoxic mode of action. Therefore it is assumed that the (local) genotoxicity contributed to the observed tumours response […]*".

In agreement with the view of the DS, RAC considers it possible that genotoxicity could have contributed to the tumour response in the rodent forestomach. RDGE belongs to the group of diglycidyl ethers and is electrophilic carrying two (DNA)-reactive epoxide groups and has a harmonised classification as Muta. 2 (H341). This endpoint was not addressed in the CLH report, but summaries of several available *in vitro* and *in vivo* genotoxicity studies in somatic cells were presented to support the carcinogenicity evaluation. *In vitro*, RDGE induced gene mutations in bacteria (*S. typhimurium* TA100 and TA1535 strains) with and without metabolic activation (purity 87.9%; NTP, 1986) and in mammalian L5178Y cells (mouse lymphoma study, tk locus) (unknown purity and solvent; McGregor 1988, 1996). Exposure to RDGE resulted also in chromosome aberrations and sister chromatid exchanges with and without metabolic activation (purity >87.95%; Gulati, 1989). *In vivo*, positive results in the bone marrow micronucleus induction test were limited to a single *intraperitoneal* injection of 90 to 270 mg/kg bw RDGE of unknown purity to male B6C3F1 mice (Shelby, 1993). Single oral doses of 300 mg/kg bw and 600 mg/kg bw (acutely toxic with 1/4 death) of rather pure RDGE (purity >98%) were negative for bone marrow micronucleus induction in male ICR mice (Seiler, 1984b). RAC concludes that the *in vitro* data suggest RDGE being a direct-acting mutagen, as expected for a reactive epoxide compound, and which is consistent with the group of structurally similar glycidyl ethers. No metabolic activation was necessary. *In vivo*, RDGE was negative in the oral micronucleus study. Several limitations of the study and its

[2] Overview provided in: Maronpot, R. R., NTP/NIHS: "Xenobiotic-induced Rodent Tumors of Questionable Relevance to Human Cancer Risk", https://focusontoxpath.com/rodent-tumors-of-questionable-relevance-to-man/

reporting are noted by RAC in a preliminary assessment: only two dose levels with four animals in each group were analysed, no repeated administration of less toxic doses was performed, the MTD was clearly exceeded at the high dose as one animal died, and no data on controls was reported. Finally, no information on the number of cells analysed or on PCE toxicity is available. Due to these limitations definite conclusions on systemic genotoxicity is hampered. RAC further notes, that the oral study was performed using a rather pure material, while the purity of the test material used in the *i.p*. study is unknown. Impact of differences in purity in particular considering the low purity grade technical RDGE of the NTP carcinogenicity studies, cannot be assessed. However, the result of the oral study might also be attributed to the high chemical reactivity whereby local damage is produced and only low concentrations of reactive compound remain available for distribution to distant sites. However, the data at hand do not provide information on initial site-of-contact genotoxicity. Efficient first pass metabolic inactivation in the liver, i.e. hydrolysis by epoxide hydrolase, could have limited the systemic availability and bone marrow exposure to RDGE when administered by the oral route. Some limited information on the toxicokinetics of RDGE is available. After a single dose of 1000 mg/kg bw in mice (Seiler *et al*., 1984), overall 50% of the administered dose (radioactivity measured) was recovered within 4 hours showing absorption and systemic bioavailability. The study indicates rapid conversion to the bis-diol metabolite (64% of radioactivity) and thus inactivation of the DNA-reactive epoxy-groups. 4% of radioactivity attributed to the phenol-diol, while no bis-epoxide or diol-epoxide had been detected. 21% of the metabolites have not been identified. *In vitro* incubation with liver S9 homogenates containing epoxidase hydrolase showed a first order kinetics and a half-life of about six minutes. The diol-epoxide was formed as an intermediate before transformation to the bis-diol. These data suggest that RDGE is rapidly inactivated at least *in vitro*. The available *in vivo* information, again, is too limited to draw any definitive conclusions on systemic availability of the reactive compounds. Effects of RDGE on kidneys in the 14-days NTP studies in rats and mice suggest at least some systemic availability of toxic species. Overall, contribution of genotoxicity to forestomach tumour development was neither demonstrated nor ruled out. The positive micronucleus outcome via *intraperitoneal* administration however shows the intrinsic potential for *in vivo* mutagenicity in the absence of an effect on bioavailability by oral absorption or gastric degradation rates and in the absence of a first pass effect.

The DS acknowledged that the repeated dose toxicity as well as carcinogenicity studies suggest that irritation and chronic tissue damage resulting in hyperplasia may have contributed to the mode of action. In the view of RAC, forestomach lesions associated with RDGE technical grade clearly assemble local irritation, hyperplasia and neoplasia. RDGE is a skin irritant and classified as Skin Irrit. 2. (H315). Prior to the 2-year studies, NTP conducted repeated dose toxicity studies, 14-days and 90-days studies in rats and mice with the same test material by oral gavage administration (NTP, 1986).

<u>In the 14-day studies</u>, the compound was administered for 14 consecutive days to rats at 190, 380, 750, 1500, 3000 mg/kg bw/d and to mice at 90, 190, 380, 750, 1500 mg/kg bw/d. Marked mortality and body weight reduction were observed. Stomach lesions including reddened mucosa and papillary growth of the forestomach were identified at these toxic doses. The DS presented another <u>14-day</u> gavage study with RDGE in male F344 rats administered the same concentrations as in the 2-year NTP study; 12 and 25 mg/kg bw/d (purity not specified) (Ghanayem et al., 1986). In this study multifocal

hyperkeratosis and mucosal cell proliferation were reported at 25 mg/kg bw/d but not at 12 mg/kg bw/d.

In the 90-day study in rats (10/sex/dose) RDGE was administered at 12.5, 25, 50, 100 and 200 mg/kg bw/d by oral gavage. At 12.5 and 25 mg/kg bw/d histopathological findings in the forestomach included inflammation (6/10 to 9/10), basal cell hyperplasia (2/10 to 5/10) and fibrosis (up to 2/10) without ulceration. Squamous papilloma were reported at higher doses (1, 1, 3 in males and 0, 1, 2 in females at 50, 100, 200 mg/kg bw/d, respectively). Also incidences of hyperkeratosis and hyperplasia were markedly increased at these higher doses, ulceration only appeared at higher doses (higher than those in the chronic studies). According to the NTP study author, the hyperkeratosis, hyperplasia and squamous papilloma in the 2-year rat study appeared to be identical lesions to those found in the 90-day study. In the 90-day study in mice (25, 50, 100, 200 and 400 mg/kg bw/d), compound-related lesions were found in the forestomach and liver. Forestomach lesions resembled those seen in rats, i.e. squamous papilloma, diffuse hyperkeratosis, basal cell hyperplasia, and inflammation. Mucosal ulceration only appeared at the high dose where only 4/10 animals survived and body weights and weight gain was markedly reduced.

The NTP study author concluded that the whole sequence of stages occurring during pathogenesis of rodent malignant forestomach neoplasia was observed and the whole process was clearly a function of time. RAC therefore concludes that irritation-related inflammation and regenerative cell proliferation of the forestomach, the first site of contact after oral gavage dosing, are consistently reported in the short-term repeated dose toxicity studies and the 2-year carcinogenicity studies.

Assessment of human relevance

The Guidance on the Application of the CLP Criteria (version 5.0, section 3.6.2.3.2a) states the following with respect to tumours occurring in tissues with no human equivalent: "*Forestomach tumours in rodents following* ***administration by gavage of irritating or corrosive, non-mutagenic substances****. In rodents, the stomach is divided into two parts by the muco-epidermoid junction separating squamous from glandular epithelium. The proximal part, or forestomach, is non-glandular, forms a continuum with the oesophagus, and is lined by keratinized, stratified squamous epithelium. While humans do not have a forestomach, they do have comparable squamous epithelial tissues in the oral cavity and the upper two-thirds of the oesophagus. See also this Section (k), IARC (2003), and RIVM (2003). Tumours occurring in such tissues indicate that the substance has the potential to induce carcinogenic effects in the species tested. It cannot automatically be ruled out that the substance could cause similar tumours of comparable cell/tissue origin (e.g. squamous cell tumours at other epithelial tissues) in humans. Careful consideration and expert judgement of these tumours in the context of the complete tumour response (i.e. if there are also tumours at other sites) and the assumed mode of action is required to decide if these findings would support a classification. However,* ***tumours observed only in these tissues, with no other observed tumours are unlikely to lead to classification****. However, such determinations must be evaluated carefully in justifying the carcinogenic potential for humans; any occurrence of other tumours at distant sites must also be considered*."

A working group of IARC concluded that "*carcinogens that are* ***DNA reactive*** *and cause* *forestomach tumours in rodents – **even if they only caused tumours at this site – should be evaluated as if they presented a carcinogenic hazard to humans [...]** agents that only produce tumours in the forestomach in rodents after prolonged treatment through non-DNA reactive mechanisms maybe of less relevance to humans, since human exposure to such agents would need to surpass time-integrated dose thresholds in order to elicit the carcinogenic response* (IARC, 2003). This conclusion is based on the fact that although humans do not have a forestomach, they do have comparable squamous epithelial tissues in the oral cavity and the upper two-thirds of the oesophagus. Besides, the target tissues for carcinogens may differ between experimental animals and humans and a forestomach carcinogen in rodents may target a different tissue in humans. It is also considered that genotoxic carcinogens are likely to target a number of sites.

In the view of the DS, "*RDGE acts (at least partly) via an indirect mode of action (i.e. a prolonged proliferation stimulus). However, as resorcinol diglycidyl ether was also found to be a mutagenic substance, though probably acting at the site of contact and not via systemic exposure due to inactivation, it may be considered that the resorcinol diglcidyl ether-induced forestomach tumours are induced via a (local) genotoxic mechanism. Taking into account the considerations of RIVM (2003) and IARC (2003), the forestomach tumours as observed in F344/N rats and B6C3F1 mice of both sexes (NTP 1986; Krishna-Murthy et al., 1990) should be taken forward for classification of resorcinol diglycidyl ether for the endpoint carcinogenicity. A potential irrelevance for humans is not clearly demonstrated for the resorcinol diglycidyl ether-induced forestomach tumours*."

In line with the CLP criteria, for the evaluation of human relevance, RAC considers 1) whether genotoxicity contributed in the MoA, 2) whether irritation and related inflammation and hyperplasia as early lesions were observed, 3) whether effects are considered specific to high dose gavage administration, and 4) whether tumours at other distant or site-of-contact tissues occurred:

1) <u>Genotoxicity</u>: RDGE is genotoxic and has a harmonised classification as Muta. 2. Considering the CLP criteria, IARC (2003) and Proctor *et al. (2007)*, the most pertinent question is whether genotoxicity was an <u>essential</u> property for tumour induction. Ultimately, this question cannot be answered based on the available data. In the view of RAC, a) the reactive epoxide groups of the molecule, b) the positive *in vitro* mutagenicity data in the absence of metabolic activation, and c) the positive *in vivo* micronucleus induction assay after *i.p.* injection suggest that local site-of-contact genotoxicity is likely, and its contribution to forestomach tumour development cannot be excluded.

2) <u>Irritation</u>: Irritation-related hyperplasia and hyperkeratosis observed in short-term studies are early non-neoplastic changes in pathogenesis of rodent forestomach neoplasia and indicate a role for irritation in the malignant tumour transformation induced by RDGE. Progression of early inflammatory stages to benign and to malignant invasive and metastasising lesions was a function of time and the severity was depending on the amount of the test substance administered. No NOAEL is available for non-neoplastic inflammatory changes or for neoplastic lesions after the 13-week or 2-year repeated dosing, respectively. Cytotoxic precursor lesions were observed Therefore, RAC considers that the concern for the observed forestomach tumours is increased, as a genotoxic MoA is of relevance for humans. In line, the DS concluded that the human relevance of the observed carcinogenic effect cannot be excluded.

3) <u>Dose and route extrapolation</u>: RDGE has been shown to induce forestomach tumours

upon gavage administration. Considering real life exposure, oral gavage dosing is of less relevance for humans. Resulting tissue concentration are much greater after gavage administration as compared to dietary intake and thus are more likely leading to sustained irritation and tissue inflammation. Forestomach tumours, however, can also be induced, although less commonly, by diet administration and even in seldom cases via other routes of exposure (IARC, 2003; NTP). No other oral dosing method was tested, the dermal studies were considered by RAC as invalid and carcinogenicity was not tested via the inhalation route. Then, another important factor that RAC takes into consideration when assessing the relevance of the tumour-inducing doses and exposure method, is whether the maximum tolerated dose (MTD) was exceeded. According to the CLP guidance (section 3.6.2.2.9.), "*the highest dose needs to induce minimal toxicity, such as characterised by an approximately 10% reduction in body weight gain (maximum tolerated dose, MTD). Excessive toxicity, for instance toxicity at doses exceeding the MTD, can affect the carcinogenic responses in bioassays. Such toxicity can cause effects such as cell death (necrosis) with associated regenerative hyperplasia, which can lead to tumour development as a secondary consequence unrelated to the intrinsic potential of the substance itself to cause tumours at lower less toxic doses.* RAC notes that the dose of 12 mg/kg bw/d of the supplemental study in rats, although administered by gavage, was a rather low dose and cannot be seen as an excessive bolus administration. This dose did not exceed the (systemic) MTD and was associated with a marked tumour response. RDGE is an irritant. All testing concentrations in the 2-year carcinogenicity studies have been shown to produce signs of irritation and regenerative cell proliferation in the short-term repeated dose toxicity studies. Conclusion on the intrinsic potential at lower less toxic (local) doses cannot be drawn.

RAC concludes that route- and gavage-specificity of forestomach neoplasia has not been proven, nor has it been ruled out. RAC acknowledges the consistent association of local cytotoxicity/irritation with related hyperplasia and neoplasia, as frequently observed after gavage administration, suggesting a role for secondary mechanisms in the study outcome, which reduces the concern.

4) <u>Organ-specificity and tissue-concordance:</u> The NTP study author concluded that RDGE was toxic/carcinogenic to the stratified squamous epithelium inducing forestomach neoplasia. Direct contact might be required because tissues of the same type but distant to the site of exposure (i.e. oral cavity) did not show lesions. In rats, the squamous epithelium of the oesophagus and nasopharynx was hyperkeratotic in some animals, but no tumours were found. No other non-neoplastic lesions were observed at distant sites. RAC agrees with the DS, that the forestomach tumours occurring only at the initial site of contact after gavage administration of such a DNA-reactive epoxy-compound could be a result of its high chemical reactivity causing only local damage due to limited systemic availability. There is no human organ counterpart to the forestomach, but humans possess histologically related organs such as the oesophagus and oral cavity with similar growth control mechanisms as the stratified squamous epithelium. Such tissues might be affected in a similar way as a function of dose, concentration and exposure duration. In humans, the exposure time could be markedly limited considering that chemicals pass through the oesophagus quickly. Compared to that, the rodent forestomach has a reservoir function resulting in a tissue dose that is not equivalent. Importantly however, in the view of RAC, despite a low probability for sustained inflammation of the human oesophagus due to these differences in gastro-oesophageal transit, the short half-life of the substance together with its high chemical reactivity raises a particular concern for

genotoxic site-of-contact effects in the oesophagus / upper GIT and/or via inhalation. A lower residence time may not be crucial in the light of high reactivity and short half-life of the substance.
In any case, classification is based on the intrinsic properties of the substance and not on exposure scenarios.
In relation to site-of-contact carcinogenicity, RAC also takes into consideration the read across to another, probably the most comparable glycidyl ether, phenyl glycidyl ether (PDGE) (CAS 122-60-1), proposed by the DS. PDGE contains one instead of two glycidylether side chains, and it has a harmonised classification as Carc. 1B and Muta. 2. In the view of the DS, the read-across to PDGE provides some support for the proposed classification of RDGE as Carc. 1B. The carcinogenicity classification was based on an increase in nasal tumours (epidermoid carcinomas) in one inhalation carcinogenicity study on rats. RAC notes that similar to RDGE, the available *in vitro* mutagenicity data for PDGE suggest the substance being a direct acting mutagen, while the *in vivo* micronucleus assay by oral gavage was negative (Seiler, 1984). Glycidyl ethers are irritants and skin sensitisers with a certain chemical reactivity attributed to epoxy groups. However, read-across to other glycidyl ethers as regards carcinogenicity does not seem straight-forward. For instance, another diglycidyl ether with some structural similarities is bisphenol A diglycidyl ether (BPDGE), containing two epoxy groups. For this substance there is insufficient evidence for carcinogenicity classification so far[3]. However, both PDGE and RDGE are site-of-contact carcinogens and the data on PDGE raises the question whether RDGE could exhibit local carcinogenicity not exclusively to the forestomach but also to other tissues following other routes of exposure. No inhalation studies on RDGE are available and the available dermal studies are considered unreliable by RAC.

In summary, RAC considers that human relevance of the rodent forestomach tumours cannot be excluded, and there is no reliable data to conclude if other routes of exposure cause carcinogenicity.

Comparison with the classification criteria

RAC concludes, in line with the DS, that the observed forestomach tumours in rodents warrant classification of RDGE for carcinogenicity. The Guidance on the Application of the CLP Criteria (version 5.0) indicates that forestomach tumours induced by gavage administration of irritating non-mutagenic substances, with no other tumours observed, are unlikely to lead to classification. This **condition for no classification is not met for RDGE** for two reasons:

- RDGE is mutagenic, and mutagenicity could have contributed to the tumour response,
- Site-of-contact carcinogenicity in the forestomach has not been proven to be specific to the gavage administration, since reliable data for other methods and

3 EFSA, 2004: Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related to 2,2-bis(4-hydroxyphenyl)propane bis(2,3-epoxypropyl)ether (Bisphenol A diglycidyl ether, BADGE). http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/86.pdf

The EFSA panel concluded that BADGE and its chlorohydrins do not raise concern for carcinogenicity and genotoxicity in vivo, respectively. BADGE.2HCl has been tested negative for in vivo mouse bone marrow micronucleus induction. RAC took note that BADGE is subject to REACH substance evaluation decision, requested information: Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays (TGR) OR *in vivo* mammalian alkaline comet assay. https://echa.europa.eu/documents/10162/173e7abd-4787-b5b2-c10a-9d5c882ce31b.

routes of administration are essentially lacking.

RAC is of the opinion that classification in category **Carc. 1A is not warranted**. According to the CLP criteria for Carcinogenicity Category 1A, *known to have carcinogenic potential for humans*, classification is largely based on human evidence. For RDGE no information on carcinogenicity in humans is available.

According to the CLP criteria (Annex 3.6.2.2.3) for Category 1B *"sufficient evidence of carcinogenicity", a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence […]".*

RAC considers the following factors in support of classification as Carc. 1B:

- Forestomach neoplasia was consistently observed in two rodent species and in both sexes,
- Forestomach tumours progressed to high grade malignancy with metastasis at several distant sites in both species and both sexes,
- Data indicate a high carcinogenic potency under the conditions of the studies. Control incidences for forestomach neoplasms were zero, while for RDGE an extremely high rate of forestomach neoplasia (benign and malignant) was evident. In addition, squamous cell carcinomas were observed very early in deceased animals before scheduled terminal sacrifice,
- RDGE is a direct-acting mutagen. Genotoxicity likely contributed to forestomach tumour development and a genotoxic mode of action is considered relevant for humans,
- Humans have comparable squamous epithelial tissue in the oesophagus and oral cavity which might be affected in a similar way as a function of dose, concentration and exposure duration,
- Forestomach-specificity has not been demonstrated mainly due to lack of reliable data on toxicokinetics, mutagenicity, and carcinogenicity via relevant and realistic exposure pathways. In particular, RAC is concerned that site-of-contact carcinogenicity following inhalation or dermal exposure could not be ruled out,
- Structure activity: RDGE belongs to the group of diglycidyl ethers and is electrophilic carrying two (DNA)-reactive epoxide groups

Placing of a substance **in category 2** is done on the "*basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies".*

In the view of RAC, there are factors that could in certain conditions reduce the concern for carcinogenicity in humans:

The rodent forestomach has no human organ counterpart and RAC acknowledges that forestomach tumours observed for RDGE were associated with inflammation and regenerative cell proliferation, suggesting a role for a secondary mechanism in the study outcome. This could be a factor reducing the concern for carcinogenicity in humans. However, the questionable human relevance of forestomach neoplasia is limited for non-mutagenic irritants administered by gavage (the Guidance on the Application of the CLP Criteria 3.6.2.3.2 (a). There is no data on the intrinsic potential of lower (genotoxic) RDGE doses for carcinogenicity in the absence of marked cytotoxicity and accompanied hyperplasia. RDGE is mutagenic and it is recognised that genetic events are central in the overall process of cancer development thereby increasing the concern for carcinogenicity in humans.

In a weight-of-evidence approach, RAC agrees with the DS´s proposal and concludes that **classification of RDGE as Carc. 1B, H350 "May cause cancer" is warranted.** As it has not been conclusively proven that no other routes of exposure cause the hazard, the route of exposure should not be stated in the hazard statement.

10.10 Reproductive toxicity

This hazard class has not been evaluated.

10.11 Specific target organ toxicity-single exposure

This hazard class has not been evaluated.

10.12 Specific target organ toxicity-repeated exposure

This hazard class has not been evaluated.

However, in support of the evaluation of the endpoint carcinogencity, a summary table and a short summary of the oral repeated dose studies are presented below. The individual studies can be found in Annex I.

Table 18: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Oral route			

Rat, Fischer 344, male 8-16 animals/group Study focussed on forestomach effects Non-guideline, Non-GLP	Resorcinol diglycidyl ether Oral via gavage 0-12-25 mg/kg bw/d 5 days/week, 2 weeks Vehicle: corn oil	Significant increase in the incidence and severity of mucosal cell proliferation and hyperkeratosis at the high dose of 25 mg/kg bw/d.	Ghanayem et al. (1986) Klimisch score 2
Rat, F344/N, male and female 5/sex/dose	Resorcinol diglycidyl ether Oral via gavage 0-190-380-750-1500-3000 mg/kg bw/d Daily for 14 consecutive days Vehicle: corn oil	Mortality: All males and females that received 750, 1500 or 3000 mg/kg bw/d and 2/5 males that received 380 mg/kg bw/d died before the end of the study. BW: All rats receiving 380 mg/kg bw/d and 2/5 males and 1/5 females receiving 190 mg/kg bw/d lost weight during the study. Macroscopy: Kidney: reneal medullae were red and more prominent than usual; Stomach: forestomach showed reddened mucosae and early development of small papillary-like growths. No histopathology performed	NTP (1986); Krishna-Murthy et al., 1990 Klimisch score 2
Rat, F344/N, male and female 10/sex/dose	Resorcinol diglycidyl ether Oral via gavage 0-12.5-25-50-100-200 mg/kg bw/d 5 days per week, for 13 weeks Vehicle: corn oild	Compound-related lesions were observed in the forestomach (squamous cell papilloma, hyperkeratosis, and basal cell hyperplasia) and the liver (minimal to mild centrilobular fatty metamorphosis). Chronic inflammation in the mesenteric lymph nodes was probably secondary to the inflammation or ulceration of the forestomach. Compared with the controls, the three male rats with fatty metamorphosis in the liver had decreased final body weights. However, lower mean body weight gains were also found in other male and female rats administered 200 mg/kg bw/d which did not show hepatic fatty metamorphosis. At necropsy, the wall of the forestomach was sometimes thickened and the mucosal surface contained small, white papillomatous nodules. When examined microscopically, some nodules and squamous papillomata, having localized acanthosis and papillary projections of the epidermis covered by thick layers of keratinized cells. The basal layer of the epithelium was hyperplastic, sometimes showing finger-like projections into the submucosa. Diffuse hyperkeratosis, focal basal cell hyperplasia, or both were usually present in the forestomach of rats without discrete squamous papillomata. In some rats that received 200 mg/kg bw/d, ulceration in the forestomach had completely eroded the epithelium and extended into the muscularis. A few rats without ulcers had circumscribed areas of inflammation in the stomach.	NTP (1986); Krishna-Murthy et al., 1990 Klimisch score 2

Mouse, B6C3F1, male and female 5/sex/dose	Resorcinol diglycidyl ether Oral via gavage 0-90-190-380-750-1500 mg/kg bw/d Daily for 14 consecutive days Vehicle: corn oild	Mortality: Five of five males and 4/5 females receiving 1500 mg/kg bw/d and 2/5 males receiving 750 mg/kg bw/d died BW: Weight loss was observed in all mice that received 750 mg/kg bw/d or more and in 4/5 males en 1/5 females thet received 380 mg/kg bw/d. Weight loss occurred in mice in the 90 mg/kg bw/d groups (4 males and 5 females), but not in animals administered 190 mg/kg bw/d. Macroscopy: kidney: reddened medullae; stomach: reddened mucosae. No histopathology performed	NTP (1986); Krishna-Murthy et al., 1990 Klimisch score 2
Mouse, B6C3F1, male and female 10/sex/dose	Resorcinol diglycidyl ether Oral via gavage 0-25-50-100-200-400 mg/kg bw/d 5 days per week, for 13 weeks Vehicle: corn oild	Mortality: Nine of ten males and 7/10 females receiving 400 mg/kg bw/d died; BW: Final mean body weight compared to controls was depressed 10-25% in groups that received 400 mg/kg bw/d Histopathology: - Forestomach: squamous papillomata, diffuse hyperkeratosis, basal cell hyperplasia, and inflammation, mucosal ulcers (high dose females). - Testis: Slight to mild focal tubular atrophy of the testes in three mice that died during weeks 9 or 10 (Lesion not seen in mice that survived to the end of the study). The testicaly atrophy was, based on accompanying reduced BW, interpreted as being the result of morbidity rather than a direct effect or resorcinol diglycidyl ether. - Liver (high dose): Hepatic necrosis, minimal to mild fatty metamorphosis in periportal areas of the liver (only in animals that died).	NTP (1986); Krishna-Murthy et al., 1990 Klimisch score 2

Summary on repeated dose toxicity data:

For the oral exposure route, multiple gavage studies in rat and mouse were available with exposure periods of 2 weeks and 13 weeks. Resorcinol diglycidyl ether was found to induce mainly effects in the forestomach of F344/N rats and B6C3F1 mice of both sexes, causing mucosal cell proliferation, hyperkeratosis, hyperplasia and papillary growth, mucosal ulcers of the forestomach (Ghanayem et al., 1986; NTP, 1986). When comparing the type/severity of stomach effects with respect to dose levels, mainly local effects with limited severity were noticed in these repeated dose studies. Severe type of effects such as ulceration was only observed at high dose levels (i.e. higher than the dose levels as applied in the carcinogenicity studies).

10.13 Aspiration hazard

This hazard class has not been evaluated.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

This hazard class has not been evaluated.

12 EVALUATION OF ADDITIONAL HAZARDS

Not relevant

13 ADDITIONAL LABELLING

Not relevant

14 REFERENCES

Canter DA, Zeiger E, Haworth S, Lawlor T, Mortelmans K, Speck W. Comparative mutagenicity of aliphatic epoxides in Salmonella. Mutat Res 1986; 172(2): 105-138.

CDC International Chemical Safety Cards (ICSC) diglycidyl resorcinol ether. https://www.cdc.gov/niosh/ipcsneng/neng0193.html Consulted: May 2017.

Chemiekaarten. Ed 32. Den Haag. TNO/SDU uitgevers, 2017

DFG. Diglycidyl resorcinol ether - MAK value. 1992

Duuren BL van, Orris L, Nelson N. Carcinogenicity of epoxides, lactones, and peroxy compounds. II. J Natl Cancer Inst 1965; 35(4): 707-717.

Ghanayem BI, Maronpot RR, Matthews HB. Association of chemically induced forestomach cell proliferation and carcinogenesis. Cancer Letters 1986; 32: 271-278

Gulati DK, Witt K, Anderson B, Zeiger E, Shelby MD. Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. III. Results with 27 chemicals. Environ Mol Mutagen 1989; 13(2): 133-193.

Health Council of the Netherlands (1995). Scientific documents of the Dutch list of occupational carcinogens (I). The Hague: Sdu Servicecentrum Uitgeverijen; 1995: publication no. RA 1/95

Health Council of the Netherlands (1999). Diglycidyl resorcinol ether : health-based calculated occupational cancer risk values. The Hague: Health Council of the Netherlands; 1999: publication no. 1999/09OSH.

Health Council of the Netherlands (2016). Resorcinol diglycidyl ether. Evaluation of the carcinogenicity and genotoxicity. The Hague: Health Council of the Netherlands, 2016; publication no. 2016/03.

Hine CH, Kodama JK, Anderson HH, Simonson DW, Wellington JS (1958). The toxicology of epoxy resins. AMA Arch Ind Health 17, 129-144.

IARC (1985). IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Allyl compounds, aldehydes, epoxides and peroxides. IARC Monogr Eval Carcinog Risk Chem Hum 1985; 36: 181-8.

IARC (1999). IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Reevaluation of some organic chemicals, hydrazine and hydrogen peroxide. IARC Monogr Eval Carcinog Risk Chem Hum 1999; 71: 1417-20.

IARC (2003). Predictive value of rodent forestomach and gastric neuroendocrine tumours in evaluating carcinogenic risks to humans. Views and expert opinions of an IARC Working Group, Lyon, 29 November – 1 December 1999. IARC Technical Publication No. 39, Lyon, France, p.1-73.

Kotin P, Falk HL. Organic peroxides, hydrogen peroxide, epoxides, and neoplasia. Radiat Res 1963; Suppl 3: 193-211.

Matthews EJ, Spalding JW, Tennant RW. Transformation of BALB/c-3T3 cells: IV. Rank-ordered potency of 24 chemical responses detected in a sensitive new assay procedure. Environ Health Perspect 1993a; 101 Suppl 2: 319-345.

Matthews EJ, Spalding JW, Tennant RW. Transformation of BALB/c-3T3 cells: V. Transformation responses of 168 chemicals compared with mutagenicity in Salmonella and carcinogenicity in rodent bioassays. Environ Health Perspect 1993b; 101 Suppl 2: 347-482.

McCammon CJ, Kotin P, Falk HL. The cancerogenic potency of certain diepoxides. Proc Am Assoc Cancer Res 1957; 2: 229-30.

McGregor DB, Brown A, Cattanach P, Edwards I, McBride D, Riach C et al. Responses of the L5178Y tk+/tk- mouse lymphoma cell forward mutation assay: III. 72 coded chemicals. Environ Mol Mutagen 1988; 12(1): 85-154.

McGregor DB, Riach C, Cattanach P, Edwards I, Shepherd W, Caspary WJ. Mutagenic responses of L5178Y mouse cells at the tk and hprt loci. Toxicol In Vitro 1996; 10(5): 643-647

Murthy AS, McConnell EE, Huff JE, Russfield AB, Good AE. Forestomach neoplasms in Fischer F344/N rats and B6C3F1 mice exposed to diglycidyl resorcinolether--an epoxy resin. Food Chem Toxicol 1990; 28(10): 723-729.

NTP Toxicology and Carcinogenesis Studies of Diglycidyl resorcinol ether (Technical Grade) (CAS No. 101-90-6) In F344/N Rats and B6C3F1 Mice (Gavage Studies). Natl Toxicol Program Tech Rep Ser 1986; 257: 1-222

National Toxicology Program. Diglycidyl resorcinol ether. Rep Carcinog 2011; 12: 163-4.

Proctor DM, Gatto NM, Hong SJ, Allamneni KP. Mode-of-action framework for evaluating the relevance of rodent forestomach tumors in cancer risk assessment. Toxicol Sci 2007; 98(2): 313-326.

RIVM (2003) report 601516012: Factsheets for the (eco)toxicological risk assessment strategy of the National Institute for Public Health and the Environment - Part IV. Editors: C.E. Smit and M.T.M. van Raaij

Seiler JP (1984a). Uptake, metabolism and mutagenic activity of aromatic glycidyl compounds. Chem Biol Interact; 51(3): 347-356.

Seiler JP (1984b). The mutagenicity of mono- and di-functional aromatic glycidyl compounds. Mutat Res 1984; 135(3): 159-167.

Shelby MD, Erexson GL, Hook GJ, Tice RR. Evaluation of a three-exposure mouse bone marrow micronucleus protocol: results with 49 chemicals. Environ Mol Mutagen 1993; 21(2): 160-179.

Westrick ML and Gross P (1960). Industrial Hygiene Foundation of America, Inc. As cited in: Gardiner TH, Waechter JM, Wiedow MA, Solomon WT (1992). Glycidyloxy compounds used in epoxy resin systems: a toxicology review. Reg Tox Pharmacol 15: S1-S77.

15 ANNEXES

- *Annex 1 to the CLH report: contains a description of the evaluated studies. See separate document*
- *Original classification proposal of* ***resorcinol diglycidyl ether*** *from 1997: see below*
- *Original classification proposal of phenyl diglycidyl ether from 1997: see below*

Additional references
EFSA, 2004: Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related to 2,2-bis(4-hydroxyphenyl)propane bis(2,3-epoxypropyl)ether (Bisphenol A diglycidyl ether, BADGE). IARC Monograph: Diglycidyl Resorcinol Ether. Volume 71. 1999

IARC Technical Publication No 39: Predictive Value of Rodent Forestomach and Gastric Neuroendocrine Tumours in Evaluating Carcinogenic Risks to Humans. 2003.

Maronpot, R. R., NTP/NIHS: "Xenobiotic-induced Rodent Tumors of Questionable Relevance to Human Cancer Risk", https://focusontoxpath.com/rodent-tumors-of-questionable-relevance-to-man/

NIOSH 1978: NIOSH criteria for a recommended standard. Occupational exposure to Glycidyl Ethers. National Institute for Occupational Health and Safety, Publication No. 78-166.

REACH Registration dossier Epichlorohydrin: https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15559/7/7/3/?documentUUID=220840b4-1516-4b6f-a625-0331037030da

Resorcinol diglycidyl ether. Evaluation of the carcinogenicity and genotoxicity. No. 2016/03, The Hague, February 29, 2016

Original classification proposal of resorcinol diglycidyl ether from 1997

FORM XI/396/93

Commission of the European Communities DG XI

CLASSIFICATION AND LABELLING OF DANGEROUS SUBSTANCES
Recommended form to be used for the proposed classification and labelling of Dangerous Substances in order to update Annex I of Directive 67/548/EEC

Date: 20 March 1997	Prepared by: Health and Safety Executive, UK

The information contained in this form is not regarded as confidential ECBT ü

1. IDENTIFICATION OF THE SUBSTANCE

INDEX No. 603-065-00-9	EC No. 202-987-5 CAS No. 101-90-6 ID No. U 043
1.1 EINECS Name	m-bis(2,3-epoxypropoxy)benzene
1.2 Synonyms (state ISO name if available)	**Resorcinyl diglycidyl ether (RDGE)** Diglycidyl resorcinol ether; 1,3-diglycidyloxybenzene; 2,2'-[1,3-phenylenebis(oxymethylene)]bisoxirane; 1,3-bis(2,3-epoxypropoxy)benzene; meta-Bis(2,3-epoxypropoxy)benzene; meta-Bis(glycidyloxy)benzene
1.3 Molecular formula	$C_{12}H_{14}O_4$
1.4 Structural formula	
1.5 Purity (w/w)	
1.6 Significant impurities or additives, their concentrations (w/w)	
1.7 Known uses	**Industrial:** liquid spray epoxy resin, as a dilutant in the production of other epoxy resins used in electrical tooling, adhesive and laminating applications, and as a curing agent for polysulphide rubber (Lee & Neville, 1967) **General public:** Not used
1.8 Proposed classification	Carc Cat 3; Muta Cat 3; R40 Xn; R21/22: Xi; R36/38: R43

EXISTING LABEL	In Annex 1 Yes	Provisional No
Symbol(s)	R-Phrase(s)	S-phrase(s)
T, Xn	R23/24/25-40-43	S(1/2-)23-24-45

2. PHYSICO-CHEMICAL CHARACTERISTICS

From: ICSC: 0193 (1991)

2.1 Physical form	Colourless solid or yellowish viscous liquid, with characteristic odour
2.2 Molecular weight	222.2
2.3 Melting point/range (°C)	32-33
2.4 Boiling point/range (°C)	208-210
2.5 Decomposition temperature	
2.6 Vapour pressure (Pa(°C))	0.05 mm Hg @ 150°C
2.7 Relative density	1.21
2.8 Vapour density (air = 1)	7.95
2.9 Fat solubility (mg/kg, °C)	
2.10 Water solubility (mg/kg, °C)	
2.11 Partition coefficient (log Pow)	
2.12 Flammability **flash point (°C)** **explosivity limits (%,v/v)** **auto-flammability temp. (°C)**	**open cup:** 177 **closed cup:** **lower limit:** **upper limit:**
2.13 Explosivity **danger of explosion as a result of: explosive properties at high temperature**	Reacts with strong oxidants Presumed to form explosive peroxides.
2.14 Oxidising properties	
2.15 Other physico-chemical properties **(eg. liberates toxic gas on heating or in contact with water or acids)**	Solubility: miscible with acetone, chloroform, methanol, benzene and most organic resins.

3. OBSERVATIONS ON HUMANS

Where available, human data are considered to be of more relevance in determining the potential effects of chemical substances on the human population. (Annex V, Directive 67/548/EEC).

3.1 Occupational exposure

Substance claimed to produce severe skin burns and skin sensitisation in a limited number of cases - however, these data are of doubtful quality and reliability (Hine & Rowe, 1963).

This information is regarded insufficient to justify classification.

3.2 Exposure of the general public

No data available.

4. TOXICOLOGICAL DATA

4.1 ACUTE TOXICITY

4.1.1 Oral

Species	LD_{50} (mg/kg)	Observations and remarks
Rat (Long-Evans) (male)	2,570	(Reported in summary form). No information on the number of animals was provided. Slight dyspnea, and, in surviving animals, loss of weight were reported, although the doses at which these clinical signs were seen were not detailed. Although the cause of death was not established, it was possibly due to irritant effects on the stomach lining. (Hine *et al*., 1958)
Mouse/ Webster (male)	980	
Rabbit/ albino (male)	1,240	**Data support classification as: Harmful if swallowed (R22)** (see Annex A)

4.1.2 Inhalation

Species	LC_{50} (mg/l)	Exposure time (h/day)	Observations and remarks
Rat	Not determined	4	Concentrated aerosol of 44.8 mg/l (60% RDGE in xylene). It is not clear whether the concentration quoted refers to RDGE or RDGE and xylene. All rats died within 5 days post exposure - number of animals not stated. (Westrick & Gross, 1960; reported in summary form). Xylene is classified for acute toxicity via inhalation (R20). No conclusion with respect to acute toxicity of RDGE can be drawn from this study.
Rat (Long Evans)	Not determined	8	The LC_{50} was greater than the highest vapour concentration attained (exposure concentration not determined analytically, but likely to be very low) - no deaths, number of animals not specified. (Hine *et al*., 1958).
Mouse (Webster)	Not determined	8	**Data do not support classification**

4. TOXICOLOGICAL DATA (continued)

4.1.3 Dermal

Species	LD50 (mg/kg)	Exposure period	Observations and remarks
Rabbit	2420 (2.0 ml/kg)	7 hours	RDGE applied as a 60 % solution in xylene. Not occluded. Number of animals, decedents, clinical signs not detailed. (Westrick & Gross, 1960; reported in summary form). Xylene is classified for acute toxicity via the dermal route (R21) therefore no conclusion with respect to acute toxicity of RDGE can be drawn from this study.
Rabbit	744 (0.64 ml/kg)	Continuous	No more detail provided. (Westrick & Gross, 1960; reported in summary form). Although it is unclear for how long the RDGE was applied to the skin, this LD_{50} value for acute toxicity suggests that classification may be appropriate. **Classification with R21 is proposed (see Annex A).**

4.1.4 Skin irritation

Species	No. of animals	Exposure time	Conc. (w/w)	Dressing: (semi-occlusive, occlusive, open)	Observations and remarks (specify degree and nature of irritation and reversibility)
Rabbit/ albino	Not stated	24hrs	Unknown concentration	Occlusive	(Reported in summary form, limited details of results provided). Draize method; irritation scored at 24 and 72 h. Moderate irritation with Draize score of 5 out of possible 8. (Hine *et al.*, 1958)
Rabbit	5	Not stated	0.01 ml of 10% solution in acetone	Not stated	(From secondary source, reported in summary form). Scar tissue formation in one animal, with definite erythema and oedema in the other 4 rabbits (Westrick & Gross, 1960)
Rabbit	4	20 daily applications of 7 hr periods (removal with acetone)	1 ml of unknown concentration	Not stated	Irritation scored (Draize) at 24 hour intervals (prior to subsequent application). Severe irritation reported, score 8/8 (individual daily scores not reported). Three animals died by day 8. The authors concluded that death was most likely due to the severe irritation rather than systemic effects. (Hine *et al.*, 1958) **The data support classification with R38 (see Annex A)**

4. TOXICOLOGICAL DATA (continued)

4.1.5 Eye irritation

Species	**No. of animals**	**Exposure time (hours)**	**Conc. (w/w)**	**Observations and remarks (specify degree and nature if irritation, any serious lesions, reversibility)**
Rabbit	>1 (but actual number not stated)	Not stated	0.1 ml of 20% suspension in propylene glycol	(Reported in summary form, limited details of results provided). Draize method. Average score from readings at 1, 24 and 48 h was 45 (max. possible =110), indicating a moderate effect. (Hine *et al.*, 1958). **Data support classification with R36.**

4.1.6 Irritation of respiratory tract

Species	**No. of animals**	**Exposure time (h/days)**	**Conc. (w/w)**	**Observations and remarks (specify degree and nature if irritation, any serious lesions, reversibility)**
Rat	Not stated	7 hr/day (50 exp)	Air saturated with vapour; control group	(Reported in summary form, limited details of results provided). Exposure concentration not determined analytically, but likely to be very low. No gross or microscopic lesions seen at necropsy. Although the tissues studied at necropsy were not detailed, since this was an inhalation study it is assumed that the lungs and/or respiratory tract would have been examined. (Hine *et al.*, 1958). ***Data do not support classification***

4.1.7 Skin sensitisation

Species	**Type of test**	**No. of animals**	**Incidence of reactions observed**
			No data available. **Arguments for classification with R43 are given in Annex A.**

4. TOXICOLOGICAL DATA (continued)

4.2 REPEATED OR PROLONGED TOXICITY GROUPED ACCORDING TO SUBACUTE AND SUBCHRONIC TOXICITY

4.2.1 Oral

Species/ strain	Dose (mg/kg bw)	Duration of treatment	Observations and remarks (specify group size, NOAEL, effects of major toxicological significance)
Rat (F344/N) [Males, 8/treatment group, 16 controls]	0, 12, 25 (gavage in corn oil)	5 d/wk for 2 weeks	Limited study in which only the stomach was examined at necropsy. There was no evidence of cell proliferation or hyperkeratosis in rats dosed at 12 mg/kg or in control rats. At the higher dose there was incidence of multifocal forestomach epithelial cell proliferation (4/8 rats) and incidence of multifocal hyperkeratosis (5/8 rats). (Ghanayem *et al*., 1986).
Rat (F344/N) 5/sex/ treatment group	0, 190, 380, 750, 1500 and 3000	14 consecutiv e daily doses	All rats dosed at 750 mg/kg or more, and 2/5 males at 380 mg/kg died before end of study. Cause of death was not established; in most cases, stomach lesions seen were not considered severe enough to result in death. Mean body weight decreased in nearly all treatment groups. Reddening of the renal medulla, and lesions and papillary growths in stomach were observed at necropsy in many of the survivors (dose not stated). (NTP, 1986).
Rat (F344/N) 10/sex/ treatment group	0, 12.5, 25, 50, 100, and 200	5 days/ week for 13 weeks	At 200 mg/kg, 1 male died during 8th week of study; cause of death not established but the animal was emaciated. Mean body weight was depressed 10% or more in male rats dosed at 100 mg/kg and above, and in females dosed at 200 mg/kg. Histopathological examination of the stomach was performed on animals dosed at 12.5, 25 or 50 mg/kg. Compound related changes observed in forestomach at 12.5 mg/kg and above, were inflammation, ulceration, squamous cell papilloma, hyperkeratosis and basal cell hyperplasia. Histopathology changes in the liver were necrosis and minimal to mild centrilobular fatty metamorphosis at 200 mg/kg. (NTP, 1986).
Rat (F344/N) 100/sex controls, 50/sex/ treatment group	0, 12, 25, 50	5 days/ week for 103 weeks	Decreased survival was dose-related. None of the males at 50 mg/kg and only 10% of the males at 25 mg/kg survived until the end of the study, compared to 46% at 12 mg/kg and 78% of controls. In females, survival rates were 2%, 32%, 70% and 74%, respectively. Most of the early deaths (occurring from week 30 onwards) were attributable to non-treatment-related bronchopneumonia. Body weight gain was not affected in the low dose group. Wheezing and respiratory distress possibly relating to bronchopneumonia, were the only compound-related clinical signs observed. Histopathology revealed hyperkeratosis, hyperplasia and neoplasms of the squamous epithelium of the forestomach at all dose levels. The squamous epithelium of the oesophagus and nasopharynx was hyperkeratotic in some rats (NTP, 1986).
Mouse ($B6C3F_1$) 5 / sex / group	0, 90, 190, 380, 750 and 1500 mg/kg	14 days	9/10 mice receiving 1500 mg/kg and 2/5 males receiving 750 mg/kg died before the end of the study. The deaths were attributed to RDGE. Mean body weight decreased in all mice dosed at 750 mg/kg and 5/10 dosed at 380 mg/kg. Gross pathology revealed reddening of stomach mucosae and renal medulla (dose-levels not stated) (NTP, 1986).

4.2.1 Oral continued

Species/ strain	Dose (mg/kg bw)	Duration of treatment	Observations and remarks (specify group size, NOAEL, effects of major toxicological significance)
Mouse ($B6C3F_1$) 10 / sex / group	0, 25, 50, 100, 200 and 400	13 weeks	16/20 mice dosed at 400 mg/kg died during the study. The deaths were attributed to RDGE. Mice at 400 mg/kg had depressed body weights (10-25%). Histopathological examination of the stomach, liver kidneys and testes was performed on animals at all doses. Compound related changes in forestomach were inflammation, ulceration, squamous cell papilloma, hyperkeratosis and basal cell hyperplasia at 25 mg/kg and above. Histopathology changes in the liver in mice dosed at 400 mg/kg included focally extensive necrosis and, in decedents only, fatty metamorphosis was seen. Slight to mild focal tubular atrophy of the testes was seen in 3/10 mice dosed at 400 mg/kg that died during weeks 9-10. This lesion was not seen in the mice in this dose level that survived until the end of the study, therefore it is considered to be secondary to general systemic toxicity. (NTP, 1986).
Mouse ($B6C3F_1$) 50/sex/ group	0, 50, 100	5 days/ week for 103 weeks	In males 68% of the mice at 100 mg/kg and 52% at 50 mg/kg survived until the end of the study, compared to 60% of the controls. In females, survival rates were 20%, 26% and 40%, respectively. Most of the deaths in female mice in all groups were attributable to non-treatment-related necrosuppurative lesion of the ovary which spread through the abdomen. Histopathology revealed hyperkeratosis, hyperplasia and neoplasms of the squamous epithelium of the forestomach at both dose levels. Minimal mineralization was found in the kidneys of 30 high dose males, and 18 low dose males compared to 8 control group males.(NTP, 1986).

Note: In the NTP studies, RDGE was administered 81% pure by gavage in corn oil.

4.2.2 Inhalation

Species	Conc. mg/l	Exposure time	Duration of treatment	Observations and remarks (specify group size, NOAEL, effects of major toxicological significance)
Rat	Air saturated with vapour + control group	7 hr/day, (5 day/wk)	10 weeks (50 exp.)	(Study reported in summary form). No treatment-related deaths. No treatment-related clinical effects were seen. No significant gross or microscopic lesions were found in survivors at necropsy. (Hine *et al.*, 1958) **Data do not support classification.**

4.2.3 Dermal

Species	Dose mg/kg	Exposure time	Duration of treatment	Observations and remarks (specify group size, NOAEL, effects of major toxicological significance)
				Data presented in section 4.3.3 **Data do not support classification.**

4. TOXICOLOGICAL DATA (continued)

4.3 CARCINOGENICITY (INCLUDING CHRONIC TOXICITY STUDIES)

On the basis of the data presented below it is proposed that this substance be classified as a category 3 carcinogen. See also Annex A.

4.3.1 Oral

Species/ strain	D ose mg/kg bw	Duration of treatment	Observations and remarks (specify group size, effects of major toxicological significance)
Rat (F344/N) 50/sex/ treatment group 100 controls/sex	0, 12, 25 & 50	5 days/week for 103 weeks	Survival rates are given under Section 4.2.1. Most of the early deaths were attributable to bronchopneumonia. Wheezing and respiratory distress were the only compound-related clinical signs observed (however, it is not clear whether this was in the same animals that had bronchopneumonia). Histopathology revealed hyperkeratosis, hyperplasia and neoplasms of the stratified squamous epithelium of the forestomach at all dose levels. In some animals (control 0/99, low dose 66/100, mid dose 72/100, high dose 7/99), squamous cell carcinomas were observed on the non-glandular stomach mucosa. The large number of early deaths in high dose animals explains the low incidences of benign and malignant neoplasms found in this group compared with the lower dose groups. (NTP, 1986; Murthy *et al.*, 1990).
Mouse ($B6C3F_1$) 50/sex/group	0, 50 & 100	5 days/ week for 103 weeks	Survival rates are given under Section 4.2.1. Most of the deaths in female mice were attributable to a non-treatment-related necrosuppurative lesion of the ovary which spread through the abdomen. Histopathology revealed hyperkeratosis, hyperplasia and neoplasms (carcinomas: low dose 26/98, high dose 48/99) of the stratified squamous epithelium of the forestomach at both dose levels. The incidences of females with either primary hepatocellular adenomas or carcinomas had a significant positive trend (control 3/48, low dose 1/50, high dose 7/49, historical 6/98); However, liver tumors are not rare in this species of mouse. (NTP, 1986; Murthy *et al.*, 1990). **Data support classification as Carc Cat 3; R40 (see Annex A).**

Note: In these NTP studies, RDGE was administered 81% pure by gavage in corn oil.

4.3.2 Inhalation

Species	conc. mg/l	Exposure time	Duration of treatment	Observations and remarks (specify group size, effects of major toxicological significance)
				No data available.

4. TOXICLOGICAL DATA (continued)

4.3.3 Dermal

Species	Dose	Exposure time	Duration of treatment	Observations and remarks (specify group size, NOEL, effects of major toxicological significance)
Mouse (C57BL)	Not stated	Skin painted 3 times per week	Not stated	Papillomas of the skin were reported. - results only reported in abstract form. (McCammon *et al.*, 1957)
Mouse (C3Hf/Bd)	0.45, 0.9, or 1.8 mg/week	Not stated (skin painting)	2 year 3 applications/ week on shaved skin.	Skin changes included mild hyperkeratosis, depigmentation and follicle depletion. No evidence of skin neoplasms. (Holland *et al.*, 1981)
Mouse (Swiss Mellerton) Groups of 30	100 mg of a solution (1% in benzene)	painted 3 times per week	Lifetime (median survival time, 70 weeks)	No evidence of benign or malignant skin tumors was observed in 30 treated mice. Moderate to severe crusting and/or scarring, and hair loss were observed at the application site. Mean survival times: treated group 70 wk, benzene control 71 wk and untreated control 63 wk. (Van Duuren *et al.*, 1965)
Mouse (C3H) Number unspecified	50 mg/kg (81% pure product as 5% in methyl ethyl ketone)	2/ wk	Assumed lifetime	Preliminary studies identified 50 mg/kg to be MTD. Median survival time was 46 wks. After 36 wks a benign papilloma appeared on one mouse that survived for an additional 15 weeks. In week 48 a subdermal growth that was identified as a squamous cell cancer appeared on another mouse. This study is difficult to interpret since there were no details of a concurrent control group (summary of unpublished study cited by Hine et al, 1958).

4.4 GENOTOXICITY

4.4.1 *In vitro* studies

Test	Cell type	Conc. range	Observations and remarks
Reverse mutation (Ames)	*S.typhimurium* TA 98, TA 100, TA 1535, TA 1537	5-5000 µg/plate	**Positive.** Reproducible, dose-related increases in revertants in strains TA100 and TA1535 observed without metabolic activation and in the presence of Aroclor-induced hamster or rat liver S9 (Canter *et al*, 1986).
Reverse mutation (Ames)	*S.typhimurium* TA100	50-1000 µg/plate	**Positive.** Reproducible, dose-related increases in revertants observed without metabolic activation. (Seiler, 1984).
Cytogenetics-chromosome aberrations	CHO cells	0, 8 and 25 µg/ml	**Positive.** Two separate experiments without metabolic activation performed, with exposure/fixation times 6 hr in first assay and 24 hr in second assay. A statistically significant dose-related increase in aberration frequency (excluding gaps) was observed at both fixation times (mean values of 8, 24 & 44% at 6 hrs, and 9, 48, and 93% at 24 hrs at 0, 8.25 mg/kg respectively). (Seiler, 1984).

4. TOXICOLOGICAL DATA (continued)

4.4.2 *In vivo* studies (somatic cells)

Test	Species	Tissue	Harvest time	Observations and remarks (include route of administration)
Micronucleus	Mouse, (ICR) strain. Male and female. (4 animals / dose group)	Bone marrow	24 h (all doses), 48 & 72 h (controls and top dose only)	**Negative.** Oral gavage (0, 300, 600 mg/kg as aqueous solution in polyethylene glycol-400). Systemic toxicity reported but not detailed, one of 4 in top dose group died. The P/N ratio was unaffected by treatment. No increases in MN frequency observed. A positive result was demonstrated for another substance, diglycidylaniline, within the same study (Seiler, 1984).

4.5 FERTILITY

Species	Route	Dose	Exposure time	Number of gen. exposed	Obsevations and remarks
					No data available.

4.6 DEVELOPMENTAL TOXICITY

Species	Route	Dose	Exposure	Observations and remarks
				No data available.

5. ECOTOXICOLOGICAL STUDIES

Data not reviewed for Health Effects Working Group

6. ENVIRONMENTAL FATE

Data not reviewed for Health Effects Working Group

7. ADDITIONAL ENVIRONMENTAL EFFECTS

Data not reviewed for Health Effects Working Group

8. REFERENCES

Canter, D.A., Zeiger, E., Haworth, S., Lawlor, T., Mortelmans, K. & Speck, W. (1986) Comparative mutagenicity of aliphatic epoxides in Salmonella. Mutation Res., 172, 105-138.

Ghanayem, B.I., Maronpot, R.R. & Matthews, H.B. (1986). Association of chemically induced forestomach cell proliferation and carcinogenesis. Cancer Lett., 32, 271-278.

Hine, C.H., Kodama, J.K., Anderson, H.H., Simonson, D.W. & Wellington, J.S. (1958). AMA Arch. Ind Health, 17, 129.

Hine, C.H. & Rowe, V.K. (1963) Resorcinyl diglycidyl ether. In Patty, F.A., ed., Industrial Hygiene and Toxicology, 2nd rev. ed., Vol. 2, New York, Interscience, pp. 1648-1649.

Holland, J.M., Gipson, L.C., Whitaker, M.J., Eisenhower, B.M., Stephens, T.J. (1981). Chronic dermal toxicity of epoxy resins. I. Skin carcinogenic potency and general toxicity. ORNL-5762, Oak Ridge National Laboratory, Oak Ridge, TN. [cited in: NTP (1986), National Institute of Health, Technical Report Series No 257, NIH publication 87-2513].

Lee, H. & Neville, K. (1967). Handbook of epoxy resins. San Francisco, McGraw-Hill, pp. 4-59.

McCammon, J.C., Kotin, P. & Falk, H.L. (1957). The cancerogenic potency of certain diepoxides. Proc. Am. Assoc. Cancer Res., 2, 229-230.

Murthy, K.A.S., McConnell, E.E., Huff, J.E., Russfield, A.B. & Good, A.E. (1990) Forestomach neoplasms in Fischer rats and B6C3F1 Mice exposed to diglycidyl resorcinyl ether - an epoxy resin. Fd. Chem. Toxicol., 28, 723-729.

NTP (1986). National Institute of Health, Technical Report Series No 257, NIH publication 87-2513. Diglycidyl resorcinol ether.

Seiler, J.P. (1984). The mutagenicity of mono- and di-functional aromatic glycidyl compounds. Mutation Res., 135, 159-167.

Van Duuren, B.L., Orris, L. & Nelson, N (1965). Carcinogenicity of epoxides, lactones and peroxy compounds, Part II. J. Natl Cancer Inst, 35, 707-717.

Westrick, M.L. & Gross, P. (1960). Industrial Hygiene Foundation of America, Inc. *[cited in: Gardiner et al., (1992) Reg. Tox. Pharmacol., 15, S1-S77].*

ANNEX A

EC CLASSIFICATION AND LABELLING

RESORCINYL DIGLYCIDYL ETHER (RDGE)

(EINECS name: m-bis(2,3-epoxypropoxy)benzene)

Background information on metabolism of RDGE

Glycidyl ethers, in common with other epoxides, may be metabolised throught the following pathways:

(i) expoxide hydrolase activity, leading to hydrolysis of the epoxide group under formation of the corresponding diol;
(ii) conversion to glutathione conjugates by glutathione-S-epoxide conjugases; and
(iii) non-enzymic covalent binding with proteins, RNA and DNA.

RDGE has been shown to conjugate with glutathione via glutathione-S-epoxide transferase *in vitro*. (Boyland & Williams, 1965). When RDGE was administered (route not detailed) to mice it was metabolised to bis-diol compounds (Seiler, 1984). No further data on the pharmacokinetics and tissue distribution were located in the literature.

Boyland, E. & Williams, K. (1965) An enzyme catalysing the conjugation of epoxides with glutathione. Biochem., 94, 190-197.
Seiler (1984) The mutagenicity of mono- and di-functional aromatic glycidyl compounds. Mutat. Res., 135, 159-167.

Arguments for classification: Physico-chemical effects

No classification is proposed.

Arguments for classification: Health effects

1) *Acute oral toxicity*
A single oral LD_{50} value in the rat, of 2570 mg/kg is available. Although this is outside the range for classification for acute toxicity, LD_{50} values in two other species (mice, 980 mg/kg and rabbits, 1240 mg/kg) support classification with R22. There is no evidence available to support the existing classification with R25.

Acute inhalation toxicity
In rats and mice exposed to a saturated vapour of RDGE for 8 hr there were no signs of toxicity. We propose that no classification is justified.

Acute dermal toxicity
No data are available to support the existing classification with R24. A rabbit LD_{50} value of 744 mg/kg supports our proposal to classify with R21.

2) *Skin irritation*
In a skin irritation study in which RDGE was applied to rabbit skin for 24 hours, RDGE was a moderate irritant (Draize score 5/8). In another study in which 4 rabbits were treated with

20 daily applications, severe irritation was reported (Draize score 8/8). Within this study, 3 deaths, which occurred by treatment-day 8, were attributed to severe irritation. RDGE has been reported to produce "severe skin burns" in humans, although these data are of unreliable quality; the actual exposure circumstances and the precise nature of the effects seen are not clear. Although there are no studies conducted to current regulatory guidelines, overall the available data indicate that RDGE is a skin irritant. These data support R38.

3) *Eye irritation*
In an eye irritation study (reported in summary form), a Draize score of 45/120 was obtained indicating that RDGE is a moderate eye irritant. These data support R36.

4) *Respiratory irritation*
In an inhalation study, fifty 7hr exposures to air saturated with RDGE vapour produced no evidence of respiratory irritation. No classification is proposed for this relatively non-volatile substance.

5) *Skin sensitisation*
RDGE is presently classified with R43. Although, there appear to be no animal studies available to support classification, a single report providing human anecdotal data was found. While the quality of this information is data is limited, it does suggest that RDGE might have skin sensitisation properties. Given that the structurally similar glycidyl ethers (allyl-, *n*-butyl-, and phenyl-) are skin sensitisers, we propose that RDGE is likely to have the same property and propose classification with R43.

6) *Respiratory Sensitisation*
No animal or human data are available. Consequently, classification is not justified.

7) *Repeated Dose Toxicity*
In repeated oral gavage studies in rats and mice, lesions of the forestomach were observed including ulceration, inflammation, hyperplasia and hyperkeratosis. A clear NOAEL for these effects could not be identified from 90-day or 2-year studies; a LOAEL of 12.5 mg/kg was found in rats exposed for 90 days or 2 years; in mice, LOAELs of 25 mg/kg and 50 mg/kg were identified in 90-day and 2 year studies respectively. No stomach lesions were seen in rats at 12 mg/kg in a 14 day oral gavage study.
No other effects indicating serious damage to health were seen at exposure levels within the classification range.

The lesions seen in the forestomach in these experiments are considered to be the result of repeated irritation due to the effect of a concentrated bolus delivery of test substance to forestomach epithelium. The bolus delivery of an irritant test substance is not considered relevant to normal occupational exposure conditions. Therefore, no classification is proposed.

8) *Mutagenicity*
RDGE gave positive results in the absence of exogenous metabolic activation in two bacterial mutagenicity assays and in an *in vitro* cytogenetics assay. From these results it is concluded that this epoxide is a direct-acting mutagen. This electrophilicity is consistent with that of the structurally similar substances, allyl-, *n*-butyl and phenyl- glycidyl ethers.

Only one study in somatic cells *in vivo* is available. In this mouse micronucleus assay, although RDGE gave a negative result following oral administration, it is considered likely that the target tissue (bone marrow) was not adequately exposed and that the result may have been a false negative. Not only were there no signs of systemic toxicity in this assay, but the LD_{50} of RDGE has been shown to be increased 10-fold in rodents when administered by i.p. injection rather than oral gavage (Hine et al, 1958). Either RDGE is poorly absorbed via the gastrointestinal tract or it is metabolised to a less toxic metabolite. The concept that the oral route of administration might have lead to a false negative result is supported by the finding that *n*-butyl glycidyl ether, a structurally similar electrophilic substance, gave a positive result in the bone marrow micronucleus test following i.p. but not oral administration (Gardiner et al, 1992).

Concern remains that RDGE may cause mutagenic effects in somatic cells at sites of initial contact in the body. Classification with Muta Cat 3; R40 is proposed since this substance is a reactive epoxide, mutagenic in vitro without exogenous activation, and has not been tested in a relevant in vivo test (i.e. at a site of initial contact).

Gardiner TH, Waechter JM Jr, Wiedow MA and Solomon WT (1992). Glycidyloxy compounds used in epoxy resin systems: a toxicology review. Regul Toxicol Pharmacol, 15, S1-S77.
Hine CH, Kodama JK, Anderson HH, Simonson DW & Wellington JS (1958). AMA Arch. Ind Health, 17, 129.

9) *Carcinogenicity*

In the absence of observations in humans, a decision regarding classification must be made on the basis of animal carcinogenicity studies.

In an oral gavage NTP study, RDGE was carcinogenic in mice and rats; the stratified squamous epithelium of the proximal alimentary tract was the primary target tissue affected. A high incidence of benign and malignant neoplasms was observed in the non glandular stomach of both male and female mice and rats. In both species, histopathology revealed also hyperkeratosis, hyperplasia and neoplasms of the stratified squamous epithelium of the forestomach at both dose levels tested (12, 25 mg/kg in rats; 50, 100 mg/kg in mice).

The malignant tumours reported in these oral gavage studies developed at the site of initial contact and were associated with hyperkeratosis and hyperplasia of the forestomach epithelium. This suggests that chronic tissue damage with resultant hyperplasia may have contributed to the carcinogenic response seen. As such, there is some uncertainty about the mechanism of tumour induction; both the genotoxic and irritant properties of RDGE may have played a role.

There is no good evidence from skin painting studies in mice that RDGE causes tumours when applied dermally.

Overall, it is possible that genotoxicity and/or chronic cell proliferation may underlie the mechanism of the carcinogenic response seen in rodents in oral gavage studies. We believe that there is considerable uncertainty about the relevance to humans of repeated bolus administration of an irritant substance that results in tumour formation at the site of contact. Furthermore, the tumour formation was in the forestomach, which is also of uncertain relevance for human health. Therefore, in view of this uncertainty, we propose that classification with Car Cat 2; R45 is not justified. We propose Carc Cat 3; R40.

10) *Reproductive Toxicity*

No data available. No classification proposed.

UK proposal

Carc Cat 3; R40: Muta Cat 3; R40: Xn; R21/22: Xi; R36/38: Xi; R43

HSE Toxicology Unit
20 March 1997

Original classification proposal of phenyl diglycidyl ether from 1997

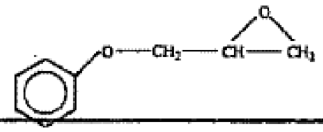
Commission of the European Communities DG XI

CLASSIFICATION AND LABELLING OF DANGEROUS SUBSTANCES
Recommended form to be used for the proposed classification and labelling of Dangerous Substances in order to update Annex 1 of Directive 67/548/EEC

Date: 29th July 1997	Prepared by: Health and Safety Executive, UK

The information contained in this form is not regarded as confidential. ECBI 21 19

1. Identification of the substance

INDEX No. 603-067-00-X	**EC No.** 204-557-2 **CAS No.** 122-60-1 **ID No.** UC40
1.1 EINECS Name **If not in EINECS IUPAC Name**	2,3-epoxypropyl phenyl ether
1.2 Synonyms (state ISO name if available)	**Phenyl glycidyl ether** 1,2-Epoxy-3-phenoxypropane, glycidyl phenyl ether, phenol glycidyl ether, phenoxypropylene oxide, 3-phenoxy-1,2-epoxypropane, phenoxypropene oxide phenyl-2,3-epoxypropyl ether, oxirane (phenoxymethyl)-, PGE
1.3 Molecular formula	$C_9H_{10}O_2$
1.4 Structural formula	
1.5 Purity (w/w)	
1.6 Significant impurities or additives, their concentrations (w/w)	
1.7 Known uses	**Industrial:** A chemical intermediate; a monofunctional reactive modifier. **General public:** Not used.
1.8 Proposed classification	Carc Cat 2; R45 Muta Cat 3; R40 Xn; R20 Xi; R37/38 R43

EXISTING LABEL	In Annex I Yes	Provisional No
Symbol(s)	R-Phrase(s)	S-phrase(s)
Xn	R21-43	S(2-)24/25

2. PHYSICO-CHEMICAL CHARACTERISTICS

(References: IARC, 1989; MAK, 1992)

2.1 Physical form	Colourless liquid		
2.2 Molecular weight	150		
2.3 Melting point/range (°C)	3.5		
2.4 Boiling point/range (°C)	245		
2.5 Decomposition temperature			
2.6 Vapour pressure (Pa(°C))	0.013 hPa at 20°C		
2.7 Relative density			
2.8 Vapour density (air = 1)	4.37 at 25°C		
2.9 Fat solubility (mg/kg, °C)			
2.10 Water solubility (mg/kg, °C)	Nearly insoluble in water		
2.11 Partition coefficient (log Pow)			
2.12 Flammability			
flash point (°C)	**open cup:**	**closed cup:**	
explosivity limits (%,v/v)	**lower limit:**	**upper limit:**	
auto-flammability temp. (°C)			
2.13 Explosivity			
danger of explosion as a result of:	**shock:**	**friction:**	**ignition:**
explosive properties at high temperature			
2.14 Oxidising properties			
2.15 Other physico-chemical properties (eg. liberates toxic gas on heating or in contact with water or acids)	Viscosity: 6cP at 25°C (cited in IARC, 1989) Conversion Factor: 1 ppm = 6.23 mg/m³ (MAK, 1992)		

3. OBSERVATIONS ON HUMANS

Where available, human data are considered to be of more relevance in determining the potential effects of chemical substances on the human population. (Annex V, Directive 67/548/EEC).

3.1 Occupational exposure

There are several reports of allergic contact dermatitis caused by occupational exposure to phenyl glycidyl ether.

Three individuals (aged 15, 19 and 45 years, respectively) developed allergic contact dermatitis from PGE used in an adhesive tape (the epoxy adhesive contained approx. 10% PGE). The allergenicity of phenyl glycidyl ether was confirmed by patch testing (Nakagawa et al, 1991).

Among 58 exposed workers with dermatitis, phenyl glycidyl ether was identified as the primary allergen in 9 cases. Sensitivity to both phenyl glycidyl ether and another epoxy resin used in the workplace was observed in an additional 26 workers (Rudzki and Krajewska, 1979).

Fifteen individuals with symptoms suggestive of occupational eczema were patch tested with the suspect allergen, phenyl glycidyl ether. Positive results were obtained with 8 cases. The aetiology of the eczema observed in the remaining 7 cases was not further characterised. Among the 15 cases, there were no responses to another potential sensitiser in the workplace (a chloroparaffin) or a standard series of allergens. Patch testing of phenyl glycidyl ether in 58 non-exposed controls gave negative results (Zschunke and Behrbohm, 1965).

A review of medical records from 1947-1956 indicated 13 cases of dermatitis at one workplace where PGE and other glycidyl ethers were used. In one case, second-degree chemical burns occurred 5 days after an accidental splash of phenyl glycidyl ether on the foot. In 5 of the remaining cases, dermatitis was related to (minimal) phenyl glycidyl ether exposure - clinical signs included itching, erythema, blisters and papules. It is not clear whether the dermatitis was irritant or allergic in nature (Hine et al, 1956).

Seven out of ten marble workers who developed contact dermatitis as a result of handling epoxy resin and cresyl glycidyl ether were found also to have developed skin sensitivity to PGE (Angelini et al, 1996).

These data indicate that PGE has the potential to cause skin sensitisation in humans and, together with the positive evidence from studies in animals (see below), support the existing classification with R43.

3.2 Exposure of the general public

No data available.

4. TOXICOLOGICAL DATA (indicate conclusions and biographical references)

4.1 ACUTE TOXICITY

4.1.1 Oral

Species	LD50 (mg/kg)	Observations and remarks
Rat (Long-Evans) [5 or 6/ dose group]	3850	Males only. (Hine et al, 1956).
Rat	4260	(Smyth et al, 1954; Weil et al, 1963).
Rat	2600	(Czaijkowska and Stetkiewicz, 1972).
Mouse (Webster) [5 or 6/ dose group]	1400	Males only. (Hine et al, 1956). **No classification is proposed.**

4.1.2 Inhalation

Species	LC50 (mg/l)	Exposure time (hours)	Observations and remarks
Rat (Long Evans) [6/ group]	not determined	4	The LC50 was greater than the highest vapour concentration attained (100 ppm*: 0.06 mg/l). Dyspnea, lacrimation, salivation and nasal discharge observed following exposure (Hine et al, 1956).
Mouse (Webster) [5 or 6/ group]	not determined	8	The LC50 was greater than the highest vapour concentration attained (100 ppm*: 0.06 mg/l). Dyspnoea, lacrimation, salivation and nasal discharge observed following exposure (Hine et al, 1956).
Rat (Sprague-Dawley) [6 males/ group]	not determined	4	Rats were exposed to a vapour aerosol mixture and observed for up to 14 days. Approximate lethal concentration of 323 ppm (approx 2 mg/l) was determined. Loss of body weight and severe irritation of the scrotum were observed in surviving rats (Terrill and Lee, 1977). **In view of the deaths reported at approx 2 mg/l, classification with R20 is proposed (see Annex A).**

* There is some doubt about the value of 100 ppm given as the exposure level in this study. Several authors have subsequently noted that Hine et al (1956) misquoted the vapour pressure of phenyl glycidyl ether and suggest that the saturated vapour level employed was probably about 10 ppm (approx 0.06 mg/l).

4. TOXICOLOGICAL DATA (continued)

4.1.3 Dermal

Species	LD50	Exposure period	Observations and remarks
Rabbit	2990 mg/kg	7 hours	Undiluted test substance applied to the skin of rabbits under rubber sleeves. Rabbits wrapped in toweling to further minimise evaporation (Hine et al, 1956).
Rabbit	1500 microlitres/kg		No further details available. (Smyth et al, 1954; Weil et al, 1963).
Rat	2160 mg/kg		Necrotic changes observed at the site of application (Czajkowska and Stetkiewiez, 1972). **These data do not support the existing classification with R21; no classification is proposed.**

4.1.4 Skin irritation

Species	No. of animals	Exposure time (h/day)	Conc. (w/w)	Dressing: (occlusive, semi-occlusive, open)	Observations and remarks (specify degree and nature of irritation and reversibility)
Rabbit	3	4 hours	100%	semi-occlusive	Annex V method. Mean scores at 24, 48 and 72 h for individual animals were 0.67, 0.67 and 1.0 for erythema, and 0, 0 and 0.33 for oedema. Slight scales were observed in all animals at end of study (day 7). (RCC, 1988a)
Rabbit	not stated	24 hours	100%	not stated: 3 layers of gauze were secured by adhesive tape.	Draize method; limited details of results provided. Irritation scores noted at 24 and 72 h. Mild irritation was observed and given an average score (all readings) of 0.7 (max. possible = 8) (Hine et al, 1956).
Rabbit	5	24 hours	various	not stated	Non-standard method designed for potency ranking of irritant substances. Grade 5 (max. possible =10) in at least 1 of 5 rabbits. (Smyth et al (1954; Weil et al, 1963)
Rabbit	4	24 hours	not stated	not stated; 2 layers of gauze were secured by adhesive tape	Draize method; hyperpigmentation and drying of the skin noted together with necrosis of the dermis and subcutaneous tissue. Necrotic changes persisted for up to 2 months, after which scars remained (Czajkowska and Stetkiewiez, 1972) **Classification with R38 is proposed.**

4. TOXICOLOGICAL DATA (continued)

4.1.5 Eye irritation

Species	No. of animals	Exposure time (hours)	Conc. (w/w)	Observations and remarks (specify degree and nature if irritation, any serious lesions, reversibility)
Rabbit	3	>24 hours	100%	Annex V method. No significant ocular lesions were observed. Mean scores of individual animal readings at 24. 48 and 72 h were 0, 0.67 and 1.0 for corneal opacity, zeros for iris lesions. 0, 0.67 and 1.0 for conjunctival redness, and 0, 0.33 and 0.33 for conjunctival oedema. (RCC, 1988b).
Rabbit	not stated	not stated	100%	Draize method; limited details of results provided. Average score from readings at 1, 24 and 48 h was 8 (max. possible =110), indicating a minimal effect (Hine et al, 1956).
Rabbit	not stated	not stated	various	Non-standard method designed to enable potency ranking of potential eye irritants. Score at 18-24 hours was 2 (max. possible=10) (Smyth et al, 1954; Weil et al, 1963).
Rabbit	4	not stated	100%	Three drops of test substance applied to the conjunctival sac. Signs of irritation (congestion of the conjuncti . ı, lacrimation) were not described fully, although no effects persisted at 4 days post-exposure (Czajkowska and Stetkiewiez, 1972). **No classification is proposed.**

4.1.6 Irritation of respiratory tract

Species	No. of animals	Exposure time (h/days)	Conc. (w/w)	Observations and remarks (specify degree and nature if irritation, any serious lesions, reversibility)
				On the basis of observations in single and repeated exposure (carcinogenicity) studies in rats, **classification with R37 is proposed (see Annex A).**

4. TOXICOLOGICAL DATA (continued)

4.1.7 Skin sensitisation

Species	Type of test	No. of animals	Incidence of reactions observed
Guinea pig	M&K	20	Induction: 3% intradermally and 5% topically. Challenge: 3% topically. One animal died on day 6 and one on day 11; these deaths not substance-related. On challenge, 13/18 animals responded with a positive result (RCC, 1988c).
Guinea pig	non standard	10	Topical induction: 5% solution; non-irritating; 6 days/week; 34 days. All 10 guinea pigs responded to topical challenge with 1% phenyl glycidyl ether (Rudzki and Krajewski, 1979).
Guinea pig	non standard	18	Animals received 8 intradermal injections of diluted phenyl glycidyl ether (conc. not specified) 3 times/week for 3 weeks. Challenge at 24 and 48 hours after the last dose indicated sensitisation in 1/18 animals. From the very limited reporting of this study, no definite conclusions can be reached (Weil et al, 1963).
Guinea pig	non standard	not stated	Topical induction with phenyl glycidyl ether (conc. not stated) daily for 7 days (erythema observed on day 7). On weeks 5, 9 and 12 of the study, single applications of phenyl glycidyl ether to previously untreated skin produced oedema and erythma within 4 days. Although this study was non-standard and poorly reported, the results appear to indicate a sensitisation response (Zschunke and Behrbohm, 1965). **These data and the observations of skin sensitisation in exposed humans, justify retention of the existing classification with R43.**

4. TOXICOLOGICAL DATA (continued)

4.2 REPEATED OR PROLONGED TOXICITY GROUPED ACCORDING TO SUBACUTE AND SUBCHRONIC TOXICITY

4.2.1 Oral

Species/strain	Dose	Duration of treatment	Observations and remarks (specify group size, NOAEL, effects of major toxicological significance)
			No data available.

4.2.2 Inhalation

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/ dose group]	0 & 100* ppm (0.06 mg/l)	7 hours/ day	50 days	Minimal signs of eye irritation and respiratory distress in exposed animals. No other significant gross or microscopic findings (Hine et al, 1956).
Rat (Sprague-Dawley) [6 males and 6 females/ group]	0 & 29 ppm (0.18 mg/l)	4 hours/ day	14 days	At 29 ppm , rats exposed to a vapour-aerosol mixture. Signs of toxicity included decreased weight gain, "atrophic changes" in kidney, liver, spleen, thymus and testes, and chronic catarrhal tracheitis. The nature and extent of these changes was not further described (Terrill and Lee, 1977).
Rat (Sprague-Dawley) [6 males and 6 females/ group/ time point]	0, 1, 5 & 12 ppm (0.005, 0.03 & 0.07 mg/l)	6 hours/ day	30, 60 or 90 days (maximum of 63 exposures)	At 5 ppm and 12 ppm, mild patchy hair loss predominantly on the head, neck and shoulder region of the animals with perifollicular inflammation, keratotic vesicles and disturbances of keratinization. These effects were more common in female animals. No other clinical signs of toxicity. No changes in biochemical, blood or urinary parameters. No gross or microscopic signs of toxicity in the tissues. (Note: No histological examination of nasal tissue). (Terrill and Lee, 1977; Lee et al, 1977).
Dog (Beagle) [6 males/ dose group]	0, 1, 5 & 12 ppm	6 hours/ day	90 days	No clinical signs of toxicity were observed. No significant haematological, biochemical, gross tissue or microscopic findings (Terrill and Lee, 1977; Lee et al, 1977). **No classification is proposed (see Annex A).**

* There is some doubt about the value of 100 ppm given as the exposure level in this study. Several authors have subsequently noted that Hine et al (1956) misquoted the vapour pressure of phenyl glycidyl ether and suggest that the saturated vapour level employed was probably about 10 ppm (0.06 mg/l).

4.2.3 Dermal

				No data available.	

4. TOXICOLOGICAL DATA (continued)

4.3 CARCINOGENICITY (INCLUDING CHRONIC TOXICITY STUDIES)

4.3.1 Oral

			No data available.

4.3.2 Inhalation

Species	conc. mg/l	Exposure time	Observations and remarks (specify group size, effects of major toxicological significance)
Sprague-Dawley rats (100 males & 100 females per group)	0, 1 & 12 ppm (0, 0.006, 0.07 mg/l)	6 h/ day 5 days/wk 24 months	Carcinomas of the nasal epithelium observed in 1/89 male and 0/87 female controls, 0/83 male and 0/88 female low dose and in 9/85 male and 4/89 female high dose rats. The findings at the high dose were statistically significant. At the high dose only, squamous cell metaplasia, rhinitis, epithelial desquamation, regeneration, hyperplasia and dysplasia of the respiratory epithelium were observed, especially in the anterior regions of the nasal cavity (Lee et al, 1983). The carcinogenic response observed in rats supports the proposal to classify with Carc Cat 2 R45. **Arguments for classification are provided in Annex A.**

4.3.3 Dermal

			No data available.

4.3.4 In vitro transformation assays

Test system	Exposure levels	Observations	Remarks
SA7 virus transformation of primary hamster embryo cells Transformation of secondary hamster embryo cells.	0, 1.6, 8 and 40 micrograms/ml (toxicity at higher levels) 0, 6.25 and 12.5 micrograms/ml (toxicity at higher levels)	Dose-dependent enhancement of viral transformation Dose-dependent increase in transformation index.	It is unclear how these non-validated studies performed by Greene et al (1979) should be evaluated. Without validation these results do not contribute to assessment of the carcinogencity of phenyl glycidyl ether.

4. TOXICOLOGICAL DATA (continued)

4.4 GENOTOXICITY

4.4.1 In vitro studies

Test	Cell type	Conc. range	Observations and remarks
Reverse mutation (Ames)	*S.typhimurium* TA97, TA98, TA100, TA1535	3-1000 micrograms/ plate	**Positive.** Reproducible, dose-related increases in revertants for TA97, TA100 and TA1535 observed without metabolic activation and in the presence of Aroclor-induced hamster or rat liver S9 (Canter et al, 1986).
	S.typhimurium TA100, TA1535,	5-100 micrograms/ plate	**Positive.** Reproducible, dose-related increases in both tester strains in absence of metabolic activation (Neau et al, 1982).
	S.typhimurium TA100	50-1000 micrograms/ plate	**Positive.** Dose-related increase in revertants in absence of metabolic activation (Seiler, 1984).
	S.typhimurium TA98, TA100, TA1535, TA1537, TA1538	0.5-500 micrograms/ plate	**Positive.** Dose-related increases in revertants for TA100 and TA1535 with and without rat liver S9 (Greene et al, 1979).
Cytogenetics-chromosome aberrations	CHO cells	0, 8 & 25 micrograms/ ml	**Positive.** Two experiments without metabolic activation were performed, with exposure/fixation times 6h and 24 h. A slight dose-related increase in aberration frequency at the shorter fixation time was observed (mean values of 1, 5 & 8%). In the other experiment, the mean aberration frequency was 2% in control and exposed cultures. No toxicity reported at top dose. Study included resorcinol diglycidyl ether as a positive control (aberration frequencies > 40%). (Seiler, 1984).
Mammalian cell gene mutations	CHO cells (*hprt* locus)	0, 20, 30, 40 & 50 micrograms/ ml	**Negative.** In the absence of serum (5-6 h exposure), assays with and without Aroclor-induced rat liver S9 showed no increases in mutant fraction. No toxicity reported at top dose. EMS gave a clear positive response. A comparable assay in the presence of serum (18-24 hours exposure) without activation also gave a negative result (Greene et al, 1979).
UDS	Rat hepatocytes	0.45, 4.5, 45 micrograms/ ml	**Negative.** Reproducible result. Exposure time was 20 h. Cytotoxicity prevented assessment of UDS at the top dose. Positive control, 2AAF, gave a clear increase in UDS (Von der Hude et al, 1990).

4. TOXICOLOGICAL DATA (continued)

4.4.2 In vivo studies (somatic cells)

Test	Species (tissue)	Dose groups	Harvest time	Observations and remarks (include route of administration)
Micronucleus	ICR-mice	0, 400, 500, 800 & 1000 mg/kg gavage.	24 h (all doses), 48 & 72 h (top dose only)	**Negative.** Male and female rats employed. Deaths occurred at the top dose (1 animal/group). No increases in MN frequency observed. Another substance, diglycidylaniline, gave a positive result in the same study (Seiler, 1984). Note that the oral route of administration was employed.
Chromosome aberration	Sprague-Dawley rat (bone marrow)	0, 1, 5 & 12 ppm inhalation (0, 0.006, 0.03 & 0.07 mg/l)	21 h after last exposure	**Negative.** Groups of 6 male rats exposed by inhalation 6 h/day for 19 days. No positive control group. No deaths and no effects on body weight observed. Fifty or more metaphases scored per animal. No significant increases in breaks or rearrangements above relatively high control level of 7.9% of cells with aberrations (Terrill et al, 1982). Note that the dose levels used were low, compared with those that can be administered via orla or parenteral routes. **Despite the negative results ob..ined in these particular assays, classification with Muta Cat 3; R40 is proposed (see Annex A).**

4.4.3 In vivo studies (germ cells)

Test	Species	Dose groups	Exposure conditions	Observations and remarks
Dominant lethal	Sprague -Dawley rats (8 males and 8 females per group)	0, 2, 6 & 11 ppm	Inhalation exposure of males 6 h/ day for 19 days.	**Negative.** Not performed to current standards. No clinical signs of toxicity in exposed males; no positive control group. Mating in each of 6 consecutive weeks following exposure. No significant effect on the number of litters with early resorptions or on mean postimplantation loss, as determined at gestation day 18 (Terrill et al, 1982).

4. TOXICOLOGICAL DATA (continued)

4.4.4 Other mutagenicity/ genotoxicity studies

Test	Species	Dose groups	Exposure conditions	Observations and remarks
Host-mediated	C57B1/6 x C3H mice	0, 2500 mg/kg (all animals also received S.typhimurium TA1535 by ip. injection).	Oral gavage, intramuscular or ip. injection. Exposure time 3h	A mutagenic response seen in bacteria from 2/5 and 1/5 mice that received phenyl glycidyl ether by oral and intramuscular routes, respectively. No criteria for interpretation of the results were provided and no positive controls were included in this experiment (Greene et al, 1979).
Inhibition of murine testicular DNA synthesis	C57B1/6 x C3H mice	0 or 500 mg/kg	Oral gavage. Exposure time 3.5 hours	5 mice per dose group. No effect on rate of testicular DNA synthesis in this limited investigation (Greene et al, 1979).

4.5 FERTILITY

Species	Dose groups	Exposure conditions	Number of gen. exposed	Obsevations and remarks
Rat (Sprague-Dawley)	0, 2, 6 & 11 ppm	Inhalation exposure of males 6 h/ day for 19 days.	1 F_0 males only	A small, non-standard reproductive toxicity test. No clinical signs of toxicity in exposed males (8/ group). Each F_0 male mated in each of 6 consecutive weeks following exposure with groups of 3 females (included 1 female for dominant lethal test). For each mating group, 8 F_1 males mated with 24 F_1 females. The percentage of females that became pregnant was significantly reduced in the high dose group in the first breeding week only. No other significant changes in fertility, progeny numbers and survival, or lactational performance. (Terrill et al, 1982). The isolated observation of decreased pregnancy in the high dose group occurred only on the first mating and is believed to have occurred by chance. The functional capacity of all the male rats was demonstrated by subsequent matings. **No classification is proposed.**

4.6 DEVELOPMENTAL TOXICITY

Species	Route	Dose groups	Exposure conditions	Observations and remarks
Rat (Sprague-Dawley)	Inhln.	0, 1, 5 & 12 ppm	25 rats/ group, 6 h/ day gestation days 4-15	**Negative.** Teratogenicity study. No clinical signs of toxicity in dams. Numbers of implantations, live fetuses and resorptions similar in all groups. No effects on fetal length or weight, gross observations or internal and skeletal development. Exposure not continued throughout entire period of organogenesis (Terrill et al, 1982). **No classification is proposed.**

5. ECOTOXICOLOGICAL STUDIES

Data not reviewed for Health Effects Working Group

6. ENVIRONMENTAL FATE

Data not reviewed for Health Effects Working Group

7. ADDITIONAL ENVIRONMENTAL EFFECTS

Data not reviewed for Health Effects Working Group

8. REFERENCES

Angelini G, Rigano L, Foti C, Grandolfo M, Vena GA, Bonamonte D, Soleo L and Scorpiniti AA (1996). Occupational sensitisation to epoxy resin and reactive diluents in marble workers. Contact Dermatitis, 35, 11-16.

Canter DA, Zeiger E, Haworth S, Lawlor T, Mortelmans K and Speck W (1986). Comparative mutagenicity of aliphatic epoxides in *Salmonella*. Mutation res., 172, 105-138.

Czajkowska T and Stetkiewicz J (1972). Evaluation of the acute toxicity of phenyl glycidyl ether with particular regard to percutaneous absorption. Medycyna Pracy, 23, 361-371.

Greene EJ, Friedman MA, Sherrod JA and Salerno AJ (1979). In vitro mutagenicity and cell transformation screening of phenyl glycidyl ether. Mutation Res, 67, 9-19.

Hine CH, Kodama JK, Wellington JS, Dunlap MK and Anderson HH (1956). The toxicology of glycidol and some allyl glycidyl ethers. AMA Arch Ind Health, 14, 250-264.

IARC (1989). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 47, 237-261.

Lee KP, Schneider PW and Trochimowicz HJ (1983). Morphologic expression of glandular differentiation in the epidermoid nasal carcinomas induced by phenyl glycidyl ether inhalation. Am J Pathol, 3, 140-148.

Lee KP, Terrill JB and Henry NW (1977). Alopecia induced by inhalation exposure to phenyl glycidyl ether. J Toxicol Environ Health, 3, 859-869.

MAK (1992). Occupational Toxicants; Critical Data Evaluation for MAK Values and Classification of Carcinogens, 4, 305-311.

Nakagawa K, Kawai K and Kawai K (1991). Three cases of allergic contact dermatitis from phenyl glycidyl ether. Skin Res. 33 (supp 10), 88-95.

Neau SH, Hooberman BH, Frantz SW and Sinsheimer JE (1982). Substituent effects on the mutagenicity of phenyl glycidyl ethers in *Salmonella typhimurium*. Mutation Res, 93, 297-304.

RCC: Research & Consulting Company AG (1988a). Primary skin irritation study with phenyl glycidyl ether in rabbits. RCC Project 214503.

RCC: Research & Consulting Company AG (1988b). Primary eye irritation study with phenyl glycidyl ether in rabbits. RCC Project 214492.

RCC: Research & Consulting Company AG (1988c). Contact hypersensitivity to phenyl glycidyl ether in albino guinea pigs, maximisation test. RCC Project 214525.

Rudzki E and Krajewska D (1979). Contact sensitivity to phenyl glycidyl ether. Dermatosen, 27, 42-44

Seiler JP (1984). The mutagenicity of mono- and di-functional aromatic glycidyl compounds. Mutation Res, 135, 159-167.

Smyth HF, Carpenter CP, Weil CS and Pozzani UC (1954). Range-finding toxicity data list V. Arch Ind Hyg Occup Med, 10, 61-68.

Terrill JB and Lee KP (1977). The inhalation toxicity of phenyl glycidyl ether. 90-Day inhalation study. Toxicol Appl Pharmacol, 42, 263-269.

Terrill JB, Lee KP, Culik R and Kennedy GL Jr (1982). The inhalation toxicity of phenylglycidyl ether: reproduction, mutagenic, teratogenic and cytogenic studies. Toxicol Appl Pharmacol, 64, 204-212.

Von der Hude W, Mateblowski R and Basler A (1990). Induction of DNA-repair synthesis in primary rat hepatocytes by epoxides. Mutation Res, 245, 145-150.

Weil CS, Condra N, Haun C and Striegel JA (1963). Experimental carcinogenicity and acute toxicity of representative epoxides. Amer Ind Hyg Ass J, 24, 305-325.

Zschunke E and Behrbohm P (1965). Eczema through phenoxypropylene oxide and similar glycidyl ethers. Dermatologische Wochenschrift, 19, 480-484.

ANNEX A

EC CLASSIFICATION AND LABELLING:

PHENYL GLYCIDYL ETHER

(EINECS name: 2,3-epoxypropyl phenyl ether)

Background information on metabolism of phenyl glycidyl ether

Glycidyl ethers, in common with other epoxides, may be metabolised through the following pathways:

(i) epoxide hydrolase activity, leading to hydrolysis of the epoxide group under formation of the corresponding diol;
(ii) conversion to glutathione conjugates by glutathione-S-epoxide conjugases; and
(iii) non-enzymic covalent binding with proteins, RNA and DNA.

The observation of a urinary cysteine conjugate, and the depletion of hepatic glutathione levels in animals exposed to phenyl glycidyl ether via the oral route, confirm that this substance can be metabolised by conjugation with glutathione (MAK, 1992). It is unclear how phenyl glycidyl ether is metabolised in humans. The potential for metabolic detoxication at sites of initial contact in the body has not been established.

MAK (1992). Occupational Toxicants; Critical Data Evaluation for MAK Values and Classification of Carcinogens, 4, 305-311.

Arguments for classification: Physico-chemical effects No classification is proposed.

Arguments for classification: Health effects

1) *Acute oral toxicity*

Acute toxicity studies in rats have consistently given LD_{50} values >2000 mg/kg. A mouse LD_{50} of 1400 mg/kg is available, however we propose that this isolated value does not support any classification given that the rat is the species specified in the criteria for classification.

Acute inhalation toxicity

In rats, deaths have been reported at exposure levels of approx. 2mg/l phenyl glycidyl ether (vapour-aerosol mixture). This supports the proposal to classify with Xn; R20.

Acute dermal toxicity

LD_{50} values of 2160 and 2990 mg/kg in rabbits indicate that no classification is justified. A poorly reported study gives a rabbit LD_{50} of 1500 mg/l; this is insufficient evidence to justify classification.

2) *Skin Irritation*

In the most recent study available, the only one to conform to Annex V, mean erythema and oedema values less that 2.00 were recorded in all animals. Since slight scales were observed in all animals on day 7 at study termination, the complete reversibility of effects was not established. Two relatively old studies also describe signs of only moderate and minimal/mild irritation in rabbits. In contrast to these findings in animals, there is a report of human exposure to undiluted phenyl glycidyl ether leading to second degree burns (1 individual) or erythema and blisters (5 individuals). There is also a Draize-type study describing necrotic lesions and persistent scaring in rabbit skin.

Given that there is only an isolated report of possible corrosive activity in humans, and the contrasting observations in animals (the majority of studies reporting minimal-moderate skin irritation), we propose that the most appropriate classification is Xi; R38.

3) *Eye Irritation*
In a study conforming to Annex V, there were no significant ocular lesions observed. In addition, in three non-standard studies, extrapolation of the Draize scores suggests that the responses seen were indicative of only minimal eye irritation. These findings suggest that classification is not justified.

4) *Respiratory Irritation*
In single exposure studies, exposure of rats to phenyl glycidyl ether caused dyspnea and nasal discharge. On longer term exposure, rats exhibited rhinitis and histopathological signs of respiratory irritation. On this basis, we propose classification with R37.

5) *Skin Sensitisation*
Observations of allergic responses in exposed humans and in animal tests justify the existing classification of phenyl glycidyl ether with R43.

6) *Respiratory Sensitisation*
No animal or human data available. Consequently, classification is not justified.

7) Repeated dose toxicity
No modern studies performed according to regulatory guidelines are available.

In a study from 1977, rats exposed for 14 days to a vapour-aerosol mixture of 0.18 mg/l (29 ppm) phenyl glycidyl ether for 4 hours/day were reported to show "atrophic changes" in the kidneys, liver, spleen, thymus and testes. Given that the nature and extent of these changes were not further characterised, and that similar findings were not observed in rats exposed at this level for longer periods, this study does not provide sufficient evidence to justify classification.

In a carcinogenicity study, repeated inhalation exposure of rats to 0.07 mg/l produced rhinitis and histopathological signs of upper respiratory tract irritation. We believe that these findings support classification with R37 rather than R48.

Hair loss and associated observations in rats exposed to 0.03 and 0.08 mg/l phenyl glycidyl ether vapour-aerosol mixtures would appear to indicate a local irritant effect and the significance of these findings to humans is unclear. These data do not support classification with R48.
Overall, no classification for repeated dose effects is justified.

5) *Mutagenicity*

Phenyl glycidyl ether consistently gave positive results in bacterial mutagenicity assays both with and without metabolic activation. A weak positive result was also seen in a chromosome aberration test in CHO cells without activation. Negative results have been reported in a CHO *hprt* gene mutation test and a rat liver cell UDS test, although the sensitivity of the gene mutation test might have been increased had higher concentrations of phenyl glycidyl ether been included. Nevertheless, it is concluded that phenyl glycidyl ether is an electrophilic substance with the potential to act as a direct-acting mutagen *in vitro*.

In standard *in vivo* tests (micronucleus, chromosome aberration and dominant lethal), phenyl glycidyl ether administered by either inhalation or oral gavage gave negative results. However given the electrophilicity of this glycidyl ether, and the relatively low doses administered via inhalation, it is most likely that the target tissues (bone marrow, testes) were not adequately exposed in these studies and that the results are false negatives. This concept is supported by the finding that *n*-butyl glycidyl ether, a structurally similar electrophilic substance, gave a positive result in the bone marrow micronucleus test following intraperitoneal injection but not following oral administration (Gardiner et al, 1992).

Concern remains that phenyl glycidyl ether may cause mutagenic effects in somatic cells at sites of initial contact in the body. Classification with Muta Cat 3; R40 is proposed since this substance is a reactive epoxide (an electrophile), mutagenic *in vitro*, and has not been tested in a relevant *in vivo* test (i.e. at a site of initial contact), .

Gardiner TH, Waechter JM Jr, Wiedow MA and Solomon WT (1992). Glycidyloxy compounds used in epoxy resin systems: a toxicology review. Regul Toxicol Pharmacol, 15, S1-S77.

6) *Carcinogenicity*

In the only available carcinogenicity study, a clear increased incidence of nasal epithelial carcinomas in male and female rats was associated with inhalation exposure to phenyl glycidyl ether (0.07 mg/l). No tumours were seen in control animals or in the lower exposure group (0.006 mg/l) in this study. Furthermore, historically, this type of tumour is rare in control rats.

There is some uncertainty about the mechanism of tumour induction in the phenyl glycidyl ether-exposed rats. Both of the genotoxic and irritant properties of this substance may have played a role. From the available information, the exposure level at which tumours were induced appears to have been below a maximum tolerated dose level (MTD) and there is no evidence for a secondary mechanism of action with a practical threshold. However, the findings are considered of relevance to humans.

In view of these considerations, classification with Carc Cat 2; R45 is proposed for phenyl glycidyl ether.

7) *Reproductive toxicology*

There were no toxicologically significant findings in a limited 2-generation reproductive toxicity test in which only F_0 male rats were exposed to phenyl glycidyl ether. No developmental effects were seen in a teratology study in rats. Phenyl glycidyl has not been demonstrated to pose a reproductive toxicity hazard; no classification is proposed for this endpoint.

UK proposal: Carc Cat 2; R45: Muta Cat 3; R40: Xn; R20: Xi; R37/38: R43

HSE Toxicology Unit
29 July 1997