Committee for Risk Assessment

RAC

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

Adopted

30 November 2018

30 November 2018

CLH-O-0000001412-86-250/F

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

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

Chemical name: ***m*-bis(2,3-epoxypropoxy)benzene; resorcinol diglycidyl ether**

EC Number: **202-987-5**

CAS Number: **101-90-6**

The proposal was submitted by the **Netherlands** and received by RAC on **26 February 2018.**

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

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **12 March 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **11 May 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Ruth Moeller**

Co-Rapporteur, appointed by RAC: **Peter Hammer Sørensen**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **30 November 2018** by **consensus**.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	603-065-00-9	*m*-bis(2,3-epoxypropoxy)benzene; resorcinol diglycidyl ether	202-987-5	101-90-6	Carc. 2 Muta. 2 Acute Tox. 4* Acute Tox. 4* Skin Irrit. 2 Eye Irrit. 2 Skin Sens. 1 Aquatic Chronic 3	H351 H341 H312 H302 H315 H319 H317 H412	GHS07 GHS08 Wng	H351 H341 H312 H302 H315 H319 H317 H412			
Dossier submitters proposal	603-065-00-9	*m*-bis(2,3-epoxypropoxy)benzene; resorcinol diglycidyl ether	202-987-5	101-90-6	**Modify** Carc. 1B Acute Tox. 3 Acute Tox. 4	**Modify** H350 H311 H302	**Add** GHS06 Dgr **Remove** GHS07 Wng	**Modify** H350 H311 H302		**Add** Oral: ATE = 980 mg/kg bw Dermal: ATE = 744 mg/kg bw	
RAC opinion	603-065-00-9	*m*-bis(2,3-epoxypropoxy)benzene; resorcinol diglycidyl ether	202-987-5	101-90-6	Muta. 2 Skin Irrit. 2 Eye Irrit. 2 Skin Sens. 1 Aquatic Chronic 3 **Modify** Carc. 1B Acute Tox. 3 Acute Tox. 4	H341 H315 H31 H317 H412 **Modify** H350 H311 H302	**Add** GHS06 Dgr **Remove** GHS07 Wng	H341 H315 H31 H317 H412 **Modify** H350 H311 H302		**Add** Oral: ATE = 500 mg/kg bw Dermal: ATE = 300 mg/kg bw	
Resulting Annex VI entry if agreed by COM	603-065-00-9	*m*-bis(2,3-epoxypropoxy)benzene; resorcinol diglycidyl ether	202-987-5	101-90-6	Carc. 1B Muta. 2 Acute Tox. 3 Acute Tox. 4 Skin Irrit. 2 Eye Irrit. 2 Skin Sens. 1 Aquatic Chronic 3	H350 H341 H311 H302 H315 H319 H317 H412	GHS06 GHS08 Dgr	H350 H341 H311 H302 H315 H319 H317 H412		Oral: ATE = 500 mg/kg bw Dermal: ATE = 300 mg/kg bw	

GROUNDS FOR ADOPTION OF THE OPINION

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).

HUMAN HEALTH HAZARD EVALUATION

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**.

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	0/36 (0%)	0/39 (0%)	20/35 (57%)	15/16 (94%)	1/1 (100%)
Life table test	p<0.001		p<0.001	p<0.001	p<0.001
Cochran-Armitage trend test	p=0.300				
Fisher exact			p<0.001	p=0.001	p=0.125
Incidental tumour test			p<0.001		
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 Adjusted incidence	0/47 0%	12/49 (24%) P<0.001 53.3%	23/49 (47%) 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.

Animal data – dermal route

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

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

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 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.

Animal data – inhalation route

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[1]).

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

[1] 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/

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 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).

In the 14-day studies, 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 14-day 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) Genotoxicity: 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 essential 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) Irritation: 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 observe. 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) Dose and route extrapolation: 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) Organ-specificity and tissue-concordance: 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[1]. 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 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,

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1 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.

- 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.

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

ANNEXES:

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

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