Committee for Risk Assessment

RAC

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

glyoxylic acid ...%

EC Number: 206-058-5
CAS Number: 298-12-4

CLH-O-0000001412-86-204/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

8 June 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:

Glyoxylic acid … %

EC Number: **206-058-5**

CAS Number: **298-12-4**

Index Number: **-**

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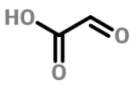
CONTENTS

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)	Glyoxylic acid
Other names (usual name, trade name, abbreviation)	Glyoxylic acid
EC number (if available and appropriate)	206-058-5
EC name (if available and appropriate)	Glyoxylic acid
CAS number (if available)	298-12-4
Molecular formula	$C_2H_2O_3$
Structural formula	
SMILES notation (if available)	C(=O)C(=O)O
Molecular weight or molecular weight range	74.036 g/mol
Degree of purity (%) (if relevant for the entry in Annex VI)	

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)
Glyoxylic acid (EC No. 206-058-5)			Skin Sens. 1; H317 Eye Dam. 1; H318 Met. Corr. 1; H290 Skin Corr. 1B; H314

Please refer to IUCLID file for further information.

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
none				

Please refer to IUCLID file for further 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
none					

Table 5: Test substances (non-confidential information) (this table is optional)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
Glyoxylic acid (CAS: 298-12-4)	**50 %**			Guillot (1984a)
Glyoxylic acid (CAS: 298-12-4)	**50 %**			Guillot (1984b)
Glyoxylic acid (CAS: 298-12-4)	**50 %**			Anderson et al. (2008)
Glyoxylic acid (CAS: 298-12-4)	**50 %**			Hoechst (1975)

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	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	none	-	-	-	-	-	-	-	-	-	-
Dossier submitters proposal	tbd	Glyoxylic acid … %	206-058-5	298-12-4	**Add:** Eye Dam. 1 Skin Sens. 1	**Add:** H318 H317	**Add:** GHS05 GHS07 Dgr	**Add:** H318 H317			**Add:** B
Resulting Annex VI entry if agreed by RAC and COM	tbd	Glyoxylic acid … %	206-058-5	298-12-4	Eye Dam. 1 Skin Sens. 1	H318 H317	GHS05 GHS07 Dgr	H318 H317			B

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	hazard class not assessed in this dossier	No
Acute toxicity via dermal route	hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	hazard class not assessed in this dossier	No
Skin corrosion/irritation		**Yes**
Serious eye damage/eye irritation		**Yes**
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation		**Yes**
Germ cell mutagenicity	hazard class not assessed in this dossier	No
Carcinogenicity	hazard class not assessed in this dossier	No
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

Glyoxylic acid is neither listed in the Annex VI of the CLP Regulation (EC) No 1272/2008 of the European Parliament and of the Council (latest consolidated version: 01.04.2016), nor has a proposal for a Harmonised Classification and Labelling in Annex VI of the CLP been submitted for this substance. Glyoxylic acid has been registered under REACH.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Reason for a need for action at Community level:

Disagreement by DS with current self-classification

Further detail on need of action at Community level

The lead registrant has classified glyoxylic acid as Eye Dam. 1 (H318) and as Skin Sens. 1 (H317). Correspondingly, as described in detail in paragraph 10, there are reliable studies available for the endpoints eye irritation/corrosion and skin sensitisation, which justify the classification of glyoxylic acid as seriously eye damaging (Eye Dam. 1 H318, Causes serious eye damage) and as a moderate skin sensitiser (Skin Sens. 1B H317, May cause an allergic skin reaction). However, while the majority of C&L notifiers classified this substance consistently, the self-classification of a substantial number of C&L notifiers is different, which justifies a proposal for harmonised classification.

Glyoxylic acid is manufactured and/or imported in the European Economic Area in 1 000 - 10 000 tons per year. It is a corrosion inhibitor, pH regulator and anti-scaling agent, which is largely used for the industrial manufacturing of cleaning products, but also furnishing products. In this context, glyoxylic acid is used as intermediate for manufacturing other substances, including bulk, large scale and fine chemicals, respectively, but also to produce and manufacture leather tanning, dye or impregnation products, as well as (fabricated) metal products. Moreover, further uses of this substance have been identified in European countries, namely its frequent use in cosmetic products as anti-static, buffering agent and for hair waving or straightening at concentrations up to 12 % (Kemper 2000; Anderson et al. 2008; Boga et al. 2014; CosIng, 2016). Thus, there is also a high potential for exposure of consumers of such cosmetic products. Exposure of glyoxylic acid can occur through inhalation and skin and eye contact e.g. while performing do-it-yourself hair applications at home or as clients of hairdressers. Moreover, exposure of professional hairdressers can be expected, as they are frequently handling products containing glyoxylic acid.

RAC general comment
Glyoxylic acid has no existing Annex VI entry to CLP. The proposal from the Dossier Submitter (DS) only addressed the endpoints eye irritation/damage, skin corrosion/irritation and skin sensitisation. The substance glyoxylic acid is supplied in the form of an aqueous solution at 50 % (v/v) according to the REACH registration dossier. All studies reported by the DS in the CLH dossier were performed on the 50 % glyoxylic acid solution. The DS also proposed to include a Note B as glyoxylic acid may be placed on the market in aqueous solutions at various concentrations and, therefore, these solutions require different classification and labelling since the hazard could vary at different concentrations. RAC supports the inclusion of a Note B as different classifications may be warranted due to the dilution rather than the intrinsic property of the substance.

RAC notes that glyoxylic acid monohydrate (CAS 563-96-2; EC 679-230-4) and its salts exist in a higher concentration than 50 %. Glyoxylic acid monohydrate is self-classified for more severe hazardous properties than glyoxylic acid i.e. as Skin Sens. 1, Eye Dam. 1, Skin Corr. 1B, Resp. Sens. 1, Met. Corr. 1. Therefore, higher concentrations of glyoxylic acid (> 50 % v/v) may lead to more severe effects (e.g. skin irritation/corrosion) but RAC had no data with which to define any cut-off value for a higher sub-classification.

5 DATA SOURCES

A literature enquiry was performed and data were obtained from the registration dossiers.

6 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Glyoxylic acid 50 % aqueous solution: Colourless to yellowish viscous liquid with pungent odour.	experimental result
	Glyoxylic acid (anhydrous): Rhombic prisms obtained from water with 1/2 mol of water of crystallization.	handbook data [CRC Handbook of Chemistry and Physics]
Melting/freezing point	Glyoxylic acid 50 % aqueous solution: solidification at -93 °C (cooling) and softening at -98 °C (heating) during cooling/ warming cycle DSC.	experimental result [OECD Guideline 102 (Melting point / Melting Range); Differential Scanning Calorimetry]
Boiling point	Glyoxylic acid 50 % aqueous solution: 111 °C at 1013 hPa. (Decomposition between 150 and 250 °C.)	experimental result [OECD Guideline 103 (Boiling point/boiling range)]
Relative density	Glyoxylic acid 50 % aqueous solution: 1.34 at 19.5 °C	experimental result [internal method]
Vapour pressure	Glyoxylic acid 50 % aqueous solution: 1.34 at 19.5 °C	experimental result [OECD Guideline 104 (Vapour Pressure Curve); dynamic method]
Surface tension	Glyoxylic acid 50 % aqueous solution: 56.3 ± 0.5 mN/m at 25 °C.	experimental result [OECD Guideline 115 (Surface Tension of Aqueous Solutions)]
Water solubility	Glyoxylic acid 50 % aqueous solution: Calculated as 1000 g/L.	calculated [using WATERNT program (2008) from Epiweb 4.0]
	Glyoxylic acid is very soluble in water.	handbook data [CRC Handbook of Chemistry and Physics]

Property	Value	Comment (e.g. measured or estimated)
	Glyoxylic acid is very soluble in water.	handbook data [The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals.]
Partition coefficient n-octanol/water	Glyoxylic acid 50 % aqueous solution: The log Kow could not be determined experimentally because glyoxylic acid 50 % reacted with n-octanol during the test forming a hemiacteal. calculated: log Kow = -0.7 (25 °C, pH: ca. 0.3)	calculated [using PC KOW WIN 1.67a (2008)]
	Glyoxylic acid (anhydrous): calculated: log Kow = -1.4 (25 °C, pH: ca. 0.3)	
Granulometry	n.a.	The substance is marketed/used as a 50 % (w/w) aqueous solution.
Stability in organic solvents and identity of relevant degradation products	Glyoxylic Acid 50 % forms a hemiacetal with n-octanol.	experimental result
Dissociation constant	Glyoxylic acid 50 % aqueous solution: Acid-base constant = 4.7.	handbook data [Ullmann's Encyclopedia of Industrial Chemistry]
	Glyoxylic acid 50 % aqueous solution: Acid-base constant = 4.6.	handbook data [The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals.]
	Glyoxylic acid: pK_a = 3.12.	calculated [using SPARC Performance Automated Reasoning in Chemistry (Release w4.5.1529-s4.5.1529; September 2009)]
Viscosity	Glyoxylic acid 50 % aqueous solution: Ca. 8.7 mPa s (dynamic) at 25°C.	experimental result [Ubbelhode tube]

7 EVALUATION OF HEALTH HAZARDS

7.1 Skin corrosion/irritation

Table 9: Summary table of animal studies on serious eye damage/eye 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
OECD TG 404 (Acute Dermal Irritation/Corrosion) GLP compliant. (study considered reliable without restrictions) Deviations from guideline: Observation period shorter (72 h versus 14d), but effects were within 48 h.	Rabbit, New Zealand white Sex: not specified; 6 animals	Glyoxylic acid (50 %) (CAS: 298-12-4)	0.5 mL non-diluted test substance/skin area (2.5 cm^2) Semi-occlusive patching Exposure duration: 4 h Washing of test substance not specified Observations: 1, 24, 48, and 72 h No controls.	Erythema score (mean of 24/48/72h) for all 6 animals (max. score: 4.0): 0; 0; 0; 0; 0; 0.33 Effects fully reversible within 48 h. Oedema sore (mean of 24/48/72h) of all 6 animals (max. score: 4.0): 0; 0; 0; 0; 0; 0 One individual showed slight erythema (barely perceptible and fully reversible within 48 h), but no other skin irritating effects were observed during the whole study period.	Guillot (1984 a)

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

There is one *in vivo* skin irritation/corrosion study available for glyoxylic acid in animals (study report 1984, unpublished). The study is performed according to OECD TG 404 and GLP and is considered relevant and reliable without restrictions. 0.5 mL of a 50 % solution of glyoxylic acid was applied to a small area (2.5 cm^2) of skin and covered with a gauze patch, which was held in place with non-irritating tape (semi-occlusive). Exposure duration was 4 h. No information is given on whether the test substance was washed after the exposure period. Examinations were carried out 24, 48 and 72 h after exposure. In one individual a very slight erythema (erythema score: 0.33) was observed, but this effect was fully reversible within 48 h. Glyoxylic acid (50 %) caused no further skin irritating effects (erythema/oedema) at any of the observation time points. Because observed effects were fully reversible within 48 h, the shortened observation period (72 h) compared to OECD TG 404 (14 d) is reasonable. Thus, this deviation from the guideline is considered not to interfere with the reliability of the study.

7.1.2 Comparison with the CLP criteria

According to the CLP Regulation (Section 3.2.1.1), skin corrosion means the production of irreversible damage to the skin, following the application of a test substance for up to 4 hours. Skin irritation means the production of reversible damage to the skin following the application of a test substance for up to 4 hours.

On the basis of the results of animal testing a substance is classified as corrosive (Category 1), as shown in Table 3.2.1 of the CLP Regulation, if it produces destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, in at least 1 tested animal after exposure up to a 4 hour duration. Three subcategories are provided within the corrosive category: subcategory 1A, where responses are noted following up to 3 minutes exposure and up to 1 hour observation; subcategory 1B, where responses are described following exposure between 3 minutes and 1 hour and observations up to 14 days; and subcategory 1C, where responses occur after exposures between 1 hour and 4 hours and observations up to 14 days (Section 3.2.2.6.2., CLP Regulation).

On the basis of the results of animal testing a substance is classified as skin irritant (Category 2) (Table 3.2.2, CLP Regulation), if

- at least 2 of 3 (3 of 5, and 4 of 6, respectively) tested animals have a mean score of ≥ 2.3 - ≤ 4.0 for erythema/eschar or for oedema from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or
- inflammation persists to the end of the observation period normally 14 days in at least 2/3 (3/5, and 4/6, respectively) animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or
- there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

A separate irritant criterion accommodates cases when there is a significant irritant response but less than the mean score criterion for a positive test (e.g. at least 1 of 3 tested animals shows a very elevated mean score throughout the study, including lesions persisting at the end of an observation period of normally 14 days). Other responses could also fulfil this criterion. However, it should be ascertained that the responses are the result of chemical exposure (Section 3.2.2.8.1, CLP-Regulation). Moreover, when inflammation persists to the end of the observation period in 2 or more test animals, then a material shall be considered to be an irritant (Section 3.2.2.8.2, CLP-Regulation).

Likewise, pH extremes like ≤ 2 and ≥ 11,5 may indicate the potential to cause skin effects, especially when buffering capacity is known, although the correlation is not perfect. Generally, such substances are expected to produce significant effects on the skin.

Due to the low pH value of ≤ 0.3 of glyoxylic acid (50 %) (see section 6), it can be expected that this substance produces significant effects on the skin and thus can be categorised as Skin Corr. 1 or Skin Irrit. 2. In the reliable *in vivo* assays performed according to OECD TG 404 and GLP, however, no skin irritating effects were observed in any of the tested animals. Hence, based on these results it can be concluded that for glyoxylic acid (50 %) no classification regarding skin corrosiuon/irritation is warranted.

7.1.3 Conclusion on classification and labelling for skin corrosion/irritation

According to CLP there is no need for classification of glyoxylic acid regarding skin corrosion/irritation.

RAC evaluation of skin corrosion / irritation
Summary of the Dossier Submitter's proposal The DS proposed not to classify glyoxylic acid for skin corrosion/irritation potential based on a GLP *in vivo* rabbit study following OECD TG 404. In this study, erythema was observed in one out of 6 animals after 4h exposure of glyoxylic acid (50 % v/v aqueous solution) under semi-occlusive conditions (Guillot, 1984a). The slight erythema was fully reversible after 48 h. No other skin irritating effects were observed during the whole study period (72 h observation period only). The DS concluded that according to the CLP criteria, no classification should be applied. Comments received during public consultation One Member State Competent Authority (MSCA) questioned the validity and reliability of the *in vivo* study from Guillot (1984a). Indeed, the MS noted that glyoxylic acid (50 % v/v)

has a very acidic pH (< 1), induced severe eye damage in an *in vivo* test guideline study and irritation in the preliminary skin sensitisation study.

The DS responded that significant irritating/corrosive effects on the skin would be expected based on the low pH value of glyoxylic acid (50 % v/v). Nevertheless, as the results of the *in vivo* study from Guillot (1984a) are considered reliable without restriction by the DS, the criteria to classify glyoxylic acid for skin corrosion/irritation are not met.

Assessment and comparison with the classification criteria

In a single skin irritation study OECD TG 404, glyoxylic acid (50 % v/v, aqueous solution) was applied to the intact skin of six rabbits. RAC considers the study reliable with limitations. Indeed, potential washing shortly after skin exposure was not reported. Moreover, the test material was applied on 2.5 cm^2 of skin instead of 6 cm^2 as recommended in the test guideline. Nevertheless, in the study, except a slight erythema score of 1 at 24 h (everage score of 0.33 for the period 24-72 h) in one out of 6 animals, reversible within 48 h, no other skin reactions were observed. Thus, glyoxylic acid does not meet the criteria for classification as skin irritant based on this study.

Irritation observed in the skin sensitisation studies should be used with care for assessing irritation potential (Guidance on the application of the CLP criteria, v. 5, July 2017; page 282). In the LLNA study performed in mice (Anderson *et al*., 2008), the use of acetone as a vehicle could have significantly enhance the penetration of the test material. In the guinea-pigs sensitisation test (Hoechst, 1975), necrosis observed after induction exposure could have been caused by intradermal injection with adjuvant. Therefore, RAC does not retain these studies to assess the irritation potential of the substance.

RAC notes as supportive information that no irritation potential was seen in an acute dermal toxicity study in rats (OECD TG 402), performed with glyoxylic acid 50 % v/v, aqueous solution (REACH registration dossier disseminated on ECHA website).

Glyoxylic acid in form of 50 % v/v aqueous solution has a reported pH of 0.3. According to the CLP criteria, extreme pH is expected to produce corrosive effects on the skin. Nevertheless, RAC consideres that existing reliable animal data should be given more weight than extreme pH in solution. Therefore, RAC concludes, in agreement with the DS proposal, that based on the standard *in vivo* rabbit study no classification for skin corrosion/irritation is warranted for glyoxylic acid 50 % v/v aqueous solution.

7.2 Serious eye damage/eye irritation

Table 10: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
OECD TG 405, GLP (study considered reliable without restrictions) Deviations from guideline: Eyes were washed out 4 s and 30 s after instillation, respectively (control eyes treated similar), evaluation of animals only once daily, no individual scoring results given, treatment about 7 days (The deviations are not considered to interfere with the reliability of the study.)	Rabbit, New Zealand White, male, 6 animals	Glyoxylic acid (50 %) (CAS: 298-12-4)	0.1 ml non-diluted test substance/eye (left eye served as control), - eyes of 3 rabbits washed out 4 seconds after instillation - eyes of other 3 rabbits washed out 30 seconds after instillation Observation period: 1h after treatment and daily until termination of study (7 days after instillation)	Chemosis score (mean of 24/48/72h and all 6 animals): 3.94 (max. score: 4.0), not reversible after 7 days of observation Conjunctivae sore/discharge (mean of 24/48/72h and all 6 animals): 1.83 (max. score: 3.0), not reversible after 7 days of observation Conjunctivae sore/erythema (mean of 24/48/72h and all 6 animals): 2.22 (max. score: 3.0), not reversible after 7 days of observation Iris score (mean of 24/48/72h and all 6 animals): 1.78 (max. score: 2.0), not reversible after 7 days of observation Cornea opacity score (mean of 24/48/72h and all 6 animals): 3.83 (max. score: 4.0), not reversible after 7 days of observation	Guillot (1984 b)

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

There is one *in vivo* eye irritation/corrosion study available for glyoxylic acid in animals (study report 1984, unpublished). The study is performed according to OECD TG 405 and GLP and is considered relevant and reliable without restrictions. 0.1 ml of a 50 % solution of glyoxylic acid was instilled into the inferior conjunctival sac of the right eye of six male Albino New Zealand rabbits. The left eye served as control. The eyes were washed out 4 seconds (3 animals) and 30 seconds (3 animals) after substance instillation. Examinations were carried out 1h after instillation and then daily until day 7 after instillation. Glyoxylic acid (50 %) caused severe damage to the treated eyes; e.g. the mean score of cornea opacity of 24 h to 72 h and all 6 animals was 3.83, the mean iris score of 24 h to 72 h and all 6 animals was 1.78 and the chemosis score of 24 h to 72 h and all 6 animals was also close to 4 (3.94). All observed effects were not reversible within the observation time of the study (7 days). Due to the severe effects observed the study was terminated before the usual observation period of 21 days. The results of the study clearly indicate that glyoxylic acid is extremely irritating (corrosive) to the rabbit eye. There are some deviations to the OECD TG 405 (Table 10), but most of them were due to the highly irritating effects of the substance. This included early eye washing already 4 and 30 s, respectively, after substance instillation and termination of the study already after 7 days. However, according to the OECD TG 405 the observed severe effects such as grade 4 corneal opacity are generally considered to not fully reverse by the end of the 21-day observation period. Individual scores for each animal are also not reported. But due to the high mean scores obtained (mean values of all 6 animals) for cornea opacity (score 3.83), iris (score 1.78) and chemosis (score 3.94) it is possible to estimate that in 5/6 animals a

mean score for cornea opacity of 4 and in 5/6 animals a mean score for chemosis of 4 was obtained. Thus, all deviations from the guideline are considered not to interfere with the reliability of the study.

No information on eye irritation/corrosion effects of glyoxylic acid is available in humans.

7.2.2 Comparison with the CLP criteria

According to the CLP Regulation (Section 3.3.2.3.) and the OECD TG 405 (testing strategy for eye irritation/corrosion) substances with extreme pH values of ≤ 2 and ≥ 11.5 are expected to produce corrosive effects on eyes. An extreme pH value of 0.3 is described (see section 6) for a 50 % solution of glyoxylic acid. Thus, a potential for irreversible eye damage for glyoxylic acid can be well expected based on the pH value.

According to Table 3.3.1 of the CLP Regulation classification criteria for irreversible eye effects are as follows:

A substance is considered to cause irreversible effects on the eye if, when applied to the eye of an animal, it produces:

- at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or
- at least in 2 of 3 tested animals, a positive response of: corneal opacity ≥ 3 and/or iritis > 1.5 (calculated as the mean score following grading at 24, 48, 72 hours after installation of the test material)

In the available reliable *in vivo* eye irritation test using rabbits treated with a 50% solution of glyoxylic acid a mean score (24, 48 and 72 hours after installation) for corneal opacity for all 6 animals of 3.83 was obtained. Individual scoring results are not documented in the study. But the given mean value implies that at least in 5/6 tested animals a corneal opacity of > 3 was obtained. Moreover, the obtained mean score (24, 48 and 72 hours after installation) for iritis of 1.7 for all 6 animals implies that in at least 5/6 animals a positive response of iritis > 1.5 was obtained.

All observed eye lesions to cornea, iris or conjunctiva in all animals were reported not to be reversible within the 7-day testing period of the study. Due to the severe effects observed the study was terminated before the 21-day observation period normally scheduled. According to OECD TG 405 the severe effects observed such as the grade 4 corneal opacity are injuries that are not expected to fully reverse by the end of the 21-day observation period.

Thus, based on data from a reliable *in vivo* study in rabbits, it can be concluded that all criteria for serious eye damage given in table 3.3.1 in the CLP Regulation are fulfilled for glyoxylic acid. This is supported by the fact that the severe effects were observed in spite of early washing of the eyes after installation of 4 and 30 s, respectively. This is in line with the extremely low pH value of 0.3, which alone was sufficient to warrant a classification as Eye Dam. 1. Moreover, a 50 % solution was used for the test.

Hence, for glyoxylic acid the classification as Eye Dam. 1 H318 (Causes serious eye damage) is justified.

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

Based on comparison of the available eye irritation/corrosion data for glyoxylic acid with the criteria laid down in the CLP Regulation it is justified to classify glyoxylic acid as Eye Dam. 1 H318 (Causes serious eye damage).

RAC evaluation of serious eye damage/irritation
Summary of the Dossier Submitter's proposal In an OECD TG 405 GLP study performed in 6 rabbits, glyoxylic acid (50 % aqueous solution) caused severe eye irritation in rabbits (Guillot, 1984b). The study is considered

reliable without restriction by the DS. The mean score for 24-72 h in the six rabbits were: 3.83 (corneal opacity), 1.72 (iritis), 3.94 (conjunctivae chemosis) and 2.22 (conjunctivae erythema). Eyes of three rabbits were washed out 4 seconds after instillation and other 3 rabbits 30 seconds after instillation. Individual data are not reported. None of the eye effects were reversible after 7 days of observation.

The mean 24-72 h scores are shown in the table below:

		Mean (24-72 h)	Max. score	Reversibility after 7 days
Conjunctivae	Chemosis	3.94	4	No
	Redness	2.22	3	No
Iris	Iritis	1.78	2	No
Cornea	Opacity	3.83	4	No

Based on the available *in vivo* study in rabbits supported by the extreme pH of glyoxylic acid (50 % aqueous solution), classification of glyoxylic acid as Eye Dam. 1; H318 is proposed by the DS.

Comments received during public consultation

One MSCA supported the DS's proposal to classify glyoxylic acid Eye Dam. 1; H318 based on the severe and irreversible eye effects.

Assessment and comparison with the classification criteria

Glyoxylic acid was tested for eye irritation in a study with 6 rabbits. RAC considers the study reliable with limitation as the observation period was only 7 days instead of 21 days recommended in the test guideline and as individual scores were not available. Nevertheless, the limitations are not considered to have compromised the positive results observed in the study.

Although individual values were not reported, it is possible to estimate, based on this *in vivo* study, that in 5/6 animals a mean score (24-48 h) for corneal opacity of 4 was obtained, meeting the criteria for serious eye damage (Guidance on the application of the CLP criteria, version 5, July 2017 (section 3.3.2.3.2.2), in case of 6 rabbits).

In agreement with DS, RAC is in the opinion that glyoxylic acid should be classified as Eye Dam. 1; H318 "Causes serious eye damage".

7.3 Skin sensitisation

Table 11: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
OECD TG 429 (Skin	Mouse, Balb/c,	Glyoxylic acid (CAS:	**Pre-Screen test:** 0,10, 20, 40 %	**Pre-screen test:**	Anderson et al. (2008)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
Sensitisation: Local Lymph Node Assay; LLNA); GLP compliance not specified (study considered reliable with restrictions) Deviations from guideline: Acetone was used as vehicle without justification; pre-screen test was different comp. to section 22 of OECD TG 429 (e.g. measurement of ear thickness only once after treatment on day ; no scoring of observed erythema)	female 5/group	298-12-4)	(v/v)* **Main test:** 0, 1.25**, 2.5, 5, 10, 20, 40 % (v/v)* Vehicle: acetone PC: hexyl cinnamic aldehyde (HCA; 30 %) * Not specified in publication whether the dose levels expressed in percentages are for the 50% v/v glyoxylic test substance or if they are already re-calculated for the 100% v/v substance ** the results for this concentration are not reported in the publication, and hence not discussed in this dossier	Irritancy: - mean ear swelling: no significant difference comp. to controls at all concentrations; but at 20 and 40 % test concentration above 25 % ear swelling of test animals (appr. 30 %); at 10 % test concentration about 18 % ear swelling of test animals. -2/5 animals at 20 % and 40 % common signs of irritation including redness and swelling -in several mice at 40 % one or both ears red and blistered after exposure (scores are not given) Body weights: - no effect up to and including 40 % **Main test:** Stimulation index (SI): 2.5 at 5 %; 10.7 at 10 %, 20.3 at 20 %, 23.9 at 40 % **estimated concentration needed to produce a stimulation index of ≥ 3 (EC3): 5.05 %** HCA (30 %): SI 23.4	
Freund's complete adjuvant test (FCAT) similar to Van der Walle et al. (1982), and Boman et al. (1988); No validated TG. (study considered not reliable) Deviations from original protocol(s) by Van der Walle et al. (1982), and Boman et al. (1988): Induction	Guinea pig, Pirbright white male, 15/group (pre-screen: 6/group)	Glyoxylic acid (50 %) (CAS: 298-12-4)	Pre-Screen test: 0, 20, 40, 60, 80, 100 % (re-calculated for the 100 % substance: 0, 10, 20, 30, 40, 50 %) Main test: - Induction exposure: 10 intracutaneous injections over 14 days (non-diluted test substance in FCA)	**Main test:** - necrosis at the test site area in 15/15 animals (100 %) after induction exposure - positive skin sensitisation response (erythema) in 15/15 treated animals (100 %) after challenge treatment; not reversible after 3 days of observation - No positive skin reaction in NC after challenge treatment (scores are not given)	Hoechst (1975)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
exposure consisted of 10 intracutaneous injections within 14 days instead of 3 (or 5) injections within 10 days; no sham injection of control animals; no PC; no scoring of observed erythema)			- Challenge (24-48 h): 80 % test substance, epicutaneously (re-calculated for the 100 % substance: 40 %) Vehicle: water, FCA NC: challenge treatment only, no vehicle control (e.g. sham injections) PC: none	**Pre-screen test:** No data given	

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

Two *in vivo* studies on skin sensitisation are available for glyoxylic acid. One study, a Local Lymph Node Assay (LLNA; Anderson et al., 2008), was performed similar to OECD TG 429 and is considered to be relevant and reliable with restrictions. The other study (Study report, 1975; unpublished) was performed on guinea pigs similar to Freund's complete adjuvant test (FCAT; Van der Walle et al., 1982; Boman et al. 1988) but not equivalent/similar to any current validated standardised test guideline. The study is considered relevant, but not reliable. In both studies a sensitising potential for glyoxylic acid was detected, and results were not contradictory.

The LLNA was performed in female Balb/c mice. 5 animals per group were included and treated with 5 concentrations (2.5, 5, 10, 20 and 40 %) of glyoxylic acid. Positive and concentration-dependent results (SI > 3) were obtained at 10, 20 and 40 %. Positive and negative controls were valid. The estimated EC3 value was 5.05 %. Based on a pre-screen test common signs of irritation including redness and swelling and also a non-significant but greater than 25 % swelling of the ears was obtained for 20 and 40 % glyoxylic acid. Erythema scores have not been calculated. Thus, excessive local skin irritation cannot be excluded for the two highest concentrations tested. Based on TG OECD 429 the highest dose selected for the main LLNA should not induce excessive skin irritation. Thus, the two highest concentrations used in the test are considered to be too high to enable reliable results. But this does not interfere with the reliability of the whole study as three additional lower concentrations (2.5, 5 and 10 %) have been tested. A positive result (stimulation index (SI) ≥ 3) was again obtained for 10 % and results for all concentrations tested were concentration-dependent. The stimulation index is a value calculated from the obtained data to assess the skin sensitization potential of a test substance. It is the ratio of the proliferation in treated groups to that in the concurrent vehicle control group. The EC3 value, on the other hand, represents the estimated concentration needed to produce a SI of ≥ 3. The estimated EC3 value of 5.05 % determined by Anderson et al. (2008) is considered to be robust. However, it is not specified in the publication whether the dose levels expressed in percentages are for the 50% v/v glyoxylic test substance or if they are already re-calculated for the 100% v/v substance. If the percentages were given for the 50% v/v glyoxylic test substance, which is not assumed to be case, the resulting estimated EC 3 value would be 2.525 %. The vehicle selected for the test was acetone. As acetone is not within the recommended vehicles in OECD TG 492 a sufficient scientific rationale is needed. This was not provided in the study. However, as

negative and positive controls are valid and solubility of glyoxylic acid in acetone has been described the vehicle is considered to be acceptable. Hence, all deviations from the guideline are considered not to interfere with the reliability of the study.

Phenotypic analysis of the draining lymph nodes identified significant increases in $B220^+$ cell populations at all concentrations tested and did not cause an elevation in the IgE^+B220^+ cells or total serum IgE levels. This result suggests that glyoxylic acid elicits a T-cell mediated hypersensitivity response (Type IV) and provides further support for the LLNA identification of glyoxylic acid as a contact sensitiser (Anderson et al., 2007).

The FCAT was performed on Pirbright white guinea pigs similar as described in van der Walle et al. (1982) and Boman et al. (1988), and did not follow any validated standardised TG. In addition, the study is considered not reliable, because:

1) a concurrent positive control (PC) was not conducted without justification and data on recent periodic PC performed in that laboratory were also not provided.

2) the negative control group received only a challenge treatment and no sham injections for induction exposure and is thus considered not valid.

3) results of the preceding range finding tests, as well as specific erythema scores are not reported.

24 h after a challenge treatment, 100 % of priorly treated animals (15/15) showed a positive skin sensitisation response (erythema), which was not reversible within 3 days. In control animals, on the other hand, no reaction was noted after the challenge treatment, indicating that glyoxylic acid might be a potential skin sensitiser. Due to the drastic deficits in study design, the results of this study have to be treated with caution. Nevertheless, because the above mentioned appropriate and reliable LLNA tests (CLP Regulation, Section 3.4.2.2.3.1) yielded in similar results, the results of this study can be taken into account as supportive data.

No information on skin sensitisation effects of glyoxylic acid is available in humans.

7.3.2 Comparison with the CLP criteria

According to the CLP Regulation (Section 3.4.1.4.) a skin sensitiser is a substance that will lead to an allergic response following skin contact. Sensitisation includes two phases: the first phase is induction of specialised immunological memory in an individual by exposure to an allergen. The second phase is elicitation, i.e. production of a cell-mediated or antibody-mediated allergic response by exposure of a sensitised individual to an allergen.

According to Sections 3.4.2.2.3.1, 3.4.2.2.3.2 and 3.4.2.2.3.3 and Tables 3.4.2, 3.4.3 and 3.4.4 of the CLP Regulation classification criteria for skin sensitising effects are as follows:

Substances shall be classified as skin sensitisers (Category 1) if:

(a) there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons; or

(b) there are positive results from an appropriate animal test.

Subcategory 1A may be appropriate for substances showing a high frequency of occurrence in humans and/or a high potency in animals. A substance is considered to cause skin sensitisation Category 1A, if it produces an $EC3 \leq 2$ in the the LLNA (OECD TG 429). For substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals, subcategory 1B may be appropriate. A substance is considered to cause skin sensitisation Category 1B, if it produces an EC3 value > 2 % in the LLNA.

In the available appropriate and reliable *in vivo* LLNA (Anderson et al., 2008), an estimated EC3 value of 5.05 % was obtained by interpolating the existing data. Based on this result, it can be concluded that the criteria for skin sensitisation Category 1B for LLNA given in Table 3.4.4 in the CLP Regulation are fulfilled for glyoxylic acid. However, as mentioned above, it is not specified in the publication whether the dose levels expressed in percentages are for the 50% v/v glyoxylic test substance or if they are already re-calculated for the 100% v/v substance. Nevertheless, even if the percentages were given for the 50% v/v glyoxylic test

substance, which is not assumed to be case, the resulting estimated EC 3 value would be 2.525 %, further supporting a classification of glyoxylic acid as Skin Sens. 1B.

Hence, for glyoxylic acid the classification as Skin Sens. Category 1B H317 (May cause an allergic skin reaction) is justified.

Based on the available data on the sensitising potency of glyoxylic acid, no SCL needs to be assigned to this substance.

7.3.3 Conclusion on classification and labelling for skin sensitisation

Based on comparison of the available skin sensitisation data for glyoxylic acid with the criteria laid down in the CLP Regulation it is justified to classify glyoxylic acid as Skin Sens. Category 1B H317 (May cause an allergic skin reaction).

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS summarised in the CLH report two *in vivo* studies on skin sensitisation properties of glyoxylic acid, a Local Lymph Node Assay (LLNA) in mice and a Freud's complete adjuvant test with open challenge in Guinea-pigs.

The LLNA was conducted with a protocol similar to OECD TG 429 (GLP status unknown). In this LLNA study, glyoxylic acid was found to be a skin sensitiser since stimulation indexes significantly above 3 were found at 10 %, 20 % and 40 % v/v concentrations (Anderson *et al*., 2008). With regards to sub-categorisation, the DS proposed a category 1B based on the EC_3 value of 5.05 % v/v calculated by Anderson *et al*. (2008). It is not specified in the publication if the dose levels were expressed for the 50% v/v glyoxylic acid or if they were recalculated for the 100 % v/v substance. The DS considered that even if the percentage were given for the 50 % v/v glyoxylic acid, which they did not assumed to be the case, the resulting EC_3 would be 2.525 %, still supporting sub-category 1B (EC_3 value > 2 %).

The results of the Anderson *et al*., (2008) study are summarised in the table below:

Concentrations (%)	SI	Mean ear swelling, 24 h post-final exposure
0	-	-
2.5	< control	Data not shown
5	2.5	Data not shown
10	10.7**	~18 %
20	20.3**	>25 %
40	23.9**	>25 %

** $p < 0.01$

In the second study, a non-guideline non-GLP Freund's complete adjuvant test, glyoxylic acid (50 % v/v) was found to be a skin sensitiser since a skin reaction was observed in 100 % of the animals in the test group after challenge (Hoechst, 1975). The DS considered this study non reliable but supportive as positive results were observed the study.

Therefore, the DS proposed to classify glyoxylic acid as a Skin Sens. 1B; H317 without an SCL.

Comments received during public consultation

One MSCA agreed with the proposed classification Skin Sens. 1; H317. Nevertheless, the MSCA commented that data are insufficient for sub-categorisation because the actual test concentrations used in the LLNA study are unknown.

In answer, DS considered that the test concentration used in the LLNA study are known and justified. According to the DS, the criteria for sub-categorisation 1B are met.

Assessment and comparison with the classification criteria

RAC considers the LLNA study (Anderson *et al*., 2008) reliable with limitations and deviations from the OECD TG 429:

- no justification of the use of acetone as a vehicle;
- results at 1.25 % v/v were not reported in the publication;
- pre-screened test differs from OECD TG 429. Animals were only exposed during 3 days instead of 6. No erythema scores have been determined.

Nevertheless, the above limitations and deviations are not considered to interfere with the reliability of the study. In this LLNA study, the EC_3 value was calculated by interpolation to be 5.05 %. At concentrations of 20 % and 40 % v/v glyoxylic acid, ear swelling scores above 25 % were observed suggesting potential irritating effect. A phenotype analysis of the lymphocytes from draining lymph nodes following exposure to glyoxylic acid was performed and resulted in statistically significant dose-related increase in $B220^+$ cell population at all tested concentration (10, 20 and 40 % v/v). The correlation of immunophenotypic marker $B220^+$ with sensitisation potential in the LLNA supports a true positive result.

In a supportive Freund's complete adjuvant test with open challenge, glyoxylic acid was found to be a potent skin sensitiser in male guinea-pigs. The study does not follow an OECD test guideline and few details are reported on the test method. Thus, in line with the DS, the study is only considered as supportive by RAC.

With regards to sub-categorisation, based on the positive results from the LLNA test, the EC_3 value > 2 % meets the criteria with sub-category 1B.

RAC agrees with the DS that the setting of an SCL is not justified.

Therefore, RAC concludes that glyoxylic acid warrants classification as Skin Sens. 1B; H317.

8 REFERENCES

Anderson, S.E., Ham, J.E., Munson, A.E., 2008, Irritancy and Sensitization Potential of Glyoxylic Acid. Journal of Immunotoxicology 5:2, p.93-98.

Anderson, S.E., Wells J.R., Fedorowicz, A., Butterworth, L.F., Meade, B.J., Munson, A.E.., 2007, Evaluation of the Contact and Respiratory Sensitization Potential of Volatile Organic Compounds Generated by Simulated Indoor Air Chemistry.Toxicological Sciences 97(2), 355–363

Kemper, F.H., Blue List, Cosmetic Ingredients. Edito Canto Verlag (ECV), 2000.

Boga, C., Taddei, P., Micheletti, G.,Ascari, F., Ballarin, B., Morigi, M., Galli, S., 2014, Formaldehyde replacement with glyoxylic acid in semipermanent hair straightening: a new and multidisciplinary investigation. International Journal of Cosmetic Science 36:5, p. 459–470.

Boman, A., Karlberg A.-T., Wahlberg, J.E., 1988, Experiences with Freund's complete adjuvant test (FCAT) when screening for contact allergens in colophony. Contact Dermatitis18:1, p. 25-29.

Budavari, S., The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals., 1996.

CosIng, 2016. CosIng is the European Commission database with information on cosmetic substances and ingredients;
http://ec.europa.eu/growth/tools-databases/cosing/index.cfm?fuseaction=search.simple. 27.10.2016.

Guillot, J., P., 1984 a, Test de tolérance locale chez le Lapin - HF 0021 (Acide Glyoxylique) Indice d'irritation primaire cutanée. Study report, unpublished.

Guillot, J., P., 1984 b, Test de tolérance locale chez le Lapin - HF 0021 (Acide Glyoxylique) Indice d'irritation primaire oculaire. Study report, unpublished.

Hoechst Pharma Forschung Toxikologie, 1975, Prüfung auf sensibilisierende Eigenschaften von Glyoxylsäure and Meerschweinchen. Study report, unpublished.

Lide, D.R., CRC Handbook of Chemistry and Physics. 72nd ed., CRC Press, p.3-262, 1991.

Lide, D.R., CRC Handbook of Chemistry and Physics. 89th ed., CRC Press, 2008.

Ullmann's Encyclopedia of Industrial Chemistry, 5th edition, 1996.

Van der Walle, H.B., Klecak, G., Geleick, H., Bensink, T., 1982, *Sensitizing Potential of 14 Mono (Meth) Acrylates in the Guinea Pig. Contact Dermatitis 8:4, p. 223-235.*