Committee for Risk Assessment

RAC

Annex 1

Background document

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

L-(+)-lactic acid; (2S)-2-hydroxypropanoic acid

EC Number: 201-196-2
CAS Number: 79-33-4

CLH-O-0000001412-86-191/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

9 March 2018

Corrigendum
3 December 2019

The statements referring to the GCL of 1% for skin corrosion/irritation and serious eye damage/eye irritation have been removed from the opinion. Formulators of mixtures containing L-(+)-lactic acid are requested to follow the CLP Regulation, to correctly classify their mixtures.

CLH report

Proposal for Harmonised Classification and Labelling

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

L-(+)-lactic acid;
(2S)-2-hydroxypropanoic acid

EC Number: **201-196-2**

CAS Number: **79-33-4**

Index Number: **-**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	L-(+)-lactic acid; (2S)-2-hydroxypropanoic acid
EC number:	201-196-2
CAS number:	79-33-4
Annex VI Index number:	-
Degree of purity:	≥ 92.95 % w/w
Impurities:	Please refer to confidential annex

1.2 Harmonised classification and labelling proposal

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

Current entry in Annex VI, CLP Regulation	No entry
Current proposal for consideration by RAC	Skin Irrit. 2, H315 Eye Dam. 1, H318 STOT SE 3 (respiratory tract irritation), H335
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Skin Irrit. 2, H315 Eye Dam. 1, H318 STOT SE 3 (respiratory tract irritation), H335

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	none	-	none	conclusive but not sufficient for classification
2.2.	Flammable gases	-	-	-	-
2.3.	Flammable aerosols	-	-	-	-
2.4.	Oxidising gases	-	-	-	-
2.5.	Gases under pressure	-	-	-	-
2.6.	Flammable liquids	none	-	none	conclusive but not sufficient for classification
2.7.	Flammable solids	-	-	-	-
2.8.	Self Reactive substances and mixtures	none	-	none	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	none	-	none	conclusive but not sufficient for classification
2.10.	Pyrophoric solids	-	-	-	-
2.11.	Self-heating substances and mixtures	-	-	-	-
2.12.	Substances and mixtures which in contact with water emit flammable gases	none	-	none	conclusive but not sufficient for classification
2.13.	Oxidising liquids	none	-	none	conclusive but not sufficient for classification
2.14.	Oxidising solids	-	-	-	-
2.15.	Organic peroxides	none	-	none	conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	none		none	data lacking
3.1.	Acute toxicity - oral	nonoe		none	conclusive but not sufficient for classification
	Acute toxicity - dermal	none		none	conclusive but not sufficient for classification
	Acute toxicity - inhalation	none (see 3.8)		none	conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Skin Irrit. 2, H315	-	none	
3.3.	Serious eye damage / eye irritation	Eye Dam. 1, H318	-	none	
3.4.	Respiratory sensitisation	none		none	data lacking

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
3.4.	Skin sensitisation	none		none	conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	none		none	conclusive but not sufficient for classification
3.6.	Carcinogenicity	none		none	conclusive but not sufficient for classification
3.7.	Reproductive toxicity-fertility	none		none	data lacking
	Reproductive toxicity-development	none		none	data lacking
	Reproductive toxicity–breastfed babies. Effects on or via lactation	none		none	data lacking
3.8.	Specific target organ toxicity –single exposure	STOT SE 3 H335 (respiratory tract irritation)		none	
3.9.	Specific target organ toxicity – repeated exposure	none		none	conclusive but not sufficient for classification
3.10.	Aspiration hazard	none		none	data lacking
4.1.	Hazardous to the aquatic environment	none		none	conclusive but not sufficient for classification
5.1.	Hazardous to the ozone layer	-	-	-	-

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Table 4: Proposed labelling based according to the CLP Regulation

	Labelling	Wording
Pictograms	GHS05 GHS07	corrosion exclamation mark
Signal Word	Danger	
Hazard statements	H315 H318 H335	Causes skin irritation Causes serious eye damage May cause respiratory irritation
Suppl. Hazard statements		

2 BACKGROUND TO THE CLH PROPOSAL

2.1 Short summary of the scientific justification for the CLH proposal

In vivo skin irritation/corrosivity studies with L-(+)-lactic acid were performed in rabbits, guinea pigs, pigs, and humans and *in vitro* with a biobarrier/chemical detection system as well as with human skin (transcuta-

neous electrical resistance, TER). In rabbits, full thickness destruction indicative of corrosivity was observed with 88 % L-(+)-lactic acid (pH 1.83) and 50 % L-(+)-lactic acid. This result was confirmed by an *in vitro* Corrositex assay which revealed a biobarrier break through at a time of only 31 minutes of 90 % L-(+)-lactic acid (< 3 min: Skin Corr. 1A; 3 min to 4 h: Skin Corr. 1B/1C). No irritation or corrosivity was observed in rabbits when a 10 % aqueous dilution of L-(+)-lactic acid was tested (Prinsen, 1995). However, experience from humans and studies in guinea pigs, pigs, and humans revealed that these species are much less sensitive to dermal exposure to L-(+)-lactic acid. In these studies, L-(+)-lactic acid was tested non-irritant in concentrations up to 88 % (pig, guinea pig) or irritant (human). From the patch test studies in humans, it is likely that dermal irritation studies in pigs underestimate the irritating potential of L-(+)-lactic acid for human skin while rabbit skin seems to be much more sensitive than human skin. Also ECETOC (2002) reported that existing data indicate that human skin is, in most cases, less sensitive than rabbit skin. Thus, the human patch test data should be used as key study (York et al. 1996) showing adequate results for classification and labelling and classification with H315, Cat. 2 (Causes skin irritation) is proposed.

Concerning eye irritation potential, concentrated L-(+)-Lactic acid has a pH < 2. Therefore, no eye irritation studies in rabbits were performed due to animal welfare considerations. Instead, a chicken enucleated eye test (*in vitro*) was performed and revealed a highly damaging potential of L-(+)-lactic acid to the eye (severe corneal opacity, corneal swelling and fluorescein retention). Thus, a classification with Eye Dam. 1, H318 (Causes serious eye damage) is proposed.

In general, a classification for corrosivity (skin, eye) is considered to cover the potential to also cause respiratory tract irritation and the additional Category 3 can be considered to be superfluous, although it can be assigned at the discretion of the DS. For precautionary reasons and based on the results observed in an acute inhalation toxicity study in rats (David, 1987), for L-(+)-lactic acid it is considered adequate to propose a classification as respiratory tract irritant STOT SE 3.

For the other toxicological hazards, either the data were conclusive but not sufficient for classification or the relevant data were lacking. Because of the high background exposure of L-(+)-lactic acid via food and endogenous metabolism, no further studies are required according to Annex II (data requirements) of Regulation (EU) No. 528/2012 concerning the making available on the market and use of biocidal products. Refer also to discussion in Section 4.1.

Additionally, the classification provided by companies to ECHA in C&L notifications identifies that this substance is corrosive to metals. Information is available that the substance corrodes metals (PA Schweitzer, Corrosion Resistance Tables (1995), Ullmann's encyclopaedia of industrial chemistry (1990)), however, no data by the designated test method are available. This substance has five active registrations under REACH, but there is no data presented, that provide test results according to the UN test method for supporting the reasons for no classification as corrosive to metals. Therefore it is necessary to have a test result for metal corrosion in accordance with the UN Manual of Tests and Criteria, Part III, section 37.4 to conclude on the harmonised classification and labelling.

2.2 Current harmonised classification and labelling

No harmonised entry in Annex VI.

2.3 Current self-classification and labelling

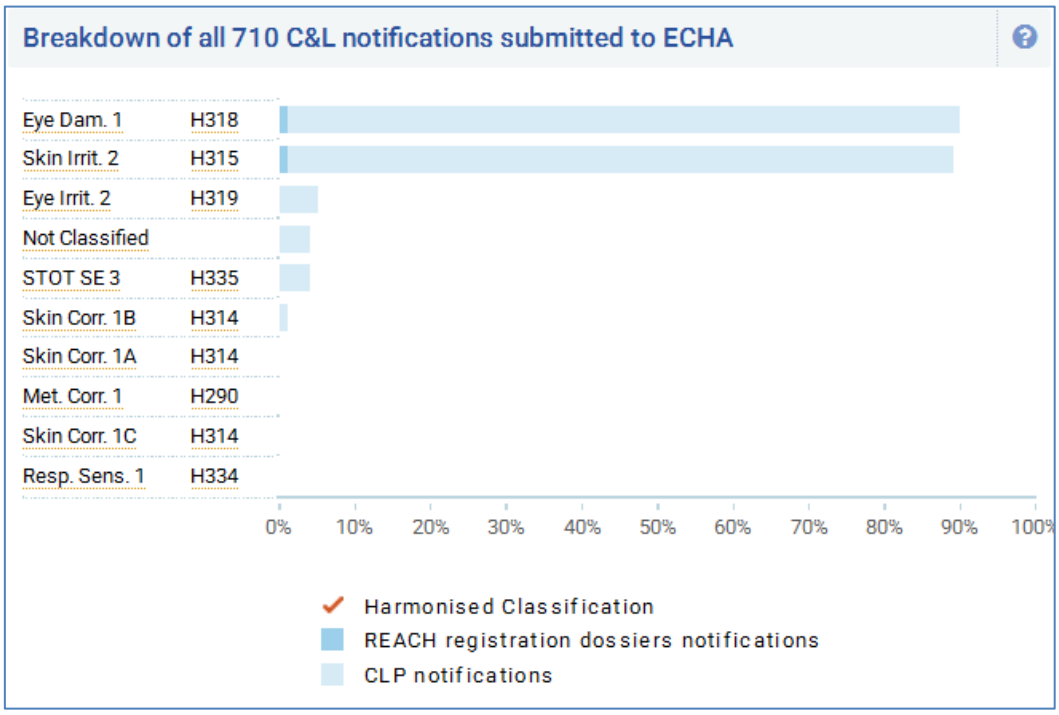


Figure 1: C&L notifications (www.echa.eu, February 2017)

RAC general comment
L-(+)-lactic acid and lactate form an integral part of normal mammalian intermediary metabolism, as they are produced by the reduction of pyruvate. Total normal lactate turnover at rest has been determined as 1.6 to 2 g/kg bw/d in humans, 4.9 to 8.1 g/kg bw/d in rats and 2.3 to 3.5 g/kg bw/d in dogs (Connor and Woods, 1982). However, it should be noted that the classification proposal concerns lactic acid, with concentrated lactic acid having a typical concentration of 92.95% (Background document [BD], table 6) and a pH of about 1.85.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

L-(+)-lactic acid is an existing active substance (evaluated under Directive 98/8/EC). It was approved by the Biocidal Products Committee (BPC) in December 2015 for its use as PT1 (human hygiene). Other uses (PTs 2, 3 and 4) are scheduled for discussion at the BPC meeting in early 2017.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

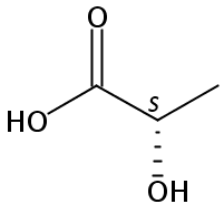
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	201-196-2
EC name:	L-(+)-lactic acid
CAS number (EC inventory):	79-33-4
CAS number:	79-33-4
CAS name:	Propanoic acid, 2-hydroxy-, (2S)-
IUPAC name:	(2S)-2-Hydroxypropanoic acid
CLP Annex VI Index number:	-
Molecular formula:	$C_3H_6O_3$
Molecular weight range:	90.08 g/mol

Structural formula:



Lactic acid will reversibly auto-polymerize to oligomeric esters, degree of polymerization depends on the concentration and temperature.

For further information: please refer to the confidential annex.

1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
L-(+)-lactic acid; (2S)-2-hydroxypropanoic acid EC No.: 201-196-2	≥ 92.95 % w/w	≥ 80 – ≤100 % w/w	For further information: please refer to the confidential annex.

Table 7: Impurities (confidential information)

Impurity	Typical concentration	Concentration range	Remarks
confidential			For further information: please refer to the confidential annex.

1.3 Physico-chemical properties

Table 8:Summary of physico - chemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance at 20°C and 101,3 kPa	liquid (aqueous solution, 88 % / 93 % L-(+)-lactic acid)	Safety data sheet of L-(+)-lactic acid (Purac, **2009**)	visual assessment
	pure: crystalline solid	C.H. Holten, *Lactic acid. Properties and chemistry of lactic acid and derivatives*, *Chapter IV: Physical properties*; Verlag Chemie, Weinheim, **1971**.	handbook data (see reference)
Melting/freezing point	53 °C (pure, crystalline solid L-(+)-lactic acid)	C.H. Holten, *Lactic acid. Properties and chemistry of lactic acid and derivatives*, *Chapter IV: Physical properties*; Verlag Chemie, Weinheim, **1971**.	handbook data (see reference)
	no solidification upon cooling until – 80 °C (93 % L-(+)-lactic acid)	study report	expert statement
Boiling point	204.2 °C (calculated) (100 % L-(+)-lactic acid)	study report	estimated by calculation (EPIWIN v. 1.4.1 (adapted Stein and Brown Method))
Relative density	1.213 (T = 20 °C, 93 % L-(+)-lactic acid)	C.H. Holten, *Lactic acid. Properties and chemistry of lactic acid and derivatives*, *Chapter IV: Physical properties*; Verlag Chemie, Weinheim, **1971**.	calculated from handbook data (see reference)
Vapour pressure	0.4 Pa (T = 20 °C, 100 % L-(+)-lactic acid, calculated)	study report	estimated by calculation (92/69/EC, A.4 (Calculation, modified Grain Method)
Surface tension	70.7 mN/m (93 % L-(+)-lactic acid (concentration: 1 g/L in water))	study report	experimental result (EU Method A.5 (Surface Tension); OECD Guideline 115 (Surface Tension of Aqueous Solutions))
Water solubility	completely miscible with water (purity not stated, crystalline L-(+)-lactic acid)	C.H. Holten, *Lactic acid. Properties and chemistry of lactic acid and derivatives*, *Chapter IV: Physical properties*; Verlag Chemie, Weinheim, **1971**.	handbook data (see reference)
Partition coefficient n-octanol/water	-0.74 (T = 20 °C) (purity not stated, crystalline L-(+)-lactic acid)	study report	experimental result (in house method ,not described in detail in literature)

Flash point	88 % solution in water: > 74 °C pure, crystalline solid lactic acid: > 150 °C 93 % L(+) Lactic acid: The steam-volatility of lactic acid is very low, therefore the solution vapour is more than 99 % water vapour, and as such the vapour is not ignitable.	C.H. Holten, Lactic acid. Properties and chemistry of lactic acid and derivatives, Verlag Chemie, Weinheim, 1971. expert judgement	handbook data
Flammability	No experimental data on flammability upon ignition for solids: The melting point of pure lactic acid was determined to be 53.0 °C, in case of the flammability test the substance is melted in contact by a hot flame from the gas burner and if brief ignition occurs, the flame will be extinguished after a short distance. The substance has no pyrophoric properties and does not liberate flammable gases on contact with water.	expert judgement	study technically not feasible or study scientifically not necessary
Explosive properties	non explosive The study does not need to be conducted because there are no chemical groups present in the molecule which are associated with explosive properties.	expert judgement	study scientifically not necessary
Self-ignition temperature	The auto-ignition temperature of a 93 % aqueous solution of lactic acid was determined to be ≥ 400 °C.	study report	experimental result (EU Method A.15, DIN 51794, IEC 79-4)

Oxidising properties	no oxidising properties The study does not need to be conducted because the organic substance contains oxygen atoms which are chemically bonded only to carbon or hydrogen and hence, the classification procedure does not need to be applied.	expert judgement	study scientifically not necessary
Corrosive to metals	It is indicated that unalloyed carbon steels to lactic acid of the entire concentration range leads to a corrosion rate of > 1.27 mm/year, therefore it is not suitable for use. From literature is known, that aqueous solutions of 80 % up to 93 % w/w L-(+)-Lactic acid is corrosive toward metallic material, normally used in production and processing, especially at high temperatures.	P.A. Schweitzer, Corrosion Resistance Tables. 4th ed. Vols. 1–3. (1995) Ullmann (1990) 5, A15, 101	handbook data handbook data (No experimental data available.)
Granulometry	The study does not need to be conducted if the substance is marketed or used in a non-solid or granular form.		study scientifically unjustified
Dissociation constant	pKa = 3.86, T = 22.5 °C (purity not stated, crystalline L-(+)-lactic acid)	C.H. Holten, *Lactic acid. Properties and chemistry of lactic acid and derivatives*, *Chapter V: Physical Chemistry*; Verlag Chemie, Weinheim, **1971**.	handbook data (see reference)

Data waiving

Information requirement: Explosives

Reason: study scientifically not necessary

Justification: The study does not need to be conducted because there are no chemical groups present in the molecule which are associated with explosive properties.

Information requirement: Flammable gases (including chemically unstable gases)

Reason: study technically not feasible

Justification: The study does not need to be conducted because the substance is a liquid.

Information requirement: Aerosols

Reason: study technically not feasible

Justification: The study does not need to be conducted because the substance is no aerosol.

Information requirement: Oxidising gases

Reason: study technically not feasible

Justification: The study does not need to be conducted because the substance is a liquid.

Information requirement: Gases under pressure

Reason: study scientifically unjustified

Justification: The study does not need to be conducted because the substance is a liquid.

Information requirement: Flammable liquids

Reason: study scientifically unjustified

Justification: Lactic acid is produced as aqueous solutions of up to 93 % lactic acid w/w. The steam-volatility of lactic acid is very low, therefore the solution vapour is more than 99 % water vapour, and as such the vapour is not ignitable.

Information requirement: Flammable solids

Reason: study technically not feasible or study scientifically not necessary

Justification: The melting point of pure lactic acid was determined to be 53.0°C, in case of the flammability test the substance is melted in contact by a hot flame from the gas burner and if brief ignition occurs, the flame will be extinguished after a short distance.

The study does not need to be conducted because the substance is a liquid.

Information requirement: Self Reactive substances and mixtures

Reason: study scientifically not necessary

Justification: The study does not need to be conducted because there are no chemical groups present in the molecule which are associated with explosive or self-reactive properties and hence, the classification procedure does not need to be applied.

Information requirement: Pyrophoric liquids

Reason: study scientifically not necessary

Justification: The study does not need to be conducted because the substance is known to be stable in contact with air at room temperature for prolonged periods of time (days) and hence, the classification procedure does not need to be applied.

Information requirement: Pyrophoric solids

Reason: study scientifically not necessary

Justification: The study does not need to be conducted because the substance is known to be stable in contact with air at room temperature for prolonged periods of time (days) and hence, the classification procedure does not need to be applied.

Information requirement: Self-heating substances and mixtures

Reason: study technically not feasible / study scientifically not necessary

Justification: The study does not need to be conducted because the substance is a liquid.

The study does not need to be conducted because the substance is a solid having a melting point <= 160°C.

Information requirement: Substances and mixtures which in contact with water emit flammable gases

Reason: study scientifically not necessary

Justification: The study does not need to be conducted because the substance is known to be soluble in water to form a stable mixture.

Information requirement: Oxidising liquids

Reason: study scientifically not necessary

Justification: The study does not need to be conducted because the organic substance contains oxygen atoms which are chemically bonded only to carbon or hydrogen and hence, the classification procedure does not need to be applied.

Information requirement: Oxidising solids

Reason: study technically not feasible

Justification: The study does not need to be conducted because the substance is a liquid.

Information requirement: Organic peroxides

Reason: study scientifically not necessary

Justification: The study does not need to be conducted because the substance does not fall under the definition of organic peroxides according to GHS and the relevant UN Manual of tests and criteria.

2 MANUFACTURE AND USES

Not addressed in this dossier.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

3.1 Physico-chemical properties

3.1.1 Summary and discussion

Lactic acid is produced as aqueous solutions of up to 93 % w/w L-(+)-lactic acid. The steam-volatility of lactic acid is very low, therefore the solution vapour is more than 99 % water vapour, and as such the vapour is not ignitable, therefore no flash point was determined up to 100 °C.

No experimental data on flammability upon ignition for solids (EEC A10) is available, but it can be concluded, that pure, crystalline solid L-(+)-lactic acid is not highly flammable, as the melting point of pure lactic acid was determined to be 53.0 °C. In case of the flammability test the substance is melted in contact by a hot flame from the gas burner and if brief ignition occurs, the flame will be extinguished after a short distance.

Experience in handling and use indicates L-(+)-lactic acid is not pyrophoric and does not react with water to liberate flammable gases.

Further, it was also tested in a standard auto-ignition temperature study (EEC A15) and no spontaneous ignition was observed below 400 °C. A study for self-heating substances/mixtures does not need to be conducted because the substance is a liquid or because the substance is a solid having a melting point ≤ 160 °C.

L-(+)-lactic acid does not contain chemical groups associated with explosive properties.

Consideration of the structure indicates that L-(+)-lactic acid will not have oxidising properties.

No experimental data on corrosion to metals is available. But it is known from literature, that the corrosion rate on unalloyed carbon steels is > 1.27 mm/year for lactic acid within the entire concentration range. Therefore it is incompatible. Aqueous solutions of 80 % up to 93 % w/w L-(+)-lactic acid are corrosive toward metallic materials, usually used in production and processing - especially at high temperatures.

3.1.2 Comparison with criteria

Substances and mixtures of hazard class corrosive to metals are classified in a single hazard category on the basis of the outcome of the UN Test C.1 (UN-MTC, Part III, Section 37, paragraph 37.4). In the test of metal corrosivity, metal pieces (steel or aluminium) are immersed in a liquid at a test temperature of 55 °C for 7 to 28 days, and if the corrosion rate exceeds 6.25 mm per year, the substance is classified as corrosive to metals. This criterion for metal corrosivity is based on Class 8, packing group III of the UN Recommendations on the Transport of Dangerous Goods, which also include skin corrosivity. However, according to the classification procedures of Class 8, it is not necessary to conduct the tests for metal corrosion for the purposes of

classification if a substance is shown to be corrosive to skin. This exemption is not allowed by the CLP regulation, testing for an appropriate classification is always required.

The hazard class corrosive to metals was not included in the DSD/DPD. Therefore, re-classification is not applicable. Furthermore, lactic acid was evaluated under the former Directive 98/8/EC, in which the test on corrosion to metals was no data requirement. Therefore no test results were presented.

Recommendation to conclude on classification: experimental results according to UN Manual of Tests and Criteria: Part III, 37.4 should be presented by the registrants.

3.1.3 Conclusions on classification and labelling

Classification is not possible, due to data lacking for the hazard class “Corrosive to metals” in Category 1; H290 "May be corrosive to metals".

RAC evaluation of physical hazards

Summary of the Dossier Submitter’s proposal

The Dossier Submitter (DS) did not propose any classification for physical-chemical hazards for L-(+)-lactic acid. However, due to lack of data for the hazard class “Corrosive to metals”, they recommended testing according to UN Manual of Test and Criteria.

The vapour of the substance contains more than 99% water and is not ignitable, therefore no flash point was determined up to 100 °C. Pure crystalline solid L-(+)-lactic acid is not a flammable solid. Since the melting point of pure lactic acid is low (53 °C), the substance will in fact melt as the flammability test (as described in Part III, sub-section 33.2.1.4.3.1, of the UN Manual of Test and Criteria [UN-MTC]) is carried out.

Experience in handling and use indicates that L-(+)-lactic acid is neither pyrophoric nor does it react with water to liberate flammable gases. Testing showed that no spontaneous ignition was observed below 400 °C. Consideration of the structure indicates further that L-(+)-lactic acid does not have explosive or oxidising properties.

Comments received during public consultation

The only comment on these hazard classes was submitted by a company/manufacturer, who provided a new study which had been completed in December 2015. Based on this study, L-(+)-lactic acid (purity: 88.2%) is not corrosive to steel and aluminium specimens according to the UN Manual of Test and Criteria (ST/SG/AC.10/11/Rev5, 2009); Test C.1.

Additional key elements

The new study mentioned above was carried out in the following way: a solution of L-(+)-lactic acid (purity 88.2%) was placed in two identical glass cup-like exposure receptacles (1.5 L per receptacle) and heated to 55 °C (± 1 °C). In each receptacle three polished, cleaned, degreased and weighed metal specimens (size 50 x 20 x 2 mm) were fixed in a way that one was fully immersed in the lactic acid, the second was half covered by the acid

and the third specimen was entirely in the gas space above the heated acid. The tests were carried out with a set of three steel specimens in one receptacle and a set of three aluminium specimens in the other. After one week, holding the temperature of the lactic acid at the same level, both sets of specimens were removed, cleaned, weighed and inspected by using a microscope to determine localised corrosion. The highest weight losses observed over seven days were < 1.63% and 0.11% for the steel and aluminium specimen, respectively, and no intrusion depths could be found microscopically.

According to the UN-MTC C.1, Part III, section 37, paragraph 37.4, the described corrosion tests are positive when after one week for any specimen the mass loss would be higher than 13.5% or when the minimum intrusion depths would be higher than 120 µm. Therefore, the substance showed a negative corrosion result in these tests.

Assessment and comparison with the classification criteria.

The outcome of the study provided during the public consultation, which was performed according to UN-MTC criteria, showed that L-(+)-lactic acid (88.2%) was not corrosive to metals. Therefore, RAC concludes that the substance does not require classification for corrosivity to metals.

Regarding the other physical hazard classes, RAC agrees with the DS **that L-(+)-lactic acid does not warrant any classification** according to CLP criteria.

4 HUMAN HEALTH HAZARD ASSESSMENT

Short summaries of the available information/data are included in this section. Longer (robust) study summaries (Doc III) are included in an appendix.

The summaries were extracted from the documentation submitted for the evaluation of L-(+)-lactic acid for biocidal products used for human hygiene purposes (Product Type 1) (i.e. first draft of the Competent Authority Report; February 2015).
REACH registration dossiers are available. Some references were also available to the Rapporteur Member State (RMS) / Dossier submitter (DS) for the biocidal evaluation in the Competent Authority Report and consequently for this CLH Report. These references are flagged with √ in the chapters, respectively.

Some references were not available for the biocidal evaluation and no original data/studies from registration dossiers for L-(+)-lactic acid were submitted to the DS. Therefore, the assessment of the registrant(s) was adopted and included in this CLH dossier. These references are mentioned in the chapters, respectively. In conclusion, no divergent proposal for classification and labelling resulted from these data.

The test substances used in the experimental studies were L-(+)-lactic acid, lactate and calcium lactate. As far as possible, the test substance characteristics, i.e. pH-values were indicated. In many publications and experimental studies no information on the pH-value of the tested material was provided.

Definitions:

- Lactic acid is an organic compound with the formula $CH_3CH(OH)CO_2H$.
- Lactate is the conjugate base of lactic acid.
- Lactic acid is chiral, consisting of two optical isomers: one is known as L-(+)-lactic acid, the other is D-(−)-lactic acid. A mixture of the two in equal amounts is called DL-lactic acid, or racemic lactic acid.
- Calcium-lactate is the calcium-salt of lactic acid. It is created by the reaction of lactic acid with calcium carbonate or calcium hydroxide and is used in foods (i.e. an ingredient in baking powder).

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Lactate/lactic acid form an integral part of normal mammalian intermediary metabolism, produced by reduction of pyruvate. Monocarboxylate transport proteins (MCT) facilitate the distribution of lactate between organs, cells and subcellular organelles and may be involved in gastrointestinal lactate absorption and renal lactate elimination. Cytosolic and mitochondrial lactate dehydrogenases (LDH/mLDH) convert lactate into pyruvate, consuming NAD+ and producing NADH. Via stepwise metabolism involving oxaloacetate and phosphoenolpyruvate as intermediates, pyruvate is utilised for gluconeogenesis ((1) in Figure 2). Alternatively, metabolites of pyruvate (oxaloacetate, acetyl-CoA) are consumed in the tricarboxylic (citric) acid cycle (TCA, (2)) generating NADH, ATP and ultimately CO2. Finally, pyruvate may be transaminated to the amino acid L-alanine (3). Gluconeogenesis occurs mainly in the liver and is energy-consuming. Increased cellular levels of lactate influence pathways of cellular metabolism, leading to a decrease in the generation of pyruvate from other sources such as glucose by reduction of glycogenolysis and glycolysis, or leading to enhanced gluconeogenesis (Gladden, 2004; Sterenborg, 2007). Total normal lactate turnover at rest has been determined as 2.3 – 3.5 g/kg bw/d and 4.9 – 8.1 in dogs and rats, respectively, supporting applicability of allometric scaling (Connor and Woods, 1982).

Following application by gavage (oral), external L-(+)-lactic acid is absorbed rapidly in rats with one half being removed from the GI-tract within 2-3 hours (Cori, 1930). Of 2 g/kg administered to rats via gavage,

42 % were converted into CO_2 and presumably exhaled within 6 hours (Andersen, 1998). Complete utilization of orally administered lactate has also been reported in dogs (Andersen, 1998). L-(+)-lactic acid that is not metabolised to CO_2 may be utilised for the synthesis of biomacromolecules, including glycogen or proteins (Cori & Cori, 1929; Andersen, 1998). Feeding of pigs and rats with a daily dose of 1.9 and 5.8 g/kg bw, which is roughly equivalent to the lactic turnover rate at rest resulted only in a slight increase of L-(+)-lactate plasma levels by 0.03 g/L (from 0.26 g/L) and 0.04 g/L (from 0.23 g/L), respectively. At the same time, elimination in urine was minimal with increases in L-(+)-lactate concentrations by 0.02 and 0.07 g/L, corresponding to < 0.01 g/kg bw/d (less than 1 % of dose) at an estimated urine volume of 100 mL/kg bw/d (Everts et al., 2000). It can therefore be concluded, that the lactate turnover rate is tightly regulated and is not saturated at total lactate load of 200 % compared to the value at rest. In contrast, Abramson & Eggleton (1927) reported between 7 and 42 % renal excretion following bolus injection of 5.4 to 30 g/kg bw in dogs. Notably, the percentage excreted with urine was correlated to urine volume, suggesting glomerular filtration as the main mechanism under these conditions.

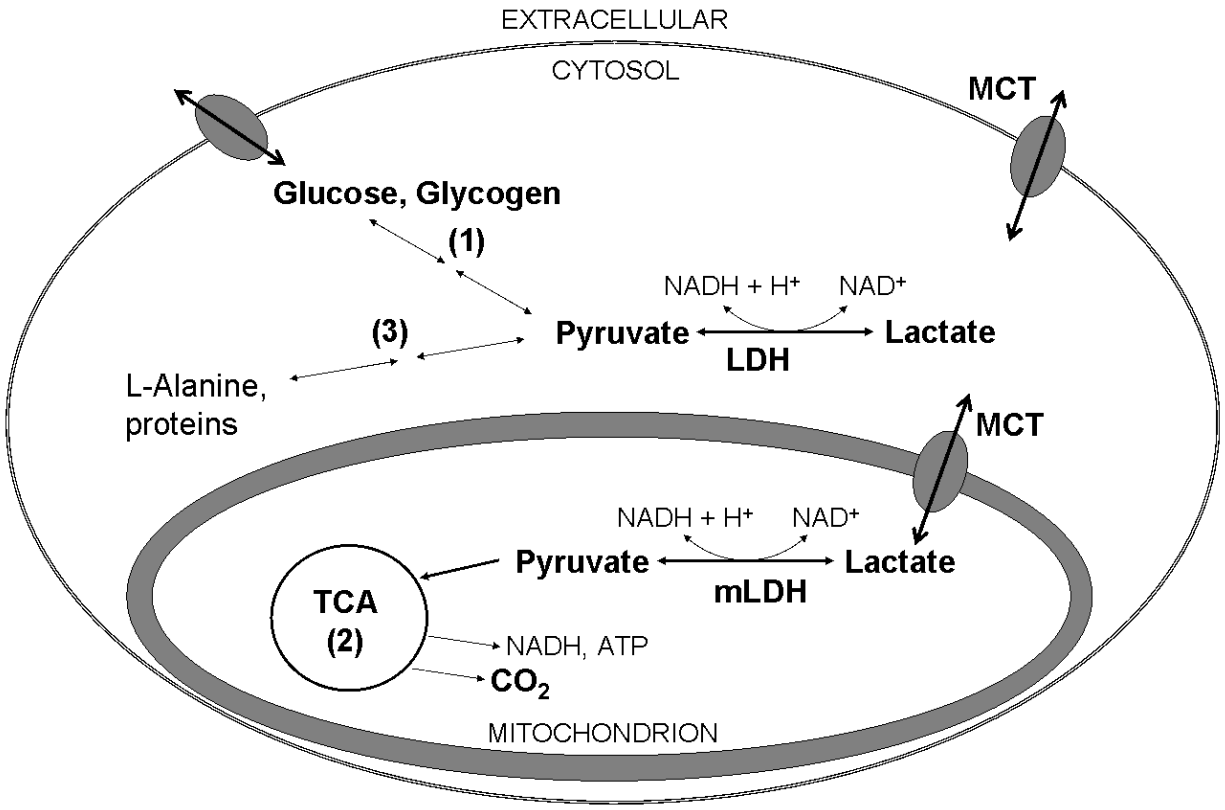


Figure 2: Lactic Acid Metabolism.

Table 9:Summary table of relevant toxicokinetic studies

Method	Results	Remarks	Reference
Metabolism and distribution, literature review non-guideline, non GLP	Normal human plasma levels: 1 (rest) to 10 (exercise) mM; facilitated diffusion into cells and subcellular organelles by monocarboxylate transporter (MCT); NADH dependent conversion to pyruvate by cytosolic and mitochondrial lactate dehydrogenase (LDH)	Secondary literature, no original data, √	Sterenborg I, 2007, ENVIRON Report No. PU-LBD-20070039
Metabolism, intra-venous; Human/ Rat/ Sheep/ Dog non-guideline, non-GLP	Lactate turnover at rest [g/kg bw/d] Human: 1.6-2 Sheep: 1.6 Dog: 2.3-3.5 Rat: 4.9-8.1 Clearance [mL/min/kg bw] Human: ~ 22	No DocIII summary, no primary data (data evaluation only), √	Connor H and Woods HF, 1982, Metabolic acidosis. Pitman Books Ltd London (Ciba Foundation symposium 87), 214-234
Rat, strain not specified, 1-3 M + 4-8 F non-guideline, non-GLP	~ 210 mg/kg bw, single dose: Oral absorption at 1h: 26 % 2h: 44 % 3h: 62 % 4h: 76 %	Lactic acid racemate; Additional reference, added by DS, no DocIII summary	Cori GT, 1930, The Journal of Biological Chemistry 87, 13-18
Toxico-kinetics and metabolism, literature review / non-guideline, non-GLP	1) Oral (gavage): Rat, F344, 5M, Dose level: ~ 2 g/kg bw: 42 % converted to CO2 within 6 h (racemic mixture) 2) Intra-venous: Human ; Dose level not stated: Vd ~ 0.5 L/kg; turnover 2.3 g/kg bw/d; 88 % conversion to CO2 3) Oral: Dog: Dose level not stated (Na-DL-lactate): 100 % oral absorption	Secondary literature, no original data	Andersen FA, 1998, International Journal of Toxicology 17, Supplement 1, 1-241
Toxicokinetics and metabolism, Oral (feeding) non-guideline, non-GLP	1) Pig, Large White, 18 M/F; Dose level: 0-1.9 g/kg bw/d, 2 weeks: Increase in plasma conc.: 0.02/0.03 g/L (D/L-lactic acid); increase in urine conc.: 0.12/0.02 g/L 2) Rat, Wistar, 12 M/F, Dose level: 0-5.6/5.8 g/kg bw/d (D/L-lactic acid), 3 weeks (gradually replacement of standard diet to experimental diet during week 1) : increase in plasma conc.: 0.23/0.04 g/L (D/L-lactic acid); increase in urine conc.: 0.84/0.07 g/L	1)None; 2)Additional reference, added by DS, no DocIII summary	Everts H et al., 2000, Journal of Animal Physiology and Animal Nutrition 83: 224-230

Method	Results	Remarks	Reference
Excretion, intravenous injection of sodium r-lactate, dogs (breed not specified) non-guideline, non-GLP	1. According to the dosage and the rapidity of the injection, lactate is excreted unchanged in the urine to about 7 to 40 % of the amount injected. The excretion is practically completed within 3 hours after the completion of the injection. 2. The excreted lactate appears to be mainly racemic. 3. There is little or no excretion of lactate into the intestinal tract. 4. The fall in concentration of blood lactic acid is synchronous with the fall in urinary lactate concentration. The kidney was able to concentrate sodium lactate about tenfold.	Doses varied between 5.4 to 30 g/kg bw	Abramson and Eggleton, 1927. Journal of Biological Chemistry 745-752
Lactate and glucose interactions during rest and exercise in men: effect of exogenous lactate infusion.	In risk assessment, the natural exposure to lactic acid in food (fruit, vegetables, sour milk products, fermented products such as sauerkraut, yogurt and beer) and via endogenous sources, as well as exposure via the use of lactic acid as a food additive (E270) should be considered. The evaluation of the human health effects of lactic acid should be based on a comparison of this background exposure and the potential contribution of lactic acid in biocidal products to these levels. An estimate of the daily consumption of lactic acid due to its natural presence in food was made using the 'FAO/WHO standard European diet'. A (minimum) daily intake of 1.175 g/person/day was calculated. A daily intake of 1.65-2.76 g/person/day was estimated based on the amount of lactic acid put onto the market (EU and USA) as a food additive. The total daily intake of lactic acid via food, both naturally and as food additive, was estimated to be 2.8 g/person/day.		Miller, B.F.et al., 2002. Journal of Physiology Vol.544, Nr.3, p.963-975
Lactate - a signal coordination cell and systemic function.			Philp, A. et al., 2005. The Journal of Experimental Biology, Vol.208, p.4561-4575

√: also mentioned in the registration dossiers

4.1.2 Human information

In principal, toxicokinetics are similar between humans and animals. Physiological plasma levels in man range between 1 mM at rest and 10 mM during exercise (Sterenborg, 2007). Very similar levels have been reported in other mammalian species. The total normal lactate turnover at rest has been determined as 1.6 – 2 g/kg bw/d in humans (Connor and Woods, 1982).

In humans, a volume of distribution of approx. 0.5 L/kg was determined after intravenous application of an unknown dose. 88 % of this dose was exhaled as CO_2 and the total turnover rate was 2.3 g/kg bw/d (Andersen, 1998), and thus similar to that reported above in animals (Connor & Woods, 1982). In humans, systemic L-(+)-lactic acid is cleared rapidly at a rate (at rest) of approx. 1.8 g/kg bw/d and absorbed L-(+)-lactic acid adds to the plasma background level of around 1 mM at rest (Sterenborg, 2007).

4.1.3 Summary and discussion on toxicokinetics

Although a OECD guideline study regarding the toxicokinetics of L-(+)-lactic acid is not available, the wealth of data generated in animal and human studies can be brought in agreement and allows for adequate characterisation of the a.s.: Oral administration of L-(+)-lactic acid is followed by fast and practically complete absorption from the GI-tract with an absorption half-life in the order of 2-3 hours. Distribution occurs into a volume of approx. 0.5 L/kg bw. In dogs and rats, normal lactate turnover rates are approx. 3 and 6 g/kg bw/d, respectively. Absorbed L-(+)-lactic acid adds to the plasma background level of > 1 mM at rest in animals. Metabolic conversion of L-(+)-lactic acid into CO_2 or biomacromolecules (glycogen etc.) account for the majority of its clearance. Significant renal elimination was observed only following bolus injection of large doses, but not after protracted application such as infusion of feeding. Minimal increases in plasma lactic acid concentrations following feeding of large doses that were in the range of the normal background turnover rate (i.e. ~ 5 g/kg in rats) suggest that the clearance capacity for lactic acid is significantly larger than this background turnover rate and that the enzymatic and transport processes involved are well regulated within this range.

4.2 Dermal absorption

Dermal absorption of various lactic acid formulations was tested on human and pig skin *ex vivo* over 6 hours (Andersen, 1998; Sah et al., 1998) and in rats *in vivo* over 3 days (Andersen, 1998). Data on the technical product was not provided. Depending on the formulation and the pH, dermal absorption *ex vivo* ranged from 10 to 30 % in human and from 7 to 32 % in pig skin. Lactic acid absorption was higher at pH 3 or 3.8 (lowest pH tested) than at pH 7 and was different depending on formulation, with a decrease in the order o/w > w/o/w > w/o. Dermal penetration of lactic acid from a 5 % o/w cream formulation through rat skin was 50 % in 3 days (Andersen, 1998). These data as well as the physicochemical properties of the technical product support the default value of 75 % dermal absorption as realistic worst-case assumption. According to the EFSA guidance on dermal absorption (2012) this default value should be used for products containing ≤ 5 % active substance. For products containing > 5 % active substance the default value of 25 % should be used.

Table 10: Summary table of dermal absorption studies

Method	Results	Remarks	Reference
Dermal absorption, literature review, non-guideline, non-GLP	1) Human, *ex vivo* (abdominal, full thickness) 5 % at pH 3 or pH 7, 6 hours: Total absorption: pH 3: 30.4 ± 3.3, pH 7: 9.73 ± 2.03 % (2 % PEG-100 stearate, 1 % laureth -4) 2) Rat, *in vivo:* 5 % in o/w [1] cream, 3 days: 50 % penetration through skin	Secondary literature, no original data, √	Andersen FA, 1998, International Journal of Toxicology 17, Supplement 1, 1-241

Method	Results	Remarks	Reference
Dermal absorption, non-guideline, non-GLP	Pig, *ex vivo* (female dermatomized skin) 8 % in o/w, w/o [2] or w/o/w [3] formulation, 6 hours: Total absorption: o/w formulation: pH 3.8: 25 % (+5 % propylene glycol: 32 %); pH 7: ~7 %; w/o formulation: pH 3.8: ~11 %; absorption is pH-and formulation-dependent: o/w > w/o/w > w/o	None, √	Sah A et al. 1998, J Cosmet Sci 49:257-273

[1] o/w: oil-in-water
[2] water-in-oil
[3] water-in-oil-in-water
√ also mentioned in the registration dossiers

4.3 Acute toxicity

4.3.1 Non-human information

4.3.1.1 Acute toxicity: oral

L-(+)-lactic acid is of low toxicity in the rat after oral exposure. The oral LD50 of lactic acid in the rat is 3543 mg/kg bw. Main effects observed in oral studies were ataxia, lethargy, prostration, irregular breathing and local irritation of the gastrointestinal tract. The LD_{50} value for guinea pigs was reported to be 1810 mg/kg bw (Smyth et al., 1941). This value would support classification in Cat. 4. The reference is an old publication and has many deficiencies, i.e. animal housing, clinical signs, body weight, necropsy and individual data are not reported, information on GLP and guideline conformity is not given. The discussion is mainly focussed on the acute oral toxicity of glycols and their esters, ethers, ether-esters, and chlorine and nitrogen compounds. Lactic acid, glycerol, methanol, ethyl alcohol and formaldehyde were tested as representatives of related non-glycols. Thus, the publication has a low reliability and it can be assumed that the acute oral toxicity in guinea pigs is also based on the local irritation in the gastro-intestinal tract. In conclusion and bearing in mind, that the preferred species for acute toxicity testing is the rat, the LD_{50} values of guinea pigs are considered not suitable for classification purposes.

Table 11: Summary table of relevant acute oral toxicity studies

Method	Results	Remarks	Reference
Oral, gavage, Rat, Albino, 1 M + 1 F (range-finding); 5 M + 5 F (main study): 3,162 (F)-3,548 (F)-3,981 (F)-4,467-5,012-5,623-6,310 mg/kg bw Similar to OECD 401, GLP	4936/3543 mg/kg bw (M/F); Lowest lethal dose: 3162 (F, 1/5); 4467 (M, 1/5). Main effects: ataxia, lethargy, prostration, irregular breathing, salivation, lacrimation, crusty eyes; discoloured lungs; erosion, ulceration and haemorrhages of stomachs	L(+) Lactic acid content of test article: 80 % (liquid), vehicle purity, source not reported, √	Wingard B & Barnes TB 1984, Toxigenics study no. 410-1369

Method	Results	Remarks	Reference
Oral, gavage, Rat, Albino, 5 M + 5 F, 5,000 mg/kg bw Similar to OECD 420, GLP	> 5000/ < 5000 mg/kg bw (M/F) Mortality at 5000 mg/kg bw: M: 1/5 F: 5/5 Main effects: lethargy, salivation, ataxia, irregular breathing, lacrimation, crusty eyes, crusty nose, prostration, black discoloration and dark contents of/in glandular stomach, discoloured lungs and trachea, dilatation of stomach	L(+) Lactic acid content of test article: 80 %; (liquid), vehicle purity, source not reported, No DocIII summary, √	Wingard B & Barnes TB 1983, Toxigenics study no. 410-1353
Oral, gavage, Rat, Wistar, 10 M; Guinea pig, strain not specified, 10 animals/ dose group Dose level: up to 5,000 mg/kg bw Pre-guideline, non-GLP	Rat: 3730 mg/kg bw Guinea pig: 1810 mg/kg bw Main effects not reported	Lactic acid, vehicle: Water, additional reference, added by DS, no DocIII summary, many deficiencies noted.	Smyth et al. 1941, J Ind Hyg Toxicol 25(6): 259-268

√: also mentioned in the registration dossiers

4.3.1.2 Acute toxicity: inhalation

L-(+)-lactic acid is of low toxicity in the rat inhalative exposure. The inhalative LC_{50} in the rat is > 7.94 mg/L air x 4 h (aerosol, nose only exposure, 4 h). Weight loss in females, rapid, shallow breathing, hunched posture, lethargy at 1 and 3 hours after exposure, and lacrimation were the main toxicological signs observed in an acute inhalative toxicity study. One female rat of the treated group died on day 5 post-treatment. This animal was hunched with laboured breathing and gasping on day 7. At necropsy no gross lesions were reported.

Findings relating to changes in respiratory pattern were transient but indicative for respiratory tract irritation (rapid breathing and eye tearing during exposure in the inhalation chamber). All animals (including the controls exposed to air only) had a hunched posture, red stained fur surrounding the eyes (tearing), ruffled fur, and appeared ungroomed with soiled fur (stained brown) one and three hours after exposure. Female rats exposed to the test article SY-83 (80-85 % L-(+)-lactic acid as aerosol) appeared lethargic at one (2/5) and three hours (5/5). The two female rats that were lethargic at one hour also had rapid, shallow breathing and appeared to be gasping at both one and three hours. The animals appeared normal by 24 hours and during the observation period. One female from the treated group had hunched posture, rapid and shallow breathing, and slight tremors, but these signs were observed only on day 5 post-treatment. All groups of male rats gained weight within the first week after exposure in comparison to pre-exposure weights (3 % for sham-exposed, 2 % for SY-83, respectively). Female rats in the sham group gained weight during the first week after exposure (less than 1 %). Female rats in the treated group lost weight during the first week after exposure (7 %). After 14 days, all surviving animals had gained weight in comparison to pre-exposure weights (14 % for males, 7 % for females). No significant differences were observed in body weight between treated and control groups. No gross lesions were observed at necropsy, histopathological evaluation was not performed. Neither information on histopathological findings nor practical observations in humans are available (see also Section 4.5.3 Respiratory tract irritation).

Table 12: Summary table of relevant acute inhalation toxicity studies

Method	Results	Remarks	Reference
Inhalative, nose-only, aerosol, Rat, Fischer 344, 5 M + 5 F Dose level: 0 and7.94 mg/L air x 4 h Similar to OECD 403; GLP	LC_{50}: > 7.94 mg/L Mortality at 7.94 mg/L: M: 0/5 F: 1/5 Main effects: eye tearing, rapid, shallow breathing, lacrimation, hunched posture, lethargy, weight loss (F)	Aerosol, L(+) Lactic acid content of test article: mean 80 %; mortalities: 1/10, √	David RM 1987, Microbiological Associates Inc. Report no. I-7083.112

√: also mentioned in the registration dossiers

4.3.1.3 Acute toxicity: dermal

L-(+)-lactic acid is of low toxicity in the rabbit after dermal exposure. The dermal LD_{50} is > 2000 mg/kg bw. Signs of corrosivity were observed in a dermal study in the rabbit (see also Table 17).

Table 13: Summary table of relevant acute dermal toxicity studies

Method	Results	Remarks	Reference
Dermal occlusive (abraded skin) Rabbit, New Zeeland White, 5 M + 5 F; Dose level: 2000 mg/kg bw Similar to OECD 402, GLP	LC_{50}: > 2000 mg/kg bw No mortality at 2000 mg/kg bw, Main local effects: severe erythema and edema, necrosis, eschar formation, blanching, denuded areas, atonia	L(+) Lactic acid content of test article: 80 %, √	Wingard B & Barnes TB 1983, Toxigenics study no. 410-1354

√: also mentioned in the registration dossiers

4.3.1.4 Acute toxicity: other routes

No studies with application via other routes were available.

4.3.2 Human information

A case report from a fatal accidental poisoning is available from the literature. A woman received ~33 g lactic acid (100 ml of a 33 % aqueous solution) via duodenum tube in a hospital. She reported immediate pain, vomited blood and had blood in the urine. She developed dyspnoea and cyanosis and died 12 h after administration. Necropsy revealed corrosion of the stomach and the duodenum with necroses, haemorrhages, bleeding, and thromboses of most blood vessels of the gastrointestinal tract. Tissue distribution 4 d post mortem revealed high lactic acid levels in the gastrointestinal tract.

Table 14: Human information

Kind of study	Examination methods, number of individuals examined	Results	Reference
Case report, fatal accidental poisoning (exposure to ca. 33 g lactic acid by stomach tube)	Clinical observation, (histo-) pathology, tissue distribution of lactic acid 4 d p.m.; 1 F	Pain, vomiting, gastrointestinal necrosis, haemorrhages and bleeding, cyanosis, dyspnoea, death within 12 h, 4 d p.m.: from analysis of lactic acid content in different organs an estimate of 17 g lactic acid is given, highest levels in the gastrointestinal tract	Führer H 1932, Arch Toxicol 3(1):71-74, in German; Additional reference, added by the DS, no DocIII summary

4.3.3 Summary and discussion of acute toxicity

L-(+)-lactic acid is of low toxicity in the rat after oral, dermal and inhalative exposure. The oral LD_{50} of lactic acid in the rat is 3543 mg/kg bw, the dermal LD_{50} in the rabbit is > 2000 mg/kg bw and the inhalative LC_{50} in the rat is 7.94 mg/L air x 4 h (aerosol, nose only exposure). Main effects observed in oral studies were ataxia, lethargy, prostration, irregular breathing and local irritation of the gastrointestinal tract. In a dermal study in the rabbit signs of corrosivity were observed. Weight loss in females, rapid, shallow breathing, hunched posture, and lacrimation were the main toxicological signs observed in an acute inhalative toxicity study.

4.3.4 Comparison with criteria

Comparison with criteria for classification and labelling and conclusion is summarised in Table 15 presenting the toxicological results in comparison with CLP criteria.

Table 15: Results of acute toxicity studies in comparison with CLP criteria

Method	Results
Oral LD_{50}, rat: 3543 mg/kg bw	Cat 4 (H302): $300 < LD_{50} \leq 2000$ mg/kg (oral)
Inhalation LC_{50}, rat: 7.94 mg/L air (nose-only, aerosol, 4-h)	Cat. 4 (H332): $10.0 < LC_{50} \leq 20.0$ mg/L (vapours) $1.0 < LC_{50} \leq 5.0$ (dusts and mists)
Dermal LD_{50}: > 2000 mg/kg bw (m/f)	Cat. 4 (H312): $1000 < LD_{50} \leq 2000$ mg/kg (dermal)

4.3.5 Conclusions on classification and labelling

In summary and based on the submitted data, L-(+)-lactic acid does not meet the criteria to be classified for acute oral, dermal or inhalative toxicity according to the criteria of the CLP regulation.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS did not propose classification for acute toxicity as all relevant LD_{50}/LC_{50} values were above the thresholds for classification for all routes of exposure.

Comments received during public consultation

There were no comments provided in the public consultation regarding this hazard class.

Assessment and comparison with the classification criteria

Acute toxicity: oral

There are three studies in rats and one in guinea pigs. Two of the rat studies were performed according to EPA´s OPP (Office of Pesticide Programs) test guidelines (1982). The other two studies are pre-guideline studies, conducted in 1941.

The lowest LD_{50} value in rats is 3543 mg/kg bw (see CLH report, Table 11), whereas the LD_{50} in guinea pigs reported in the 1941 study was 1810 mg/kg bw. Although the latter study would support a classification as Acute Tox. Cat. 4, RAC agrees not to classify lactic acid because the guinea pig study covered many substances with focus on glycols and their esters and suffer from several deficiencies (e.g. necropsy and individual data were not reported). Therefore, RAC does not consider the guinea pig study relevant for classification, especially as there are two rat studies showing LD_{50} values > 3500 mg/kg that are both GLP-compliant and are similar to OECD guidelines (see CLH report, Table 11).

RAC notes that the guideline rat studies are conducted with 80% L-(+)-lactic acid instead of 93% (the highest obtainable concentration of the active substance, according to the CLH dossier). Although a higher concentration is likely to be more toxic (irritative/corrosive),

the oral LD_{50} values caused by the 80% lactic acid were so much higher than the threshold for classification that it is not expected that a higher concentration than 80% would fulfil the criteria. Therefore, **RAC does not propose a classification for acute toxicity via the oral route.**

Acute toxicity: inhalation

In one rat study conducted according to EPA´s OPP test guidelines (1985) and similar to OECD TG 403, the acute inhalation LC_{50} value was > 7.94 mg/L/4h (the only dose level tested, 1/10 animals died at this dose level) with a concentration of 76.5-83.5% lactic acid in the aerosol. The limit for classification for acute toxicity 4 via inhalation route (mists) is 1.0 mg/L/4h < ATE ≤ 5.0, therefore RAC supports the DS´s view that no classification is warranted, although the concentration of the test substance was 80% instead of 93% (see above).

Acute toxicity: dermal

In one rabbit study conducted according to EPA´s OPP test guidelines (1982) by Wingard & Barnes (1983), the acute dermal LC_{50} value is > 2000 mg/kg bw. RAC agrees with the DS that **no classification is justified**, although the concentration of the test substance was 80% instead of 93% (see above) as no mortality was observed in the tested animals.

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

Due to the irritant/corrosive nature of L-(+)-lactic acid local effects of the stomachs (erosion, ulceration, haemorrhages) were noted in rats after oral administration. These signs were accompanied by ataxia, lethargy, irregular breathing, and salivation. After acute inhalative exposure of SY-83 (80-85 % L-(+)-lactic acid in water) findings relating to changes in respiratory pattern were transient but indicative for respiratory tract irritation (rapid, shallow breathing and eye tearing during exposure in the inhalation chamber). Female rats in the treated group lost weight during the first week after exposure (7 %) (see also Section 4.5.3 Respiratory tract irritation). The administered doses in these studies were far in excess of the guidance value ranges for single-dose exposures leading to classification of STOT SE 1 / 2 (see Table 16).

4.4.1 Comparison with criteria

Table 16: Results of acute toxicity studies in comparison with CLP criteria for STOT SE

Method	Results
Oral LD_{50}- ,inhalation LC_{50}-, and dermal LD_{50}-values of lactic acid are greater than the limit dose, respectively (see Table 15). No significant and/or severe toxicity is reported in humans. Thus, classification of lactic acid with STOT SE 1 is not proposed.	Category 1 (H370) Oral (rat): C ≤ 300 mg/kg bw Dermal (rat or rabbit): C ≤ 1000 mg/kg bw Inhalative (rat, dust/mist/fume): ≤ 1 mg/L/4 h Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure - reliable and good quality evidence from human cases or epidemiological studies; or - observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations.
Oral LD_{50}- ,inhalation LC_{50}-, and dermal LD_{50}-values of lactic acid are greater than the limit dose, respectively (see Table 15). Thus, classification of lactic acid with STOT SE 2 is not proposed.	Category 2 (H371) Oral (rat): 2000 ≥ C > 300 mg/kg bw Dermal (rat or rabbit): 2000 ≥ C > 1000 mg/kg bw Inhalative (rat, dust/mist/fume): 5 ≥ C > 1 mg/L/4 h Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following single exposure - observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations
See Section 4.5.3 Respiratory Tract Irritation. Classification of lactic acid with STOT SE 3 (respiratory tract irritation) is proposed.	Category 3 (H335/H336) Guidance values do not apply (mainly based on human data) Transient target organ effects This category only includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2 indicated above. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function.

4.4.2 Conclusions on classification and labelling

Comparing the results of the toxicological studies with the guidance value ranges for single-dose exposures, no classification with STOT SE 1 / 2 is proposed. Classification of lactic acid with STOT SE 3 (respiratory tract irritation) is proposed (see Section 4.5.3 Respiratory Tract Irritation).

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

Summary of the Dossier Submitter's proposal

The DS proposed classification with STOT SE 3 (respiratory tract irritation), H335, based on transient rapid and shallow breathing and eye tearing in an acute inhalation study where rats were exposed for four hours to an aerosol consisting of 76.5-83.5% lactic acid.

Comments received during public consultation

Three industry organisations disagreed with the proposed classification for STOT SE 3. Their arguments focused on lack of human data, uncertain animal data, and that respiratory irritation is covered by classification for skin irritation and serious eye damage.

Assessment and comparison with the classification criteria

Concentrated lactic acid has a pH < 2. Substances and mixtures with a pH < 2 can be predicted to be irritating or corrosive to skin (CLP 3.2.2.1.2.3. and CLP 3.2.3.2.1.1.) and eyes (CLP 3.3.2.2.4.). Similar effects could be expected on epithelia of the respiratory system. Accordingly, the acute inhalation study in rats indicates transient respiratory effects, such as rapid and shallow breathing occurring shortly after exposure.

However, as there are neither any specific human data nor any pathological findings at necropsy in the acute inhalation toxicity rat study (histopathological evaluation was not performed) unequivocal evidence of transient irritation of the upper or lower respiratory tract has not been provided. **RAC, therefore, concludes that the DS´s proposal to classify L-(+)-lactic acid for STOT SE 3 is not justified on the basis of the available data.**

4.5 Irritation

4.5.1 Skin irritation

4.5.1.1 Non-human information

In vivo skin irritation/corrosivity studies with L-(+)-lactic acid were performed in rabbits, guinea pigs and pigs and *in vitro* with a biobarrier/chemical detection system and a skin organ culture model (rabbit and human skin).

In rabbits, full thickness destruction indicative of corrosivity was observed with SY-83 (88 % L-(+)-lactic acid (pH 1.83)) and 50 % L-(+)-lactic acid (Wingard and Barnes, 1983; van Beek, 1986).

SY-83 was evaluated for acute dermal toxicity at a dose level of 2000 mg/kg bw. The test article was applied to the skin (clipped free of hair and abraded) of 5 males and 5 females for 24 hours of exposure. Severe erythema and severe edema were observed at the test sites of all animals after removal on day 1. Erythema decreased in severity on days 12 or 14 for 3 animals; edema decreased in severity for 8 animals on day 2, and

was not observed for one female on day 12 and for one male on day 14. Thus, reversibility was only observed for edema. Other dermal reactions observed at test sites included: blanching, necrosis, eschar formation, atonia, desquamation, and fissures. Necropsy on day 14 revealed brown, crusted discolorations of the treated skin. The test article was considered to have corrosive properties (Wingard and Barnes, 1983, Report 410-1354, see also Table 13).A sample of lactic acid (50 %) was examined for acute dermal irritating/corrosive properties in an experiment with six albino rabbits.

After an exposure period of **4 hours** the patches and the material applied were removed and the resulting skin reactions were evaluated by the method of Draize et al.. The dermal effects generally observed in all rabbits consisted of very slight to slight ischemic necrosis, moderate to severe haemorrhages and slight or moderate oedema. After **28 hours** the dermal effects observed generally consisted of very slight to slight ischemic necrosis, moderate haemorrhages, slight or moderate incrustation and slight oedema. During the course of the following **two days** ischemic necrosis, haemorrhages and oedema were no longer observed. The application sites generally became crater-shaped with a central sunken area which was moderately or severely encrusted, and a surrounding, raised border of non-necrotic skin showing well-defined erythema. After **7 days** this picture had hardly changed, apart from the clearance of erythema. The central sunken areas of the application sites generally showed moderate to severe incrustation. At the end of the observation period, after **3 weeks**, some signs of healing were observed at the edges of the encrusted skin areas which had been in contact with the test material. Anyhow slight to severe incrustation, formation of scar tissue and disturbed hair growth was noted **3 weeks** post treatment.

In the new skin visible under the crust edges coming off from the treated skin, formation of scar tissue could be observed whereas hair growth was absent. There were no distinct differences between reactions of the intact skin and those of the abraded skin. On the basis of observations in earlier experiments with comparable results, performed at the same laboratory, the authors state that it is expected that the old necrotic skin will be ejected and that the formation of scar tissue will be continued. This scar tissue formed already or yet to be formed is not considered a reversible skin alteration (van Beek, 1986).

The test article, SY-83 (80-85 % L-(+)-lactic acid, pH 1.83), was evaluated for primary dermal irritation potential when applied to 2 intact and 2 abraded test sites on the skin of each of 6 albino rabbits and covered with impervious bandages for 24 hours. These 24 test sites were evaluated for erythema, edema, and other lesions at 30 to 60 minutes after test article removal. This study was designed to comply with the procedures described in the EPA/OPP Guidelines, 1982. The following clinical signs were observed at 30 to 60 minutes after test article removal: Severe erythema was observed at all test sites on 3 animals, and at both abraded sites on 2 other animals and at one intact site on one of these 2 animals. Moderate to severe erythema was observed at all test sites on one animal, and at one or both intact sites on 2 animals. Severe edema was observed at all test sites on 5 animals and at one intact site and one abraded site on the sixth animal. Slight edema was observed at the other 2 sites on the sixth animal. Blanching was observed at both abraded sites on all animals and at both intact sites on 5 of these animals. Yellow-brown color of the skin was observed at all sites on 3 animals and at either 2 or 3 test sites on 2 animals. A red exudate was observed at one intact site on one animal. Skin was missing at all test sites on one animal, at one intact and both abraded sites on one animal, and at one intact site or one abraded site on 2 other animals. No other dermal reactions were observed during the study. This study was terminated after the 30- to 60-minute evaluations upon the recommendation of an attending veterinarian due to the severity of the reactions observed. No abnormal clinical signs were observed and no mortalities occurred prior to sacrifice after the 30- to 60-minute evaluations (Wingard and Barnes, 1983).

No irritation or corrosivity was observed in rabbits when a 10 % aqueous dilution of L-(+)-lactic acid was tested (Prinsen, 1995).

Studies in guinea pigs and pigs revealed that these species are much less sensitive to dermal exposure to L-(+)-lactic acid. In these studies, L-(+)-lactic acid was tested non-irritant in concentrations up to 88 % (van Beek, 1987; Cuthbert, and Carr, 1986).

These results were confirmed by an *in vitro* Corrositex assay which revealed a biobarrier break through at a time of only 31 minutes of 90 % L-(+)-lactic acid (< 3 min: Skin Corr. 1A; 3 min to 4 h: Skin Corr. 1B/1C) (Harbell, 1994).

Lactic acid was examined for *in vitro* skin toxicity in skin organ cultures. Toxicity was determined by measuring epidermal cell proliferation and the conversion of the tetrazolium salt MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltei:razolium bromide). In rabbit skin, MTT conversion was statistically significantly reduced after exposure to HS88 (88 % L(+)-lactic acid in aqueous solution, pH not stated, but assumed to be <2). Possible species-specific irritant effects of lactic acid were tested *in vitro* by comparing rabbit skin to human skin. Based on the MTT assay and inhibition of epidermal cell proliferation, rabbit skin was clearly more sensitive to HS88 than human skin. A possible explanation for this difference is a lower skin absorption of the test substance in human skin, since rabbit skin is generally more permeable for topically applied chemicals than human skin (ECETOC, 1993). The anionic surfactant sodium dodecyl sulfate (SDS) was used as a reference substance to enable comparison of the *in vitro* results of this study to previous data obtained with the skin organ culture model. Exposure of rabbit skin for 30 minutes to 5 % SDS (5 % L(+)-lactic acid aqueous solution, pH not stated) induced a decrease of MTT conversion of approximately 15 %. Human skin was less sensitive to SDS than rabbit skin, which is in agreement with results obtained in previously performed studies (van de Sandt and Rutten (1995b) and unpublished data). It has been reported that 5 % SDS is a moderate irritant in rabbits (Gad et al., 1986) and human volunteers (Willis et al., 1988). In conclusion, this *in vitro* skin toxicity study revealed that rabbit skin was more sensitive to HS88 than human skin (Van de Sandt, 1996).

Table 17: Summary table of relevant skin irritation studies

Method	**Results**	**Remarks**	**Reference**
Rabbit, New Zealand White, 5M+5F (acute dermal toxicity) Similar to OECD 404, GLP	Average score 24, 48, 72 h: 4, 4, 4 Severe erythema and edema, blanching, necrosis, eschar formation Reversibility: Edema: Yes; other skin lesions: No **Corrosive**	88 % L(+) lactic acid, pH 1.83; No DocIII summary	TB Barnes 1983, Toxigenics Study No 410-1354 (see Table 13)
Rabbit, New Zealand White, 6M OECD 404, GLP	Average score 4, 28, 52, 76 h: 3.7, 3.5, 3.7, 3.5 Erythema (eschar, scar formation): No; Edema: Yes **Corrosive**	50 % lactic acid, pH not stated; No DocIII summary	L van Beek 1986, TNO Project No 85-0067/2
Rabbit, New Zealand White, 6M OECD 404, GLP	Average score 24, 48, 72 h: 0, 0, 0 **Not irritating**	10 % lactic acid + sodium lactate, pH 4	MK Prinsen 1995, TNO Project No 450061/12
Pig, Large White x Dutch Landrace (F1), 3M OECD 404, GLP	Average score 24, 48, 72 h: 0, 0, 0 **Not irritating**	88 % lactic acid, pH not stated (assumed to be < 2), √	L van Beek 1987, TNO Project No B 87-0405/270419
Pig, Large White x Dutch Landrace (F1), 3M OECD 404, GLP	Average score 24, 48, 72 h: 0, 0, 0 **Not irritating**	50 % lactic acid, pH not stated, no DocIII summary, √	L van Beek 1987, TNO Project No B 87-0406/270419
Guinea pig, Dunkin Hartley, 6F Similar OECD 404, GLP	Average score 24, 48, 72 h: 0, 0, 0 **Not irritating**	88 % lactic acid, pH not stated (assumed to be < 2); No DocIII summary, √	JA Cuthbert & SMA Carr 1986, IRI Report No 3625
Corrositex *in vitro* assay, biobarrier + chemical detection system Non-guideline, non GLP	**Corrosive**	90 % lactic acid, pH not stated (assumed to be < 2); No DocIII summary	JW Harbell 1994, Microbiological Associates Inc., Project No. A000449

Method	Results	Remarks	Reference
Acute dermal irritation/corrosion test with lactic acid (88 %) in albino rabbits	Corrosive	88 % lactic acid, pH not stated (assumed to be < 2), No DocIII summary, √	Van Beek, L., 1986; TNO, Report 86.016
In vitro skin irritation study in rabbit and human skin cultures after 30 minutes exposure to lactic acid and lactic acid esters.	Rabbit skin is more sensitive to HS88 than human skin.	88 %+5 % L(+)-lactic acid aqueous solution (pH not stated, (assumed to be < 2) 10 % L(+)-lactic acid buffered solution, ph 4), √	Van de Sandt, J., 1996, TNO Report No. V96.636
Primary dermal irritation study in rabbits using SY-83 (80-85 % L-(+)-lactic acid	Corrosive	SY83 (pH 1.83) no further information, √	Wingard, B. and Barnes, T.B., 1983, ToxiGenics, Inc., Study No. 410-1355.

√: also mentioned in the registration dossiers

4.5.1.2 Human information

In vivo skin irritation/corrosivity studies with L-(+)-lactic acid were performed in humans and with human skin *in vitro* (transcutaneous electrical resistance, TER). Irritation and corrosivity was observed in the human patch test and in the *in vitro* assay, respectively. The objective of the 4-hour human patch test was to determine whether the test materials (beside other L-(+)-lactic acid, positive control sodium dodecyl sulfate (SDS)) should be classified as "irritant to the skin" by exposing approximately 30 volunteers to each test material for up to 4 hours. The sequential single patch test procedure (short exposure time up to 4 hours) permits the development of a "positive" but not "severe", irritant response. It involved the application of 0.2 ml 88 % L-(+)-lactic acid on to a 25 mm Plain Hill Top Chamber containing a Webril pad. To avoid the production of unacceptably high reactions a cautious approach to testing was adopted. The test materials were applied progressively from 15 and 30 min through 1, 2, and 4 hours. The 15 and 30 min exposure periods were omitted if the study director was satisfied that excessive reactions would not occur following the 1 hour exposure. The upper outer arm was used as the treatment sites. Treatment sites were assessed for the presence of irritation potential using a 4 point scale at 24, 48, and 72 h after patch removal. A volunteer reacting with +, ++, or +++ reaction at any one of the assessment times was considered to have demonstrated a "positive" irritant reaction, and treatment was terminated on that person. In each panel 2 or 3 test materials were tested with SDS as positive control. The principle of the patch testing procedure and the testing approach to avoid the production of strong responses had been approved by local ethical review committees and the experiments were performed in accordance with the Helsinki Declaration of 1975.

Table 18: Summary table of relevant skin irritation studies in humans

Method	Results	Remarks	Reference
Human 4-hour Patch Test (upper outer arm), 26 humans (sex not stated) *in vivo* were tested sequential single patch test procedure: 0.2 ml were applied on a Plain Hill Top Chamber and applied progressively from 15-30 minutes through 1, 2, 3 and 4 hours. Non-guideline, GLP	No positive reactions were observed at assessment at 24, 48, and 72 hours after treatment when volunteers were treated for 15 minutes, 30 minutes, or 1 hour. After application times of 2, 3, and 4 hours, a total of 21 of the 26 volunteers who completed treatment had an irritant reaction to L-(+)-lactic acid at either 24, 48 or 72 h.	88 % lactic acid, pH not stated	York M et al. 1996, Contact Dermatitis 34:204-212

	Conclusion: 9/26 volunteers exhibited mild positive erythema, 12/26 volunteers exhibited moderately to strong positive reactions (Grading and description adopted from Fregert S. *Manual of contact dermatitis*, 1981) pos. control (erythema): 20 % sodium dodecyl sulfate: 15/25) Erythema was reversible. **Irritating**		
Human skin *in vitro* (Transcutaneous electrical resistance, TER) Similar to OECD 430; non-GLP	TER: 2.3 ± 0.2 kΩ/disc (≤ 11.0 kΩ/disc = corrosive) **Corrosive**	Purity lactic acid: 88 %, Test conc.: undiluted, pH not stated	York M et al. 1996, Contact Dermatitis 34:204-212

4.5.1.3 Summary and discussion of skin irritation

In vivo skin irritation/corrosivity studies with L-(+)-lactic acid were performed in rabbits, guinea pigs, pigs, and humans and *in vitro* with a biobarrier/chemical detection system as well as with human skin (transcutaneous electrical resistance, TER).

In rabbits, full thickness destruction indicative of corrosivity was observed with 88 % L-(+)-lactic acid (pH 1.83) and 50 % L-(+)-lactic acid. This result was confirmed by an *in vitro* Corrositex assay which revealed a biobarrier break through at a time of only 31 minutes of 90 % L-(+)-lactic acid (< 3 min: Skin Corr. 1A; 3 min to 4 h: Skin Corr. 1B/1C). No irritation or corrosivity was observed in rabbits when a 10 % aqueous dilution of L-(+)-lactic acid was tested.

However, experience from humans and studies in guinea pigs, pigs, and humans revealed that these species are much less sensitive to dermal exposure to L-(+)-lactic acid. In these studies, L-(+)-lactic acid was tested non-irritant in concentrations up to 88 % (pig, guinea pig) or irritant (human).

In general, substances with a pH-value ≤ 2 and ≥ 11.5 may indicate potential to cause corrosive skin effects. From the patch test studies in humans, it is likely that dermal irritation studies in pigs underestimate the irritating potential of L-(+)-lactic acid for human skin while rabbit skin seems to be much more sensitive than human skin. Also ECETOC (2002) reported that existing data indicate that human skin is, in most cases, less sensitive than rabbit skin. Also the *in vitro* human skin transcutaneous electrical resistance and the *in vivo* human 4-hour patch test (York et al. 1996) are publications, have some deficiencies (not conducted according to a guideline, lot/batch number of the test material L-(+)-lactic acid (88 %) not mentioned, test material not specified, purity and stability of the test material not mentioned), and has a reliability factor of 2, they represent direct methods of assessing skin irritation hazard to man, by using the endpoint of concern in the species of concern. From this study it is possible to assess the skin irritating potential of L-(+)-lactic acid in humans. Therefore, the human patch test and the TER data should be used as key study showing adequate results for classification and labelling.

4.5.1.4 Comparison with criteria

Table 19: Results of skin irritation studies in comparison with CLP criteria

Toxicological result	CLP criteria
In vivo, acute dermal toxicity in rabbits, Test substance: 88 % L-(+)-lactic acid (pH 1.83); Exposure: 24 hours; Observation: 14 days; Result: Full thickness destruction of the skin; corrosive (Wingard and Barnes, 1983; Report 410-1354).	Since corrosivity is assessed after a maximum of ≤ 4 hours exposure, CLP criteria do not apply to this study.
In vivo, rabbit, Test substance: 50 % L-(+)-lactic acid (pH not stated); Exposure: 4 hours; Observation: 28 hours, 2, 7 and 21 days, Result: after 4 hours: very slight to slight ischemic necrosis, moderate to severe haemorrhages,slight or moderate oedema; after 28 hours: slight ischemic necrosis, moderate haemorrhages, slight or moderate incrustation, slight oedema; after 2 days: crater-shaped skin with a central sunken area, moderately or severely encrustation, raised border of non-necrotic skin with well-defined erythema; after 7 days: moderate to severe incrustation; after 3 weeks: slight to severe incrustation, formation of scar tissue and disturbed hair growth: corrosive (van Beek, 1986).	Category 1C: Corrosive ; Exposure > 1 hour - ≤ 4 hours, Observation; 14 days
In vivo, rabbit, Test substance: 80-85 % L-(+)-lactic acid (pH 1.83); Exposure: 24 hours; Observation: 30-60 min. after test article removal; Result: Severe erythema, severe edema, blanching, yellow-brown color of the skin, red exudate (Wingard and Barnes, 1983; Report 410-1355).	Since corrosivity is assessed after a maximum of ≤ 4 hours exposure, CLP criteria do not apply to this study.
In vivo, rabbit, 10 % L-(+)-lactic acid (pH 1.83): No irritation or corrosivity (Prinsen, 1995).	CLP criteria do not apply to this study.
In vivo, pig, 88 and 50 % L-(+)-lactic acid: No irritation or corrosivity (van Beek, 1987).	CLP criteria do not apply to this study.
In vivo, guinea pig, 88 % L-(+)-lactic acid (Cuthbert & Carr 1986	CLP criteria do not apply to this study.
In vitro Corrositex assay, biobarrier + chemical detection system, 90 % L-(+)-lactic acid: Corrosive (Harbell 1994)	Category 1B: Corrosive ; Exposure > 3 minutes - ≤ 1 hour, Observation; 14 days
In vitro, rabbit and human skin organ cultures, 88 % L(+)-lactic acid: Based on MTT conversion, rabbit skin is more sensitive than human skin (Van de Sandt, 1996).	CLP criteria do not apply to this study

Toxicological result	CLP criteria
In a human 4-hour Patch Test, no positive reactions were observed at assessment at 24, 48, and 72 hours after treatment when volunteers were treated for 15 minutes, 30 minutes, or 1 hour. After application times of 2, 3, and 4 hours, a total of 21 of the 26 volunteers who completed treatment had an irritant reaction to L-(+)-lactic acid (88 %) at either 24, 48 or 72 h. Out of these, 9 volunteers exhibited mild positive reactions, and 12 volunteers exhibited moderately to strong positive reactions (Grading and description adopted from Fregert S. *Manual of contact dermatitis*, 1981) Conclusion: Mild erythema (+): 9/26 volunteers Mild to strong erythema: 12/26 volunteers	The CLP Regulation does not contain clear criteria for classification for skin irritation based on human data. Anyhow, the data obtained in the human 4-hour Patch Test are considered appropriate for classification and labelling of lactic acid.

4.5.1.5 Conclusions on classification and labelling

Based on the *in vivo* human 4-hour patch test (York et al. 1996), which is considered the key study for the proposal on skin irritation, criteria for skin corrosive effects of L-(+)-lactic acid are not given. In the study skin irritation effects in the form of mild to strong erythema was observed. Corrosive reactions, i.e. necrosis through the epidermis and into the dermis, ulcers, bleeding and bloody scabs were not noted. As discussed above, rabbit skin seems to be much more sensitive than human skin. Also ECETOC (2002) reported that existing data indicate that human skin is, in most cases, less sensitive than rabbit skin. In summary and based on the submitted data especially the above mentioned study with human volunteers, L-(+)-lactic acid should be classified for skin irritation/corrosion. (Category 2: H315, Causes skin irritation).

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed to classify L-(+)-lactic acid for skin irritation/corrosion, category 2, H315 (Causes skin irritation), based on human data which are considered to provide the key information for classifying L-(+)-lactic acid according to CLP criteria.

Comments received during public consultation

In the only comment received for this hazard class, a MSCA suggested to classify lactic acid as a corrosive substance (Skin Corr. 1C) based on the rabbit study by van Beek (1986). In addition, the MSCA pointed out that such a classification would warrant the EU supplementary hazard statement EUH071, "corrosive to the respiratory tract".

Assessment and comparison with the classification criteria

Irritation/corrosivity was tested *in vitro* with a biobarrier/chemical detection system and in a skin organ culture model with rabbit and human skin and *in vivo* in rabbits, guinea pigs, pigs and humans. More recently, three studies have been published where lactic acid has been studied using five different *in vitro* skin models (Catarino *et al*., 2018; Desprez *et al*., 2015; Alépée *et al*., 2014). Although the studies have not been analysed in detail by RAC (they were not included in the CLH proposal), they are suggestive of corrosive effects of lactic acid in *in vitro* skin models.

In the acute dermal toxicity study in rabbits (using 80% L-(+)-lactic acid), in two acute dermal irritation/corrosion tests on rabbits (using 88% (pH not stated) and 80-85% (pH 1.83) L-(+)-lactic acid) irritative and corrosive effects such as necrosis, formation of scar tissue and blanching could be observed. The CLP and OECD TG compliant rabbit study by van Beek (1986) using 50% L-(+)-lactic acid (pH not stated) showed very slight to slight ischaemic necrosis, moderate to severe haemorrhage and slight oedema after an exposure duration of four hours. After 3 weeks slight to severe incrustation, formation of scar tissue and disturbed hair growth could be observed. In addition, 88% L-(+)-lactic acid was also corrosive *in vitro* on rabbit skin.

A non-GLP, non-guideline *in vitro* Corrositex assay by Harbell (1994) revealed a biobarrier (artificial biomembrane) break through time of 31 minutes of 90% L-(+)-lactic acid, which would correspond to corrosive 1B/1C.

Neither irritation nor corrositivity, however, was found in two studies in pigs and in one study in guinea pigs, testing L-(+)-lactic acid in concentrations up to 88%. All these three studies were GLP and OECD compliant.

York *et al.* (1996), conducted an *in vitro* (Transcutaneous electrical resistance, TER) corrosivity test on human skin and a Patch Test on 26 volunteers using 88% lactic acid (pH not known but assumed to be < 2). The substance was corrosive in the *in vitro* test. In the Patch Test (0.2 mL applied in a Plain Hill Top Chamber), reversible irritative effects were seen after application times of 2, 3 and 4 hours in 21 out of 26 volunteers. However, it is acknowledged that the exposure was stopped as soon as signs of irritation were observed. Thus, the study is not really designed to assess corrosion (further information on this study is provided in the section "Supplemental information – in depth analyses by RAC").

Overall, RAC is of the opinion that for L-(+)-lactic acid (pH 1.83) a classification for **Skin Corrosion Category 1C, H314** is justified due to the outcome of the rabbit study by van Beek (1986), finding corrosive effects of 50% L-(+)-lactic acid after 4 hours exposure, supported by two studies showing corrosion after exposure to concentrated lactic acid (Barnes 1983; Wingard and Barnes 1983). Category 1C applies when corrosion has been observed after an exposure duration of 1-4 hours. Corrosive effects at high concentrations are also demonstrated in the Corrositex assay and the human *in vitro* TER assay. Category 1C might also be supported by the human patch test, where effects only were observed when the exposure time exceeded 1 hour.

In addition, RAC agrees that the supplementary labelling with **EUH071 "corrosive to the respiratory tract"** is warranted, based on the fact that the substance is corrosive and based on the possibility of exposure to aerosols (see chapter 3.2.4.2. of Guidance on the Application of the CLP Criteria).

~~The GCL was discussed, and it was noted that whereas the GCL for corrosive 1C is normally 5%, the GCL for substances with a pH ≤ 2, which is the case for concentrated lactic acid, is 1%.~~

Supplemental information - In depth analyses by RAC

According to the table below, which is taken from the original paper by York *et al.* (1996), 15 volunteers showed positive reactions (of whom nine demonstrated either "++" or "+++" reactions) after two or three hours dermal exposure to lactic acid. As individuals with positive reactions were excluded for further exposure, only 11 volunteers were tested for four hours exposure, of whom six reacted positively (three demonstrated again a response higher than "+"). Lactic acid (88%) induced more severe reactions than sodium dodecyl sulphate (SDS, 20%) which is known to cause skin irritation.

Table 4. Patch test results for lactic acid and geraniol with SDS (20%) also tested as a reference material

Test material	Exposure time/results						
	15 min	30 min	1 h	2 h	3 h	4 h	Total +ves
lactic acid	0/28**	0/28	0/27**	8/27 (5)*	7/18** (4)*	6/11 (3)*	21/26
geraniol	0/28**	0/28	0/27**	0/27	0/25**	2/25	2/25
SDS (20%)	0/28**	0/28	0/27**	3/27	2/22**	10/20 (3)*	15/25

* Figures in parenthesis indicate number of (++) reactions and (+++) reactions.
** Person(s) dropped out of panel for reasons unrelated to treatment.

It is pointed out that more than one third of volunteers were not exposed for four hours. The authors of the study suggested a minimum classification of lactic acid as "irritating to skin".

4.5.2 Eye irritation

4.5.2.1 Non-human information

A chicken enucleated eye test (CEET *in vitro*) was performed with different L-(+)-lactic acid formulations and revealed different results:

- a highly damaging potential of L-(+)-lactic acid to the eye (HS 88: severe corneal opacity, corneal swelling and fluorescein retention),
- a moderately damaging potential of L-(+)-lactic acid to the eye (H60: moderate corneal opacity and moderate fluorescein retention by damaged epithelial cells) and
- a slightly damaging potential of L-(+)-lactic acid to the eye (BF S36: maximum mean corneal swelling of 6 % at 75 min after treatment, very slight corneal opacity and slight fluorescein retention by damaged epithelial cells).

The eyes were collected from a slaughter-house for chickens (which were killed for human consumption). In this *ex vivo* bioassay, three parameters were measured to disclose possible adverse eye effects, namely corneal thickness (expressed as corneal swelling), corneal opacity, and fluorescein retention. Three different forms of L-(+)-lactic acid are tested:

- Powder H60 (L-(+)-lactic acid solid adduct with Ca-lactate);
- Lactic acid HS88 (L-(+)- lactic acid aqueous solution);
- Lactic acid buffered BF S36 (a buffered solution of BF S36 Lactic acid B).

The test substances were used undiluted, and for the solid sample 0.03 g powder was applied. Exposure period was 10 seconds. After that, the corneal surface was rinsed with 20 ml of isotonic saline. Examination time points were at 0, 30, 75, 120, 180, and 240 min after treatment. The three L-(+)-lactic acid samples caused different corneal effects in the CEET: L-(+)-lactic acid solid adduct with Ca-lactate (powder): moderate corneal effects (irritating to eyes); L-(+)-lactic acid aqueous solution: severe corneal effects (severely irritating to eye); a buffered solution of BF S36 L-(+)-lactic acid: slight corneal effects (not irritating to eyes).

A summary of the maximum mean scores for corneal swelling, opacity and fluorescein, the irritation categories assigned, and final (EC-) classification of the three lactic acid samples is presented in Table 20.

- Powder H60 (powder, undiluted, 60 % lactic acid and 40 % Ca-lactate):

After treatment, the thickness of the cornea of the test eyes gradually increased considerably; a maximum mean corneal swelling of 17 % was obtained at 240 min after treatment. In addition, moderate corneal opacity and moderate fluorescein retention by damaged epithelial cells were observed in the test eyes. The irritancy categories assigned to these findings are also presented in table 22, together with the final irritancy classification. The categories defined for corneal swelling, corneal opacity, and fluorescein retention were: II, III, and III.

- Lactic acid HS88 (88 % L-(+)-lactic acid, aqueous solution):

After treatment, severe to complete corneal opacity was observed in the three test eyes, which hampered the measurement of corneal thickness at the 30, 75 and 120 minutes after treatment. At 180 and 240 minutes after treatment, corneal thickness could be measured again and at 240 minutes a maximum mean corneal swelling of 28 % was determined. All three eyes showed severe fluorescein retention by damaged epithelial cells. The categories defined for corneal swelling, corneal opacity, and fluorescein retention were: III, IV, and IV.

- Lactic acid buffered BF S36 (liquid, undiluted, buffered):

After treatment, only a slight increase in corneal thickness of the test eyes was observed. A maximum mean corneal swelling of 6 % was obtained at 75 min after treatment. Very slight corneal opacity and slight fluorescein retention by damaged epithelial cells were observed in the test eyes. The categories defined for corneal swelling, corneal opacity, and fluorescein retention were: II/I/II.

- Control eye: The control eye did not show any unusual effects.

Table 20: Summary of the maximum mean scores for corneal swelling, opacity and fluorescein and the irritation categories assigned

Test material	Maximum mean score for [1]:			Categories [1]	Classification
	Swelling	Opacity	Fluorescein		
H60 (powder, undiluted)	17	2.0	2.0	II/III/III (moderate corneal effects)	H318
HS 88 (liquid, undiluted)	28	4.0	3.0	III/IV/IV (severe corneal effects)	H318
BF S36 (liquid, undiluted, buffered)	6	0.5	1.0	II/I/II (slight corneal effects)	no classification

[1] See OECD 438 for description of criteria

The numbers indicate the categories defined for corneal swelling, corneal opacity, and fluorescein retention

Table 21: Results of eye irritation study (CEET)

Method	Results	Remarks	Reference
In vitro Chicken Enucleated Eye test; ROSS spring chickens, 4 eyes per group Similar to OECD 438; GLP	Maximum mean score for HS88 (aqueous solution of 88 % L-(+)-lactic acid, pH 2) at 0, 30, 75, 120, 180 and 240 minutes after treatment: Severe to complete corneal opacity (30, 75, 120 min), max. corneal swelling 28 % after 240 min, severe fluorescein retention and swelling Powder H60 (powder, undiluted, 60 % lactic acid and 40 % Ca-lactate): maximum mean corneal swelling of 17 % at 240 min after treatment, moderate corneal opacity and moderate fluorescein retention by damaged epithelial cells. Lactic acid buffered BF S36 (liquid, undiluted, buffered): slight increase in corneal thickness of the test eyes, maximum mean corneal swelling of 6 % at 75 min after treatment. Very slight corneal opacity and slight fluorescein retention by damaged epithelial cell.	HS88 (aqueous solution): 88 % L-(+)-lactic acid, pH 2, pH-value of other formulations not reported, √	MK Prinsen 1996, TNO Project No. 460069/01

√: also mentioned in the registration dossiers

4.5.2.2 Human information

No information submitted by the applicants.

4.5.2.3 Summary and discussion of eye irritation

Since concentrated L-(+)-lactic acid has a pH < 2 no eye irritation studies in rabbits were performed due to animal welfare considerations. Instead, a chicken enucleated eye test (*in vitro*) was performed. The three tested formulations of lactic acid have different eye irritation properties: HS88 (aqueous solution of 88 % L-(+)-lactic acid, pH 2) revealed a seriously damaging potential of L-(+)-lactic acid to the eye (severe corneal opacity, corneal swelling and fluorescein retention), Powder H60 (powder, undiluted, 60 % lactic acid and 40 % Ca-lactate) revealed a moderately damaging potential to the eye and lactic acid BF S36 (liquid, undiluted, buffered) a slightly damaging potential to the eye. Overall, a classification of L-(+)-lactic acid with Category 1, H318: Causes serious eye damage is proposed.

4.5.2.4 Comparison with criteria

Substances that have the potential to seriously damage the eyes are classified in Category 1 (irreversible effects on the eye). Substances are classified in this hazard category only on the basis of the results of animal testing, in accordance with the criteria listed in Table 3.3.1 (Category for irreversible eye effects) of the Guidance for CLP criteria. As mentioned above, no animal data are available for L-(+)-lactic acid but an *in vitro* Isolated Chicken Eye (ICE) test (OECD TG 438; TM B.48). Therefore, the test results of the ICE-test cannot be compared with the CLP criteria.
Together with the Bovine Corneal Opacity and Permeability (BCOP) test (OECD TG 437; TM B.47) and the Fluorescein Leakage (FL) test (OECD TG 460), these tests are recommended for regulatory classification and labelling. A substance can be considered as causing serious eye damage (Category 1) based on positive results in either of the tests.

Table 22: Results of eye irritation studies in comparison with overall *in vitro* classification (EU B.48/OECD TG 438)

Toxicological result	**CLP Regulation/OECD criteria**
Maximum mean score for HS88 (aqueous solution of 88 % L(+) lactic acid, pH 2) at 0, 30, 75, 120, 180 and 240 minutes after treatment: Severe to complete corneal opacity (20, 75, 120 min), max. corneal swelling 28 % after 240 min, severe fluorescein retention. Categories III/IV/IV Maximum mean score for H60 (powder) at 0, 30, 75, 120, 180 and 240 minutes after treatment: Moderate corneal opacity, max. corneal swelling 17 % after 240 min, moderate fluorescein retention. Categories II/III/III. Maximum mean score for BF S36 (liquid) at 0, 30, 75, 120, 180 and 240 minutes after treatment: Minimal corneal opacity, max. corneal swelling 6 % after 75 min, minimal fluorescein retention. Categories II/I/II.	Irreversible effects on the eye (Category 1, H318): 3 x IV 2 x IV, 1 x III 2 x IV, 1 x II* 2 x IV, 1 x I* Corneal opacity ≥ 3 at 30 min (in at least 2 eyes) Corneal opacity = 4 at any time point (in at least 2 eyes) Severe loosening of the epithelium (in at least 1 eye)

4.5.2.5 Conclusions on classification and labelling

In summary and based on the submitted *in vitro* data and physico-chemical properties (pH < 2), L-(+)-lactic acid should be classified for eye irritation/corrosion according to OECD criteria and CLP Regulation as Category 1, H318: Causes serious eye damage.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed to classify L-(+)-lactic acid for serious eye damage, Category 1, H318, based on the pH < 2 of concentrated L-(+)-lactic acid and on the outcome of an *in vitro* Chicken Enucleated Eye Test (CEET).

Comments received during public consultation

The three comments received were in favour of the proposed classification. However, one commenter by an industrial association recommended to propose a specific concentration limit (SCL) of 10% for eye damage since the outcome of a new *in vitro* Bovine Corneal opacity and Permeability (BCOP) tests suggested no effects up to a concentration of 10% lactic acid.

DS replied that the Guidance on the Application of the CLP Criteria states that, while the possibility to use in vitro test methods as a basis for setting SCLs have not yet been explored, an SCL should apply to any mixture containing the substance. However, in this case the available data refer only to a specific solvent and not different solvents, and hence cannot be used for setting of an SCL.

The Chicken CEET is an alternative to the Draize eye irritation test with albino rabbits. According to the above mentioned Guidance, this test is one of four *in vitro* test methods adopted for the identification of substances inducing serious eye damage.

In OECD TG 437 it is clearly stated that the BCOP test is considered to evaluate the eye hazard potential of a test chemical. However, it is known that the BCOP test method can only identify correctly 31% of the chemicals that do not require classification for eye irritation or serious eye damage.

Assessment and comparison with the classification criteria

The CEET was performed with three different formulations:

a) powder consisting of 60% L-(+)lactic acid and 40% Ca-lactate,

b) 88% L-(+)-lactic acid (pH 2) and

c) a buffered solution containing 73-84% L-(+)-lactic acid and sodium lactate.

Results for corneal thickness expressed in swelling, for corneal opacity and fluorescein retention were reported. The overall test outcome described different corneal effects for each of the test substance from slight corneal effects (with the buffered solution) to severe corneal effects with the 88% concentration of L-(+)-lactic acid.

Table: Summary of the maximum mean scores for corneal swelling, opacity and fluorescein

retention and the irritation categories assigned (see table 20 in the BD).

Test material	Maximum mean score for[1]:			Categories according to OECD TG 438[1]	Classification
	Swelling	Opacity	Flurescein		
a)	17	2.0	2.0	II/III/III moderate corneal effects	No prediction can be made
b)	28	4.0	3.0	III/IV/IV severe corneal effects	H318
c)	6	0.5	1.0	II/I/II slight corneal effects	No classification

[1]The criteria can be found in OECD TG 438.

Although not mentioned in the CLH report, the REACH registration dossier mentions a published ocular tolerance study (Guillot et al., 1982) of humectants and moisturizers used in cosmetics, which included tests of lactic acid. According to the registration dossier, the test showed that 10% and 20% lactic acid provoked a significant ocular irritation in the rabbit eye, only with the lesion caused by 10% lactic acid being reversible within 7 days.

Based on the pH value of < 2, on the outcome of the CEET assay using 88% L-(+)-lactic acid, and supported by the study by Guillot, RAC is of the opinion that a classification for serious eye damage, Category 1, H318 is warranted.

With regards to setting a specific concentration limit (SCL), four new GLP-compliant BCOP tests, compliant with OECD TG 437, were submitted by industry. While a concentration of 10% of lactic acid did not induce eye irritation, concentrations of 20% and 40% resulted in mild and severe irritation, respectively. However, RAC is of the opinion that only three concentrations tested in one type of assay, using only one solvent, does not justify the setting of a SCL. ~~The GCL for eye damage (category 1) is 3%, but in the event that the pH is ≤ 2 the GCL will be 1% (CLP Regulation, table 3.3.4).~~

Overall, RAC agrees to classify L-(+)-lactic acid as **Eye Dam. 1,~~ with an GCL of 1%~~.**

4.5.3 Respiratory tract irritation

No information/studies (conducted in non-humans or humans) concerning respiratory tract irritation were available with the exception of an acute inhalation study with SY-83 (80-85 % L-(+)-lactic acid in water) in rats (see Table 12). Findings relating to changes in respiratory pattern were transient but indicative for respiratory tract irritation (rapid breathing and eye tearing during exposure in the inhalation chamber).

All animals (including the controls exposed to air only) had a hunched posture, red stained fur surrounding the eyes (tearing), ruffled fur, and appeared ungroomed with soiled fur (stained brown) one and three hours after exposure. Female rats exposed to SY-83 appeared lethargic at one (2/5) and three hours (5/5). The two female rats that were lethargic at one hour also had rapid, shallow breathing and appeared to be gasping at both one and three hours. The animals appeared normal by 24 hours and during the observation period. One female from the treated group had hunched posture, rapid and shallow breathing, and slight tremors, but these signs were observed only on day 5 post-treatment. One female rat from the treated group died on Day 8 post-treatment. This animal was hunched with labored breathing and gasping on Day 7. No gross lesions were observed at

necropsy, histopathology was not performed. Information on practical observations in humans are not available.

4.5.3.1 Comparison with criteria

Table 23: Results of an acute inhalation toxicity study in comparison with CLP criteria

Toxicological result	CLP criteria
No human data available/reported. Transient signs of respiratory irritation in rats are: rapid, shallow, labored breathing, gasping. Female rats appeared lethargic. No gross lesions at necropsy, no histopathology (David, 1987).	The criteria for classifying substances as Category 3 for respiratory tract irritation are: (a) respiratory irritant effects (characterized by localized redness, oedema, pruritis and/or pain) that impair function with symptoms such as cough, pain, choking, and breathing difficulties are included. This evaluation will be based primarily on human data.
No human data available/reported.	(b) subjective human observations could be supported by objective measurements of clear respiratory tract irritation (RTI) (such as electrophysiological responses, biomarkers of inflammation in nasal or bronchoalveolar lavage fluids).
No human data available/reported.	(c) the symptoms observed in humans shall also be typical of those that would be produced in the exposed population rather than being an isolated idiosyncratic reaction or response triggered only in individuals with hypersensitive airways. Ambiguous reports simply of "irritation" shall be excluded as this term is commonly used to describe a wide range of sensations including those such as smell, unpleasant taste, a tickling sensation, and dryness, which are outside the scope of classification for respiratory irritation.
Transient signs of respiratory irritation in rats are: rapid, shallow, labored breathing, gasping. Female rats appeared lethargic. No gross lesions at necropsy, no histopathology (David, 1987).	(d) there are currently no validated animal tests that deal specifically with RTI, however, useful information may be obtained from the single and repeated inhalation toxicity tests. For example, animal studies may provide useful information in terms of clinical signs of toxicity (dyspnoea, rhinitis etc) and histopathology (e.g. hyperemia, edema, minimal inflammation, thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above. Such animal studies can be used as part of weight of evidence evaluation.
Severe organ effects are not reported. No gross lesions at necropsy, no histopathology (David, 1987).	(e) this special classification would occur only when more severe organ effects including in the respiratory system are not observed.

4.5.3.1 Conclusions on classification and labelling

In general, a classification for corrosivity (skin, eye) is considered to cover the potential to also cause respiratory tract irritation and the additional Category 3 can be considered to be superfluous, although it can be assigned at the discretion of the DS. For precautionary reasons and based on the results observed in an acute inhalation toxicity study in rats (David, 1987), for L-(+)-lactic acid it is considered adequate to propose a classification as respiratory tract irritant STOT SE 3.

In summary and based on the submitted data, L-(+)-lactic acid meets the criteria to be classified as respiratory tract irritant STOT SE 3.

Classification in STOT SE Category 3 for respiratory irritation does not take potency into account and consequently does not have any guidance values.

4.6 Corrosivity

Please compare to section 4.5.1and 4.5.2 (Skin and Eye irritation/corrosion).

4.7 Sensitisation

4.7.1 Skin sensitisation

4.7.1.1 Non-human information

Preliminary range-finding trials revealed very slight erythema and edema at the 100 % concentration of L-(+)-lactic acid. No other dermal reactions were noted for the other concentrations (3, 10, and 30 %) Therefore, the 100 % concentration of the test article was utilized in the main study testing for contact dermal sensitization potential.

No mortalities occurred and all animals gained body weight.

In the main study, the 80 % (100 % SY-83; first 2 inductions and challenge) and 24 % L-(+)-lactic acid (30 % SY-83). produced very slight erythema at 3 sites and very slight edema at l site after the 1st induction. Erythema grades increased in severity after the 2nd induction application. One site was graded as severe erythema, however, this grade was given a 4 due to pinpoint pitting of the skin and scab formation, not for redness. Due to the increase of severity of the reactions, the concentration of the test article was reduced to 30 % and the induction site was changed to the left flank. Very slight erythema was noted after the 5th induction application. Grades ranging from very slight to severe erythema were noted from the 7th to the 9th induction applications. Again, the severe (grade 4) reactions were given this grade due to pinpoint pitting of the skin and the eschar formation, not for redness.

Both 24 and 48 hours after the challenge application, the test article (100 % SY-83 corresponding to 80 % L-(+) lactic acid)) produced grade 4 erythema in up to 6 test animals. These gradings were very similar in character as those seen during the induction applications, that is, pinpoint pitting of the skin and eschar formation, very little redness. These reactions were considered to be irritation reactions, not sensitization reactions. Other reactions noted at challenge for the test animals were very slight to moderate erythema, and very slight to moderate edema. The test article (100 %) produced grade 4 erythema in up to 8 naive control animals. These gradings were also pinpoint pitting of skin and eschar formation with very little redness. These reactions were considered to be irritation reactions, not sensitization reactions. Other reactions noted for the naive control animals were very slight to moderate erythema and very slight to moderate edema. The reactions seen in the naive control animals at challenge were similar to the reactions seen for the test group animals and L-(+)-lactic acid was not considered to be a contact dermal sensitizer.

Table 24: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Guinea pig, Hartley, 10 F Induction: 80 % L(+) Lactic acid (= 100 % SY-83) (3, 10, 30, 100 % were used in the range-finding study) Challenge: 80 % L(+) Lactic acid (= 100 % SY-83) No adjuvant used. Similar to OECD 406 (modified Buehler test), GLP	Not sensitizing	Irritating concentrations of L(+) Lactic acid were used. Signs of irritation: pinpoint pitting, eschar formation, only slight redness, √	Smith 1986, American Biogenics Corporation Study No. 480-2750

√: also mentioned in the registration dossiers

Note: SY-83 is formulated by dilution to a concentration of 80 % with water: 83.5-76.5 % lactic acid in water. The concentrations of all dilutions (10 %, 30 %) in this study relate to 100 % SY-83 which yields 80 % L(+) lactic acid.

4.7.1.2 Human information

No information submitted by the applicants.

4.7.1.3 Summary and discussion of skin sensitisation

In a modified Buehler test with 9 inductions L-(+)-lactic acid was non sensitising (0/10 animals sensitised). Induction and challenge were performed with 80 % (100 % SY-83; first 2 inductions and challenge) and 24 % L-(+)-lactic acid (30 % SY-83). While only slightly irritating in the range-finding studies, these concentrations proved to be highly irritating after repeated exposure. Irritation reactions were pinpoint pitting and eschar formation with only slight redness. The quality of these observed skin effects differ from those caused by a skin sensitising substance. Therefore, the results of the study can be interpreted as skin irritation. In addition, L-(+)-lactic acid is a metabolic intermediate and a sensitisation potential for endogenous substances which are formed in considerable amounts in the human (or animal) body is highly unlikely. Therefore, an additional skin sensitisation study is considered not necessary.

4.7.1.4 Comparison with criteria

Table 25: Results in comparison to the CLP criteria

Toxicological result	CLP criteria
24 h after challenge: 0/10 animals negative 48 h after challenge: 0/10 animals negative	Buehler assay Category 1A (H317): ≥ 15 % responding at ≤ 0.2 % topical induction dose or ≥ 60 % responding at > 0.2 % to ≤ 20 % topical induction dose Category 1B (H317): ≥ 15 % to < 60 % responding at > 0.2 % to ≤ 20 % topical induction dose or ≥ 15 % responding at > 20 % topical induction dose

4.7.1.5 Conclusions on classification and labelling

L-(+)-lactic acid did not meet the criteria to be classified for skin sensitising properties according to the criteria in CLP regulation.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Based on the results of a modified Buehler test, in which none out of 10 tested animals showed sensitising effects, the DS concluded that 80% L-(+)-lactic-acid does not meet the criteria for classification for skin sensitisation according to the CLP Regulation.

Comments received during public consultation

There were no comments provided in the public consultation regarding this hazard class.

Assessment and comparison with the classification criteria

In a Guinea Pig study conducted according to EPA´s OPP test guidelines (1982) and similar to OECD TG 406, 80% L-(+)-lactic acid was selected for induction as the range-finding trials revealed very slight erythema and oedema at this concentration after one single application. However, as after two topical induction applications this concentration proved to be highly irritating (grade 4), the test site of the animals was changed and the

concentration of the test substance was reduced to 24% L-(+)-lactic acid for the subsequent seven induction applications.

The reactions observed after 24 and 48 hours after the challenge (pinpoint pitting of the skin and eschar formation, very little redness) were very similar to those observed during the induction phase and occurred in up to six animals and in up to eight naïve control animals.

Due to the fact that the same type of effects, including scab formation, were observed in the test and control animals, RAC agrees with the DS that these effects should be considered as irritation reactions. Thus, no conclusions as to the sensitising potential of L-(+)-lactic acid can be drawn from this study. However, a sensitising potential of this endogenous substance is not expected. Based on lack of relevant data, **RAC supports no classification for skin sensitisation**.

4.7.2 Respiratory sensitisation

No data/information (from non-humans or humans) was submitted that would allow an evaluation of sensitising properties for the respiratory tract.

4.8 Repeated dose toxicity

4.8.1 Non-human information

One 13-week study in rats with repeated oral administration of L-(+)-lactic acid was available. Neither studies with other species, nor studies with other routes of administration were submitted.

The 13-week oral sub-chronic toxicity study was performed using calcium lactate instead of L-(+)-lactic acid. The study lacks some detail (published literature) and it is not clear from the data which effects are due to high calcium uptake and which might be due to lactic acid. Anyhow, calcium lactate dissociates in dilutions into calcium-ions and lactic acid. The solubility of calcium lactate is 50 g/L (Merck). That means that calcium lactate is a soluble salt and the rats were exposed to calcium-ions and lactic acid. It can be assumed that the occurrence of nephrocalcinosis in animals of the 13-week study was the result of the high calcium uptake and not due to lactic acid.

4.8.1.1 Repeated dose toxicity: oral

Table 26: Summary table of relevant repeated dose oral toxicity studies

Method	Animal species, number & strain	Doses, vehicle, duration	Result	Reference
Non-guideline, non-GLP	Rat, F344/DuCrj, 10 M + 10 F	Oral application of calcium lactate pentahydrate (food additive) via drinking water and food, 13 wk Dose levels drinking water: 0.3-0.6-1.25-2.5-5 %; Dose levels food: 0-5-10-20-30 %	Decreased bw gain, nephrocalcinosis, GI tract: necrosis, erosion, atrophy of the epithelium NOAEL: 20 % in food (~8.5 g/kg bw/d) LOAEL: 30 % in food (~12 g/kg bw/d) Effects observed might be due to high calcium intake; Report lacks some detail, √	Matsushima et al. 1989 Bulletin of the National Institute of Hygienic Sciences, Tokyo 107: 78-83

√: also mentioned in the registration dossiers

4.8.1.2 Human information

No information submitted by the applicants.

4.8.1.3 Other relevant information

No other relevant information available.

4.8.1.4 Summary and discussion of repeated dose toxicity

The 13-week repeat-dose toxicity study was performed with calcium lactate. Calcium lactate is a black or white crystalline salt made by the action of lactic acid on calcium carbonate or calcium hydroxide. It is used in foods (as an ingredient in baking powder) and given medicinally. Calcium lactate is a food additive (E327). In medicine, calcium lactate is most commonly used as an antacid and also to treat calcium deficiencies. Calcium lactate can be absorbed at various pHs and does not need to be taken with food for absorption for these reasons.

Also the presented data of the 13-week repeat dose toxicity study (published literature) with calcium lactate are of low reliability, it can be assumed that the occurrence of nephrocalcinosis in animals of the 13-week study was the result of the high calcium uptake and not due to lactic acid.

Anyhow, the results can only be used as a very rough approximation for a NOAEL for L-(+)-lactic acid because the effects observed (decrease in food consumption and body weight gain) might be due to high calcium intake, palatability problems and/or malabsorption due to local gastrointestinal irritation (provoked by calcium or lactate). Thus, it is inadequate to use of the obtained NOAEL for derivation of reference values. In addition, the administered doses in these studies were far in excess of the guidance value ranges for repeated-dose exposures leading to classification of STOT RE (see Table 27).

In the light of the low toxicity of lactic acid and the high endogenous exposure non-submission of data on repeat-dose toxicity with L-(+)-lactic acid is acceptable.

Table 27: Results of toxicity studies relevant for STOT RE in comparison to the CLP criteria

Toxicological results	CLP criteria
No human data available. One 13-week non-guideline study with calcium lactate in rats. NOAEL 8500 mg/kg bw/d LOAEL: 12000 mg/kg bw/d Effects (decreased bw gain, nephrocalcinosis, necrosis, erosion, atrophy of the epithelium of the GI tract) were due to irritating properties of the test substance.	Category 1 (H372): Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Equivalent guidance values for 28-day and 90-day studies: Oral, rat: 28-day: ≤ 30 mg/kg bw/d 90-day: ≤ 10 mg/kg bw/d
No human data available. One 13-wk non-guideline study with calcium lactate in rats. NOAEL 8500 mg/kg bw/d LOAEL: 12000 mg/kg bw/d Effects (decreased bw gain, nephrocalcinosis, necrosis, erosion, atrophy of the epithelium of the GI tract) were due to irritating properties of the test substance.	Category 2 (H373): Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided below (see 3.9.2.9) in order to help in classification. In exceptional cases human evidence can also be used to place a substance in Category 2. Equivalent guidance values for 28-day and 90-day studies: Oral, rat: 28-day: ≤ 300 mg/kg bw/d 90-day: ≤ 100 mg/kg bw/d

4.8.2 Conclusion on classification and labelling for STOT RE

Comparing the results of the toxicological studies with the guidance value ranges for repeated-dose exposures, L-(+)-lactic acid did not meet the criteria to be classified for repeated exposure (STOT RE) according to the criteria in CLP regulation.

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

Summary of the Dossier Submitter's proposal

The DS did not propose any classification for STOT RE as no effects were observed in an oral subchronic toxicological study in rats.

Comments received during public consultation

There were no comments provided in the public consultation regarding this hazard class.

Assessment and comparison with the classification criteria

The only available subchronic study in rats is a non-guideline, non-GLP study using calcium lactate pentahydrate (used as food additive and as an antacid and as a medicine to treat calcium deficiencies). According to information in the CLH dossier, the solubility of calcium lactate is 50 g/L and calcium lactate is likely to dissociate in solution to lactic acid and calcium. The results of this study can be used for lactic acid, but calcium effects also have to be considered.

In the first setting of the study, five males and five females per group were treated with a concentration of 0, 0.3, 0.6, 1.25, 2.5 and 5% of calcium lactate pentahydrate in drinking water for 13 weeks. No effects were observed.

In second setting, the same number of rats per group was fed with a concentration of 0, 5, 10, 20 and 30% of the substance in food for 13 weeks. Nephrocalcinosis was observed, but the findings were even more pronounced in the controls. It was shown that it was the feed used in the experiment that caused nephrocalcinosis and not calcium lactate.

A 2-year study where rats were given 0, 2.5, or 5% calcium lactate pentahydrate via the drinking water showed a slightly decreased body weight gain, but no other effects, at the top dose (in the CLH report stated to be 880 mg/kg bw/day, but in the REACH registration dossier 880 mg/kg bw/day in males and 930 mg/kg bw/day in females).

As calcium lactate pentahydrate caused no effects at doses much higher than the guidance value for STOT RE, RAC agrees **not to classify L-(+)-lactic acid for specific target organ toxicity – repeated exposure (STOT RE)**.

4.9 Germ cell mutagenicity (Mutagenicity)

4.9.1 Non-human information

4.9.1.1 In vitro data

Three Ames tests revealed no genotoxic potential of L-(+)-lactic acid in the absence or presence of S9 mix. Two chromosomal aberration assays, one in Chinese hamster fibroblasts, one in human lymphocytes were negative, too. A chromosomal aberration showed cytotoxicity and clastogenic effects at unphysiologically low pH of 5.7-6.7 of L-(+)-lactic acid in Chinese hamster ovary cells. The authors judged L-(+)-lactic acid as non-clastogenic and the results as "pseudo-positive". An *in vitro* mammalian cell gene mutation test in mouse lymphoma cells was negative too.

In a reverse gene mutation assay in bacteria, S. typhimurium strains TA1535, TA1537, TA98, TA100 and E. coli strain WP2uvrA were exposed to L-(+)-lactic acid at concentrations of 0, 100, 333, 1000, 3330 and 5000 µg/plate in the presence and absence of mammalian metabolic activation. The test with metabolic activation (10 % S9) was a plate incorporation test. L-(+)-lactic acid was tested up to the limit concentration of 5000 µg/plate. The positive controls induced the appropriate responses in the corresponding strains. There was no evidence of induced mutant colonies over background. This study is classified as acceptable and satisfies the requirement for Test Guideline OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation assay) (Verspeek-Rip, CM., 2014).

In a mammalian cell gene mutation assay L5178Y TK+/--3.7.2C mouse lymphoma cells cultured *in vitro* were exposed to L-(+)-lactic acid, solved in RPMI 1640 medium at concentrations of 0.54, 1.7, 5.4, 17, 52, 164, 512

and 901 µg/mL in the presence and absence of mammalian metabolic activation. The S9 -mix was a S9 fraction derived from phenobarbital and ß-naphtoflavone treated male SD rats supplemented with cofactor mix. Metabolic activation was only performed in experiment 1 with a treatment duration of 3 hours, but not in experiment 2 with a treatment duration of 24 hours. L-(+)-lactic acid was tested to the maximum concentration of 0.01 M, equivalent to 901 µg/ml. The induced mutation frequency with and without metabolic activation was not increased compared to control in all tested concentrations. The positive controls did induce the appropriate response. The spontaneous mutation frequencies in the solvent-treated controls were within the historical control data ranges. This study is classified as acceptable. This study satisfies the requirement for Test Guideline OECD 476 for *in vitro* mutagenicity (mammalian forward gene mutation) data (Verspeek-Rip, CM., 2014).

In a mammalian cell cytogenetics assay peripheral human lymphocyte cultures were exposed to L-(+)-lactic acid, solved in RPMI 1640 cell culture medium. In the first and second experiment the doses were 0, 10, 100, 901 µg/ml for 3 hours with and without metabolic activation. In the second experiment additional treatment to doses of 0, 100, 333, 666 and 901 µg/ml was carried out for 24 and 48 hours exposure time. S9 was derived from phenobarbital plus ß-naphtoflavone treated rats and supplemented with cofactors.

L-(+)-lactic acid was tested up to 901 µg/ml which was cytotoxic based on determination of the mitotic index after an exposure time of 24 and 48 hours. The percentage of the mitotic index after 24 hours of 901 µg/ml was 55 %, that after 48 hours of 901 µg/ml 59 %. Concentrations lower than 901 µg/ml did not cause a dose-dependent decrease in the percentage of the mitotic index after 24 and 48 hours of exposure. The mitotic index after 3 hours of exposure was lower compared to control (66 % in experiment 1, 84 % in experiment 2) but did not reach the threshold value of 45 ± 5 % according to OECD guideline 473 for cytotoxicity. Positive controls induced the appropriate response. There was no evidence for a concentration related positive response of chromosome aberration induced over background.

This study is classified as acceptable and satisfies the requirement for Test Guideline *in vitro* mammalian chromosomal aberration test OECD 473 (Verbaan, 2014).

Table 28: Summary table of relevant *in vitroin vitro* mutagenicity studies

Method	**Results**		**Remarks**	**Reference**
	+ S9 +, -, +/-	**- S9** +, -, +/-		
Salmonella typhimurium TA92, TA1535, TA100, TA1537, TA94, TA98 Up to 10 mg/plate Ames test, similar to OECD 471, non-GLP	-	-	Publication (lacks details), √	Ishidate M et al. 1984, Food Chem Toxicol 22(8):623-636
Chinese hamster fibroblasts Up to 1 mg/mL Chromosomal aberration test, sim to OECD 473, non-GLP	-	-	Publication (lacks details), √	Ishidate M et al. 1984, Food Chem Toxicol 22(8):623-636
Salmonella typhimurium TA97, TA98, TA100, TA104 0.5-2.0 µL/plate (≈ 0.6-2.4 mg/plate) Ames test, similar to OECD 471, non-GLP	-	-	Publication (lacks details), √	Al-Ani FY & Al-Lami SK (1988), Mutation Research 206:467-470
Chinese hamster ovary K1 8-16 mM Chromosomal aberration test, sim to OECD 473, non-GLP	+/-	+/-	Cytotoxicity and clastogenicity observed at low pH; publication (lacks details); conclusion of the authors: non-clastogenic, pseudo-positive reactions at unphysiological pH;	Morita T et al. 1990, Mutation Research 240:195-202

			No DocIII summary, √	
E. coli B/Sd-4/1,3,4,5 & E.coli B/SD4/3,4 0.01-0.021 % lactic acid "Streptomycin" method, non-guideline, non-GLP	N/A	+/-	Cytotoxicity even at the lowest dose, weak mutagenic effect at some concentrations, not dose-dependent No DocIII summary, √	Demerec M et al. 1951, The American Naturalist 85(821): 119-137
S. typhimurium strains TA1535, TA1537, TA98, TA100 and E. coli strain WP2uvrA Doses: 0, 100, 333, 1000, 3330 and 5000 µg L-(+)-lactic acid /plate OECD 471, GLP: yes	-	-	No information on cytotoxicity, No DocIII summary, √	Verspeek-Rip, CM., 2014, WIL Research Europe B.V. Report No. 5041704
L5178Y TK+/--3.7.2C mouse lymphoma cells Doses: 0.54, 1.7, 5.4, 17, 52, 164, 512 and 901 µg L-(+)-lactic acid/mL OECD 476; GLP: yes	-	-	No information on cytotoxicity, No DocIII summary, √	Verspeek-Rip, CM., 2014, WIL Research Europe B.V. Report No. 504706
Peripheral human lymphocytes Doses: 0, 10, 100, 901 µg L-(+)-lactic acid /ml for 3 hours, 0, 100, 333, 666 and 901 µg L-(+)-lactic acid /ml for 24 and 48 hours OECD 473; GLP: yes	-	-	Cytotoxicity at the highest dose of 901 µg L-(+)-lactic acid /ml, No DocIII summary, √	Verbaan, IAJ., 2014

√: also mentioned in the registration dossiers

4.9.1.2 In vivo data

No studies available, not necessary. Refer also to discussion in Section 4.1.

4.9.2 Human information

No information submitted by the applicants.

4.9.3 Other relevant information

No other relevant information available.

4.9.4 Summary and discussion of mutagenicity

Three Ames tests revealed no genotoxic potential of L-(+)-lactic acid in the absence or presence of S9 mix (Ishidate et al. 1984 and Al-Ani & Al-Lami 1988). Two chromosomal aberration assays, one in Chinese hamster fibroblasts, one in human lymphocytes were negative, too (Ishidate et al. 1984). A chromosomal aberration assay (Morita et al. 1990) showed cytotoxicity and clastogenic effects at unphysiologically low pH of 5.7-6.7 of L-(+)-lactic acid in Chinese hamster ovary cells. The authors judged L-(+)-lactic acid as non-clastogenic and the results as "pseudo-positive". Overall, L-(+)-lactic acid proved to be devoid of mutagenic or clastogenic effects at non-cytotoxic concentrations and pH in *in vitro* tests. An *in vitro* mammalian cell gene mutation test in mouse lymphoma cells was negative too. Thus, and because of the high background exposure via food and endogenous metabolism, no further studies are required according to Annex II (data requirements) of Regulation (EU) No. 528/2012 concerning the making available on the market and use of biocidal products.

4.9.5 Comparison with criteria

Table 29: Results in comparison to the CLP criteria

Toxicological results	CLP regulation
Ames test (3): negative (± S9) Chromosomal aberration assay in Chinese hamster fibroblasts and in human lymphocytes (2): negative Chromosomal aberration assay in Chinese hamster ovary cells (1): Cytotoxicity and clastogenic effects at pH of 5.7-6.7 of L(+) Lactic acid Mammalian cell gene mutation test in mouse lymphoma cells (1): negative	The classification in **Category 1A** is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans. The classification in **Category 1B** is based on: — positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or — positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells *in vivo*, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or — positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people. The classification in **Category 2** is based on: — positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from: — somatic cell mutagenicity tests *in vivo*, in mammals; or — other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays. Note: Substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

4.9.6 Conclusions on classification and labelling

It can be concluded that L-(+)-lactic acid did not meet the criteria to be classified for mutagenicity according to the criteria in CLP regulation.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The mutagenicity potential of L-(+)-lactic acid was tested in several *in vitro* studies, covering gene mutation and chromosomal damage endpoints. Six out of eight studies were clearly negative. A chromosomal aberration assay (Moriat *et al.* 1990) showed cytotoxicity and clastogenic effects at a pH of 5.7-6.7 in Chinese hamster ovary cells and a non-guideline, non-GLP study by Demerec *et al.* (1951) showed some cytotoxic and mutagenic effects in the absence of S9 mix.

Based on the test results (the Moriat study was considered to be "pseudo-positive" due to the unphysiological pH used) the DS proposed not to classify L-(+)-lactic acid as germ cell mutagen.

Comments received during public consultation

There were no comments provided in the public consultation regarding this hazard class.

Assessment and comparison with the classification criteria

There are three Ames tests (all three are similar to OECD TG 471 but two of them were not GLP-compliant) - with and without S9 mix - which did not reveal any genotoxic potential of L-(+)-lactic acid. The doses of L-(+)-lactic acid were up to 2.4 mg/plate in one test, up to 5000 µg/plate in the other and up to 10 mg/plate in the third study.

Two out of three OECD-compliant chromosomal aberration assays were also negative. One of these negative assays was performed in human lymphocytes with a test dose up to 901 µg/mL for 3 hours as well as for 24 and 48 hours. The other negative study was performed in Chinese hamster fibroblasts with a test dose up to 1 mg/mL. In the third study, using Chinese hamster ovary cells under an unphysiological low pH, cytotoxicity and clastogenicity was observed. The study lacks details but the authors came to the conclusion that the observations should be considered as pseudo-positive due to the low pH.

The study by Demerec *et al*. (1951) was not described in detail in the CLH dossier but it is pointed out that cytotoxicity was observed even at the lowest dose as well as weak mutagenic effects at some doses, but not dose-dependently.

The last of the eight studies provided in the CLH dossier, is an OECD- and CLP-compliant mammalian cell gene mutation assay, in which mouse lymphoma cells were exposed to L-(+)-lactic acid dissolved in RPMI medium at concentrations up to 901 µg/mL. In none of the tested concentrations - with and without metabolic activation - was the induced mutation frequency increased compared to the controls.

Summing up, the results of the *in vitro* studies and the fact that there is high background exposure on L-(+)-lactic acid via food and endogenous metabolism indicate that L-(+)-lactic acid, as proposed by the DS and agreed by RAC, **does not warrant a classification for mutagenicity** according to CLP criteria.

4.10 Carcinogenicity

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

One 2-year study in rats with repeated oral administration via drinking water of calcium lactate was available. Neither studies with other species, nor studies with other routes of administration were submitted.

Calcium lactate dissociates in dilutions into calcium-ions and lactic acid. The solubility of calcium lactate is 50 g/L (Merck). That means that calcium lactate is a soluble salt and the rats were exposed to calcium-ions and lactic acid. It can be assumed that the quantity of water intake of the animals during the 2-year study was high enough for lactic acid liberation and thus for an adequate long-term exposure with lactic acid.

Male and female specific-pathogen-free (SPF) Fischer (F344) rats were randomly allocated to three groups, each consisting of 50 males and 50 females. Calcium lactate was dissolved in distilled water at levels of 0 (control), 2, 5 or 5 %. These doses were selected after a 13-week subchronic toxicity study (Matsushima *et al*, 1989). Rats were given these solutions *ad libitum* in their drinking-water. Administration of the compound ended after 104 weeks, and the rats were then given distilled water for a recovery period of 9 weeks. At week 113, all surviving animals were killed and autopsied. Throughout the administration period, a 13 % decrease in body-weight gain was observed in male and female rats of the high-dose group. In females, the mortality rate in the 5 % group was slightly higher than those in the other two groups. Tumours were found in many organs and/or tissues in all groups including the controls. None of the experimental groups showed a significant increase in the incidence of any specific tumour. A number of non-neoplastic lesions (e.g. myocardial fibrosis, bile-duct proliferation, hepatic microgranulomas and chronic nephropathy) were observed in all groups, with no difference in their incidences and/or degrees. No specific dose RElated changes were observed in any of the haematological and biochemical parameters. It was concluded that calcium lactate had neither toxic nor carcinogenic activity in rats.

Table 30 Summary table of relevant carcinogenicity study

Method	Animal species, number & strain	Doses, vehicle, duration	Result	Reference
Non-guideline, non-GLP	Rat, F344, 50 m / 50 f	Oral application of calcium lactate pentahydrate (food additive) via drinking water, 2-yr, dose level: 0-2.5-5 %	Decreased food intake, decreased bw gain (87 % of controls), calcium lactate had no carcinogenic activity. NOAEL: 2.5 %, ~460 mg/kg bw/d LOAEL: 5 %, ~880 mg/kg bw/d Effects observed might be due to high calcium intake; Report lacks some detail, √	Maekawa et al. 1991, Food Chem Toxicol 29(9):589-594

√: also mentioned in the registration dossiers

4.10.2 Human information

No information submitted by the applicants.

4.10.3 Other relevant information

No other relevant information available.

4.10.4 Summary and discussion of carcinogenicity

Based on the information given in the study summary and the absence of genotoxic potential of L-(+)-lactic acid, Calcium lactate did not induce tumours in rats. However, the limitations of the available study need to be taken into account. In the light of the low toxicity of L-(+)-lactic acid and the high endogenous exposure, non-submission of data according to Annex II (data requirements) of Regulation (EU) No. 528/2012 concerning the making available on the market and use of biocidal products on chronic toxicity / carcinogenicity with L-(+)-lactic acid is acceptable.

4.10.5 Comparison with criteria

Table 31: Results in comparison to the CLP criteria

Toxicological results	CLP criteria
No data on carcinogenicity of calcium lactate or L-(+)-lactic acid in humans, e. g. in form of epidemiological studies, are available. A 2-year rat study with calcium lactate did not show evidence of a carcinogenic potential.	See Table 3.6.1 (Hazard categories for carcinogenes) of Guidance on the Application of the CLP Criteria

4.10.6 Conclusions on classification and labelling

It can be concluded that calcium lactate / L-(+)-lactic acid did not meet the criteria to be classified for carcinogenicity according to the criteria in CLP regulation.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

According to the information provided in a summary of an oral chronic non-guideline, non-GLP rat study, using the food additive calcium lactate (pH 6.0-8.0) dissolved in distilled drinking water, decreased food intake and decreased body weight gain but no significant dose-related increase in the incidence of any specific tumour was observed.

Based on the limited information in the study summary, the absence of genotoxic potential of L-(+)-lactic acid and based on the high background exposure levels on L-(+)-lactic acid via food and endogenous metabolism in mammals and humans, the DS concluded that L-(+)-lactic acid do not meet the criteria to be classified for carcinogenicity according to the CLP criteria.

Comments received during public consultation

There were no comments provided in the public consultation regarding this hazard class.

Assessment and comparison with the classification criteria

In the long-term carcinogenicity study by Maekawa *et al*. (1991), 50 male and 50 female F344 rats were treated with a concentration of 0, 2.5 and 5% of calcium lactate in drinking water *ad lib*. for two years.

According to information in the CLH dossier, the solubility of calcium lactate is 50 g/L, and calcium lactate is likely to dissociate in solution to lactic acid and calcium. The results of this study can be used for lactic acid but calcium effects also have to be considered.

At week 113, the surviving animals (the number of the surviving animals was not reported) were sacrificed and histologically examined. Haematological and biochemical parameters were also measured but no details of the results are provided. A dose-dependent 13% decrease in body weight gain was observed in both sexes of the high-dose group (in the CLH report it was stated to be 880 mg/kg bw/day, but in the registration dossier 880 mg/kg bw/day in males and 930 mg/kg bw/day in females).

Overall, based on the summary of the chronic carcinogenicity study on calcium lactate, RAC concludes that the available data indicated neither toxic nor carcinogenic effects of the substance in F344 rats. As calcium lactate was administered in the diluted form, the study can partly be used for assessment of the carcinogenic potential of lactic acid and therefore RAC agrees with the conclusion in the CLH dossier, that **L-(+)-lactic acid should not be classified for carcinogenicity**.

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

4.11.1.1 Non-human information

No studies were submitted for this endpoint. However, in the view of the DS no further studies according to Annex II (data requirements) of Regulation (EU) No. 528/2012 concerning the making available on the market and use of biocidal products are required, based on the fact that L-(+)-lactic acid is an endogenous mammalian metabolite and a common, naturally occurring food constituent and physiological exposure and nutritional uptake is likely to exceed exposure via the biocidal product by far. Refer also to discussion in Section 4.1.

4.11.1.2 Human information

No information submitted by the applicants.

4.11.2 Effects on or via lactation

No information was submitted for this endpoint.

4.11.3 Developmental toxicity

4.11.3.1 Non-human information

Two publications investigating potential developmental effects of L-(+)-lactic acid are available. Colomina et

al. (1992) investigated the developmental toxicity of 570 mg/kg bw/d lactic acid in mice. They observed a slight albeit not statistically significant decrease in foetal weight and a statistically significant delayed ossification of parietal bones which might be due to the decreased foetal weight. In the dams, there was a statistically significant treatment related decrease in food consumption of 15 % during treatment. Since no compensation (higher food consumption than control animals) was observed during the post-treatment period and no statistically significant decrease in weight gain it can be assumed that the lactic acid given by gavage partly covered the daily energy requirement of the dams. Thus, this finding was not considered adverse. Anyhow, the decrease in food consumption and slight decrease in body weight gain (no statistical significance) might be the reason for the delay in parietal ossification in combination with a slightly decreased foetal weight. Thus, it was not considered to represent a specific substance related effect. No further treatment related effects were observed.

Thus, these findings were not considered as a substance-specific developmental toxicity effect (in accordance with Carney & Kimmel, 2007).

In the study of D'Amour (1934) only the effects of lactic acid on the sex ratio in rats were investigated (no effects observed). The publication lacks details.

Table 32:

TABLE 3. Reproductive and fetal data of mice given oral $Al(OH)_3$, $Al(OH)_3$ and lactic acid, aluminum lactate, or lactic acid on gestation days 6-15

	Control	$Al(OH)_3$	$Al(OH)_3$ + lactic acid	Aluminum lactate	Lactic acid
No. of litters	13	11	13	10	12
No. of implantation sites/ litter	14.83±3.01	12.70±4.27	12.15±4.46	14.70±2.16	13.92±1.67
No. of live fetuses	14.17±3.29	11.90±4.90	10.85±4.37	13.80±2.34	13.00±1.88
No. of resorptions	0.66±0.77	0.80±1.03	1.23±1.73	0.70±0.66	0.76±1.01
No. of dead fetuses	0.00±0.00	0.00±0.00	0.07±0.27	0.20±0.63	0.16±0.38
Postimplantation loss/ litter (%)	4.45±6.53	6.29±7.92	10.69±12.91	6.12±7.24	6.61±8.13
No. of litters with dead fetuses	0	0	1	1	2
Sex ratio (M/F)	0.88±0.24	0.93±0.46	0.86±0.19	0.89±0.37	0.82±0.30
Fetal body weight/ litter (g)	1.24±0.14	1.26±0.11	1.27±0.15	1.04±0.18**	1.19±0.12

Asterisks indicate significantly differents from control, **P < 0.01.

Table 33:

TABLE 4. Summary incidence of malformations and variations in fetuses from dams given oral doses of $Al(OH)_3$, $Al(OH)_3$ and lactic acid, aluminum lactate, or lactic acid on gestation days 6-15

	Control	$Al(OH)_3$	$Al(OH)_3$ + lactic acid	Aluminum lactate	Lactic acid
Internal examination					
No. of fetuses (litters) examined	54 (13)	40 (11)	50 (13)	53 (10)	47 (12)
Cleft palate	0 (0)	0 (0)	0 (0)	7 (4)*	0 (0)
Skeletal examination					
No. of fetuses (litters) examined	74 (13)	55 (11)	53 (13)	52 (10)	66 (12)
Assymetrical sternebrae	3 (2)	4 (3)	9 (6)	5 (3)	8 (5)
Dorsal hyperkiphosis	0 (0)	0 (0)	0 (0)	7 (4)*	1 (1)
Parietal, delayed ossification	0 (0)	0 (0)	0 (0)	8 (5)**	10 (4)*
Sternebrae, reduced ossification	0 (0)	0 (0)	0 (0)	7 (3)	3 (1)
Total skeletal defects	3 (2)	4 (3)	9 (6)	11 (5)	17 (6)

Asterisks indicate significantly different from control: *$P < 0.05$, **$P < 0.01$, respectively. The litter was the statistical unit of comparison.

Table 34: Summary table of relevant developmental toxicity studies

Method	Results	Remarks	Reference
Mouse, Swiss albino (CD-1), 13 F (control), 12 F (lactic acid) Oral, gavage, GD 6-15, cesarian section on day 18 of gestation, Dose level: 0-570 mg/kg bw/d Similar to OECD 414, non-GLP	No adverse effects were observed in dams and fetuses.	Publication lacks details,	Colomina et al., 1992, Res Comm Chem Pathol Pharmacol 77(1):95-106
Rat, strain not specified, 10 F (2.5 %); 28 F (5 %) Oral, gavage, GD 0-22 Dose level: 2.5-5 % in food (1,250-2,500 mg/kg bw/d) Non-guideline, non-GLP	Investigation of effects of lactic acid on the sex ratio: no effects observed.	Additional reference, added by DS, publication lacks details, no DocIII summary	D'Amour 1934, Science 79(2038):61-62
Review article	Delayed (or incomplete) ossification of developing fetal bones and wavy ribs are some of the most common skeletal variations developmental toxicity studies.	Additional reference, added by DS, no DocIII summary	Carney and Kimmel, 2007; Birth Defects Research (Part B) 80:473–496

4.11.3.2 Human information

No information submitted by the applicants.

4.11.4 Other relevant information

No other relevant information available.

4.11.5 Summary and discussion of reproductive toxicity

No studies were submitted for reproductive toxicity, including fertility. In two reports from open literature, no adverse effects of L-(+)-lactic acid on developmental toxicity in mice (Swiss albino (CD-1)), and no effects on the sex ratio in rats (strain not specified) were reported.

The publications lack detail and only a few reproductive or developmental endpoints are addressed. However, in the view of the DS no further studies are required according to Annex II (data requirements) of Regulation (EU) No. 528/2012 concerning the making available on the market and use of biocidal products, based on the fact that L-(+)-lactic acid is an endogenous mammalian metabolite and a common, naturally occurring food constituent and physiological exposure and nutritional uptake is likely to exceed exposure via the biocidal product by far. Refer also to discussion in Section 4.1.

4.11.6 Comparison with criteria

Table 35: Results of studies on sexual function and fertility in comparison to the CLP criteria

Toxicological results	**CLP criteria**
No toxicological studies submitted.	Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility - where the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

Table 36: Results of developmental toxicity studies in comparison to the CLP criteria

Toxicological results	CLP criteria
No adverse effects were observed in dams and foetuses (Colomina et al., 1992). No effects of L(+) Lactic acid on the sex ratio in rats were observed D'Amour, 1934).	Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on development in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development and - the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

4.11.7 Conclusions on classification and labelling

It can be concluded that L-(+)-lactic acid does not meet the criteria to be classified for fertility and/or embryotoxic effects according to the criteria in the CLP regulation. Furthermore, considering the high endogenous exposure and exposure via food it is highly unlikely that L-(+)-lactic acid has effects on sexual function and fertility. Thus, it can be concluded that L-(+)-lactic acid does not meet the criteria to be classified according to the criteria in the CLP regulation.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Sexual function & fertility

According to the CLH dossier, no studies are available for this hazard class.

Effects on or via lactation

Also for this endpoint, no data are available.

Developmental toxicity

There is one study investigating potential developmental effects of lactic acid in Swiss albino mice (Colomina *et al.* 1992) and another one examining the effects on sex ratio in rats (D´Amour, 1934), but both studies lack details and only few reproductive or developmental endpoints were addressed. As no adverse effects were observed in dams, foetuses, or on the sex ratio, the DS concluded that L-(+)-lactic acid does not meet the criteria to be classified for developmental toxicity.

Comments received during public consultation

There were no comments provided in the public consultation regarding this hazard class.

Assessment and comparison with the classification criteria

The non-GLP oral gavage study by Colomina *et al*. (1992) was conducted to investigate the developmental toxicity of aluminium and the modifying influence of lactate on aluminium toxicokinetics. Aluminium is of no interest in this context, but in addition to a control group (producing 13 litters), one group only received lactic acid (570 mg/kg bw/day during day 6-15 post mating) and the 12 litters produced by this group can thus provide some limited information on the potential developmental toxicity of lactic acid.

The treatment with lactic acid resulted in a decreased food consumption of 15% in the dams. It was assumed that the lactic acid treatment partly covered their daily energy requirement, and that the reduced food consumption therefore was not an adverse effect.

There was also a very slight (-4%) not statistically significant decrease in foetal weight and a statistically significant delayed ossification of parietal bones affecting 15% of the pups in contrast to 0% in the control pups (one-third of the foetuses of each group was examined for visceral anomalies). Although possibly being a substance-related effect, as indicated by the study authors, delayed ossification generally does not lead to classification.

The rat study by D´Amour was neither guideline- nor GLP-compliant. The dose levels administered (1250 mg/kg bw/day to 10 females and 2500 mg/kg bw/day to 28 females) by gavage from GD 0-22 did not show any effects of lactic acid on the sex ratio.

Although both studies lack details, RAC supports the DS´s opinion that based on the available data, **lactic acid does not warrant classification for developmental toxicology**.

4.12 Other effects

4.12.1 Neurotoxicity

No studies on neurotoxicity of L-(+)-lactic acid were submitted. From the high exposure to L-(+)-lactic acid as natural food ingredient and food additive there are no concerns about a possible neurotoxic potential. Thus, in the view of the DS no further studies according to Annex II (data requirements) of Regulation (EU) No. 528/2012 concerning the making available on the market and use of biocidal products are required and no classification according to the criteria in the CLP regulation is triggered.

4.12.2 Immunotoxicity

No special studies on immunotoxicity of L-(+)-lactic acid were submitted. From the high exposure to L-(+)-lactic acid as natural food ingredient and food additive there are no concerns about a possible immunotoxic potential. Refer also to discussion in Section 4.1.

Thus, in the view of the DS no further studies are required according to Annex II (data requirements) of Regulation (EU) No. 528/2012 concerning the making available on the market and use of biocidal products and no classification according to the criteria in the CLP regulation is triggered.

4.12.3 Specific investigations: other studies

No further studies/ information were submitted.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Information available in REACH registration dossiers has been considered.

5.1 Degradation

Table 37: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in activated sludge according to Dutch Guidelines NEN 6633 and NEN 6634	Degree of degradation (%) **2 mg a.s./L** Day 5 = 48 % Day 20 = 60 % **4 mg a.s./L** Day 5 = 50 % Day 20 = 67 %		Hanstveit and Pullens, 1993, TNO Report nr. IMW-R 92/018; Doc. IIIA 7.1.1.2.1- 01
Biochemical Oxygen Demand (BOD) Directive 92/69/EEC, C.5	**4 mg/L** Day 5 = 50 % Day 20 = 67 %		Bowmer et al., 1998, Chemosphere Vol. 37, No.7, pp. 1317-1333

5.1.1 Stability

Hydrolysis

Experimentally derived data on hydrolysis in water are not available. From the structural formula of L-(+)-lactic acid it is clear that only one hydrolysable group is present: the acid group. For the hydrolysis of the acid group, the dissociation constant (pK) of 3.86 should be taken into account (ref. Doc IIIA7.1.1.1.1). As no further hydrolysable groups are available, a test on hydrolysis in aqueous solutions is scientifically not justified.

Photolysis

The UV-spectrum of pure L-(+)-lactic acid shows that light is absorbed in the wavelength range of 210 to 250 nm, while no absorbance was observed in the range of 290 to 800 nm (Holten, 1971). Chemicals with an UV/absorption maximum of < 290 nm cannot undergo direct photolysis in sunlight and are therefore inaccessible for direct photodegradation in sunlight. Consequently, requesting experimentally derived data on phototransformation in water is scientifically not relevant.

5.1.2 Biodegradation

5.1.2.1 Screening tests

One study on ready and inherent biodegradability was submitted (Hanstveit and Pullens, 1993). Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were tested according to the dutch guidelines NEN 6633 and NEN 6634, using the supernatant from settled activated sludge from an oxidation ditch which treats domestic sewage on the premises of TNO, Delft, The Netherlands. The guideline NEN 6634 is basically comparable to the OECD guideline 301D. The degradation in the toxicity control was > 25 % after 14 days (day 5 degradation was 51 %) indicating that no inhibitory effects towards micro-organisms at the test concentration of 4 mg/L are to be expected. The pass level for ready biodegradability (60 % COD removal in 28 days) was reached. However, since oxygen depletion was only measured at three sampling points (0, 5, and

20 days) it was not possible to determine the time-point at which 10 % of the substance were degraded. Thus, neither the 10-days window nor the 14-day window could be safely assessed and were considered to be not fulfilled during evaluation as biocidal active substance. The BOD_5/COD ratio at the higher concentration was 0.5 based on a BOD_5 of 0.45 mg O_2-mg^{-1} and a COD value of 0.90 mg O_2-mg^{-1}.

5.1.2.1 Biodegradation estimation

QSAR calculations have been conducted by the eCA using the Biowin function of EPIWEP 4.1. The results of all seven models indicate, that the substance is readily biodegradable.

Table 38: QSAR calculations for biodegradation

Model	Prediction	Value
Biowin1 (Linear Model Prediction) :	Biodegrades Fast	0.94*
Biowin2 (Non-Linear Model Prediction):	Biodegrades Fast	0.97*
Biowin3 (Ultimate Biodegradation Timeframe):	Days-Weeks	3.52**
Biowin4 (Primary Biodegradation Timeframe):	Days	4.23**
Biowin5 (MITI Linear Model Prediction) :	Readily Degradable	0.74*
Biowin6 (MITI Non-Linear Model Prediction):	Readily Degradable	0.88*
Biowin7 (Anaerobic Model Prediction):	Biodegrades Fast	0.91*
Ready Biodegradability Prediction:	YES	

*a probability greater than or equal to 0.5 indicates -> biodegrades fast; a probability less than 0.5 indicates -> does not biodegrade fast

**result classification: 5.00 -> hours 4.00 -> days 3.00 -> weeks (primary & ultimate) 2.00 -> months 1.00 -> longer

5.1.3 Summary and discussion of degradation

Taking into account a mineralization of 67% within of 20 days in the screening test, a BOD_5/COD ratio of 0.5 and the results of QSAR estimations, L-(+)-lactic acid can be considered as rapidly degradable in the environment.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

A HPLC-screening test according to the OECD guideline 121 was submitted (Baltussen, 2008). As the substance is expected to be ionised for at least 10 % at pH 5.5 to 7.5, the pKa-value was calculated (Perrin method: pKa = 3.08) and the HPLC-analysis was performed with both the ionised (measured at neutral pH) and the non-ionised form (measured at pH 2). Instead of using a calibration graph, the retention time of L-(+)-lactic acid was compared with the retention time of phenol, one of the reference substances of the method with a low $\log K_{OC}$ of 1.32. Under the chromatographic conditions of the method, the retention time of L-(+)-lactic acid was lower than the retention time of the reference substance phenol. Therefore it was concluded, that the $\log K_{OC}$ of L-(+)-lactic acid at neutral pH, as well as at pH 2 is < 1.32 (K_{OC} < 20.9 L/kg). Although this value alone is outside of the range for which the method is applicable ($\log K_{OC}$ 1.5 to 5 L/kg, see OECD 121), this approach can be accepted under consideration of all circumstances. These circumstances are the properties of L-(+)-lactic acid e.g. high water solubility, low $\log K_{OW}$ (-0.74), biodegradability and being a naturally occurring substance. The answers of an inquiry to the electronic discussion group (2008) supported our conclusion to accept the test, due to the circumstances mentioned above. Formally a test according to OECD

TG No. 106 has to be required; but it is not expected, that the results will considerably differ from the above mentioned statements. Hence, a K_{OC}-value of 20 L/kg was applied for the environmental exposure assessment during the approval of L-(+)-lactic acid in the framework of the biocidal products regulation ((EU) Nr. 528/2012).

5.2.2 Volatilisation

The vapour pressure of L-(+)-lactic acid is 0.4 Pa at 20°C. The Henry's Constant amounts to 3.6×10^{-5} Pa m^3/mol at 20°C (calculated according to eq. 21 given in the TGD; EC 2003). The photo-oxidative degradation of L-(+)-lactic acid in air was estimated by a QSAR method using the AOPWIN v1.90 (US EPA EPI Suite). The half-life of L-(+)-lactic acid in the atmosphere was estimated to be 2.71 days considering a global 24-hours mean OH-radical concentration of 5×10^5 OH radicals/cm^3.

5.3 Aquatic Bioaccumulation

Table 39: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Standard equation (formula 74), TGD on Risk Assessment (EC, 2003), part II, page 126, chapter 3.8.3.2	BCF_{Fish} = 0.048 L/kg $_{wet\ fish}$		Calculation performed by dossier submitter

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

Based on the physicochemical properties an approximate estimation of the bioconcentration factor for fish (BCF_{Fish}) was performed in accordance with the TGD on Risk Assessment (part II, chapter 3, equation 74, p. 126; EC, 2003). By applying the experimentally derived log K_{OW} of -0.74 a BCF_{Fish} of 0.048 L/kg was derived. Furthermore, no other indicators point to an intrinsic potential for bioconcentration. The surface tension, for instance, is 70.7 mN/m and thus above the trigger value of 50 mN/m. Therefore, an experimental study with fish is not required, as the estimated BCF indicates a low bioaccumulation potential of L-(+)-lactic acid in aquatic organisms.

5.3.2 Summary and discussion of aquatic bioaccumulation

An experimentally derived BCF is not available and the log K_{OW} of -0.74 is far below the trigger value of log $K_{OW} \geq 4$ for classification as bioaccumulative. Hence, the criterion is not fulfilled and L-(+)-lactic acid has to be considered as having a low potential to bioaccumulate in the environment.

5.4 Aquatic toxicity

Table 40: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
Oncorhynchus mykiss (Salmo gairdneri) EPA-660/3-75-009	96 h LC_0 = 100 mg a.s./L 96 h LC_{50} = 130 mg a.s./L 96 h LC_{100} not reported	Results based on nominal concentrations; pH not adjusted; study not reliable	Forbis et al., 1984a, ABC Inc. Report nr. #32147; Doc. IIIA7.4.1.1-02

Method	Results	Remarks	Reference
Oncorhynchus mykiss (Salmo gairdneri) EPA-660/3-75-009	96 h LC_0 = 100 mg a.s./L 96 h LC_{50} = 130 mg a.s./L 96 h LC_{100} not reported	Results based on nominal concentrations; pH not adjusted; study not reliable	Forbis et al., 1984a, ABC Inc. Report nr. #32147; Doc. IIIA7.4.1.1-02
Lepomis macrochirus EPA-660/3-75-009	96 h LC_0 = 100 mg a.s./L 96 h LC_{50} = 130 mg a.s./L 96 h LC_{100} = 180 mg a.s./	Results based on nominal concentrations; pH not adjusted; study not reliable	Forbis et al., 1984b, ABC Inc. Report nr. #32146; Doc. III A.74.1.1-03
Danio rerio (old: Brachidanio rerio) OECD 203	96 h LC50 > 320 mg/L 96 h NOEC ca. 320 mg/L	Results based on nominal concentrations; pH not adjusted; study not reliable	Bowmer et al., 1998, Chemosphere, Vol 37, No.7, pp. 1317-1333
Danio rerio (old: Brachidanio rerio) OECD 203	96 h LC50 = 320 mg/L (nominal) 96 h LC50 = 195 mg/L (real) 96 h NOEC = 180 mg/L (nominal)	pH values not adjusted	Hooftman et al., 1992, TNO report nr. R 91/29
Daphnia magna OECD 202	48 h EC_0 = 117 mg a.s./L 48 h EC_{50} = 156 mg a.s./L 48 h EC_{100} = 208 mg a.s./L	Results based on mean recovery rate of test substance; pH not adjusted; study not reliable	Hooftmann et al., 1992, TNO Report nr. IMW-91-0076-01; Doc. III A7.4.1.2-01
Daphnia magna EPA 660/3-75009	48 h EC50 ca. 750 mg/L 48 h NOEC ca. 320 mg/L	Results based on nominal concentrations; not assessable as it is only poorly documented – study not reliable?	Forbis et al., 1984c, ABC Laboratories Inc., Report nr. 32148
Daphnia magna OECD 202	48 h EC50 ca. 240 mg/L	Results based on nominal conc., pH not adjusted; study not reliable	Bowmer et al., 1998, Chemosphere, Vol. 37, No.7, pp. 1317-1333
Selenastrum capricornutum OECD 201	70.5 h NOE_rC = 1,100 mg a.s./L 70.5 h E_rC_{50} = 3,900 mg a.s./L	Results based on mean recovery rate of test substance	Hanstveit and Oldersma, 1992, TNO Report nr. IMW-91-0076-05; Doc. III A7.4.1.3-01
QSAR	Fish: LC_{50} = 177 g a.s./L Invertebrates: EC_{50} = 78.8 g a.s./L Algae: E_rC_{50} = 21.3 g a.s./L		Calculation performed by dossier submitter

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Two acute toxicity studies with fish were performed according to the US EPA standard 660/3-75-009 (Forbis et al., 1984a, 1984b). The acute toxicity of L-(+)-lactic acid to rainbow trout and bluegill sunfish, respectively, was assessed by exposing fish to concentrations of 0, 32, 56, 100, 180 and 320 mg a.s./L. Actual concentrations

of the test substance were not measured during the study. Hence, results are related to the nominal concentrations. In the highest test concentrations rather low pH values (3.5 to 4.9) were measured during the study. Therefore, it was concluded that the observed mortality of the test animals was caused by the low pH. Because of the varying pH between treatments of different L-(+)-lactic acid concentrations and the fact that no analytical monitoring of the test substance concentration was performed, both studies were assessed as not reliable. However, the test results can be used as supportive information.

Two studies with *Danio rerio* were performed according to OECD Guideline 203 (Bowmer et al., 1998 and Hooftman et al., 1992). In (Bowmer et al., 1998) Zebrafish was exposed to 320 and 560 mg/L of L-(+)-lactic acid in a semi-static test system. The pH value varied between the treatments with a pH value of 4.1 at 320 mg/L and 3.5 at 560 mg/L. Because of the varying pH between treatments of different L-(+)-lactic acid concentrations and the fact that no analytical monitoring of the test substance concentration was performed, the study was assessed as not reliable. However, the test results can be used as supportive information. Also in (Hooftman et al., 1992) the pH values depended on the concentrations of L-(+)-lactic acid (pH of medium = 8.0; pH of highest test concentration = 3.25). Test concentrations used were nominal 80, 144, and 256 mg/L. Here an analytical verification of the test concentrations was performed. There was a concentration loss of approximately 70 %. Similar to (Forbis et al., 1984a, 1984b) it is possible that the observed mortality of the test animals was caused by the low pH value.

Due to the very low toxicity of L-(+)-lactic acid on fish (LC_{50} > 100 mg/L) in the above mentioned tests without pH adjustment and reasons of animal welfare, a new fish test was not demanded. Furthermore, additional data from the scientific literature as well as from estimations by quantitative structure–activity relationship models (QSAR) are available which support the findings on the low toxicity of L-(+)-lactic acid and the concentration related pH effects on fish. Additional data on the acute toxicity of L(+) lactic acid to fish is available from a study in which semi-static bioassays were conducted according to the APHA guideline from 1995 (Saha et al., 2006). In this test no analytical measurement was conducted, but the medium was replaced every 24h. The authors stated that the pH decreased significantly in treatments of high concentrations without giving specific information on measured pH values., In this study a 96 h LC_{50} of 258 mg/L (nominal) was obtained for tilapia (*Oreochromis mossambicus*) which is in the same order of magnitude as the results provided for rainbow trout and bluegill sunfish.

The QSAR analyses for L-(+)-lactic acid were performed using the ECOSAR model (v1.11) and revealed a LC_{50} value for fish of 177 g/L for L-(+)-lactic acid (Table 38).

5.4.1.1 Long-term toxicity to fish

No data available.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Daphnia magna was exposed to six different concentrations of L-(+)-lactic acid (0, 32, 56, 100, 180, 320, and 560 mg a.s./L) for 48 hours in a static acute toxicity study according to the OECD guideline 202 (Hooftman et al., 1992). As the actual test substance concentrations were measured at the beginning and at the end of the test by enzymatic analysis, the effect concentrations (EC_x) were recalculated by the dossier submitter on the basis of the mean recovery rate. In the highest test concentrations rather low pH values (3.7 to 4.1) were measured during the test. Therefore it was concluded that the mortality of *D. magna* in these treatments was related to the low pH. As for the tests with fish this study was assessed as not reliable because the pH varied between treatments as a function of the L-(+)-lactic acid concentration. However, the test results can be used as supportive information. Normally, a new study with *D. magna* should be requested, but due to the very low toxicity of L-(+)-lactic acid against *D. magna* (EC_{50} > 100 mg/L) a new test was considered unnecessary.

Furthermore, additional data from the scientific literature as well as from estimations by QSAR support the findings on the low toxicity of L-(+)-lactic acid and the concentration related pH effects on invertebrates. Saha

et al. (2006) assessed the acute toxicity of L-(+)-lactic acid on the cladoceran crustacea *Moina micrura* using a bioassay approach according to the APHA guideline from 1995. In this test no analytical measurement was conducted, but the medium was replaced every 24h. In this study a 96 h LC_{50} of 329 mg/L (nominal) was determined for *M. micrura* which is in the same order of magnitude as the findings of the study with *D. magna*.

The QSAR estimation (ECOSAR; v1.11) were performed by the dossier submitter and revealed an EC_{50} for *D. magna* of 78.8 g/L (Table 38).

5.4.2.1 Long-term toxicity to aquatic invertebrates

No data available.

5.4.3 Algae and aquatic plants

A valid study on growth inhibition of algae according to the OECD guideline 201 was performed with *Selenastrum capricornutum* (Hanstveit and Oldersma, 1992). Algae were exposed to six nominal test concentrations of a 80 % L-(+)-lactic acid solution (0, 0.10, 0.33, 0.56, 1.0, 1.9, 2.8 g/L; these concentrations correspond to 0, 0.08, 0.26, 0.45, 0.8, 1.52, 2.24 g a.s./L). The actual test concentrations were measured by enzymatic analysis at the start and the end of the test. The pH values were adjusted at the beginning of the test and remained stable.

Since no significant inhibition of growth was observed during the test, even at the highest test concentration, the effect concentrations given in the study report were extrapolated from the available data. Although the measured concentrations were consistently lower (at least 21 %) than the nominal concentrations in all treatments, the effect concentrations presented were calculated on the basis of the nominal concentrations. Hence a recalculation of the E_rC_{50} was conducted by the dossier submitter using the mean measured concentrations from the highest treatment (nominal concentration = 2.24 g a.s./L). Out of this approach, an E_rC_{50} of 3.9 g a.s./L and a NOE_rC of 1.1 g a.s./L. was derived for *S. capricornutum*. The effect concentration (E_rC_{50}) for algae of 21.3 g a.s./L that was estimated by QSAR (ECOSAR; v1.11) indicated that algae can be considered as the most sensitive species for L-(+)-lactic acid.

5.4.4 Other aquatic organisms (including sediment)

No data available.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Degradation

A substance is classified to be rapidly degradable when it is demonstrated to be readily biodegradable in a 28-day test for ready biodegradability, while the pass level of the test (70 % DOC removal or 60 % theoretical oxygen demand) must be achieved within 10 days from the onset of biodegradation. If this is not possible, then the pass level should be evaluated within a 14 day time window if possible, or after the end of the test. Rapidly degradability is also indicated by a BOD_5/COD ratio ≥0.5. Taking into account a mineralization of 67% within of 20 days in the screening test, and a BOD_5/COD ratio of 0.5, the criteria mentioned above are fulfilled. The results of QSAR estimations further support that L-(+)-lactic acid can be classified as rapidly degradable in the environment.

Bioaccumulation

An experimentally derived BCF is not available and the log K_{OW} of -0.74 for L-(+)-lactic acid is far below the trigger value of log $K_{OW} \geq 4$ for classification as bioaccumulative. Hence, the criterion is not fulfilled and L-(+)-lactic acid has to be considered to have a low bioaccumulative potential in the environment.

Short-term (acute) aquatic hazard

For L-(+)-lactic acid acute studies are available for fish, invertebrates and algae. For all three trophic levels the available effect values are $L(E)C_{50} > 100$ mg/L.

The criterion for classification as H400 Very toxic to aquatic life is $LC_{50} \leq 1$ mg/L. Hence, L-(+)-lactic acid **does not fulfil this criterion** and no classification as H400 is necessary.

Long-term (chronic) aquatic hazard

For long-term aquatic toxicity, suitable chronic data is available only for algae. With a NOErC ≥ 1000 mg/L the effect value is far from the critical trigger value for rapidly degradable substances of NOEC ≤ 1 mg/L for classification.

Because there is no suitable chronic data available for all three trophic levels, according to CLP Annex I, figure 4.1.1 in a second step the surrogate approach has to be applied, in which data on the acute toxicity is combined with information on the fate in the environment. However, the trigger value for classification is a $L(E)_{50} \leq 100$ mg/L and as all acute effect values are $L(E)_{50} > 100$ mg/L no classification is needed.

None of the criteria for long-term (chronic) aquatic hazard classification is fulfilled and there is no need for long-term (chronic) aquatic hazard classification.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

L-(+)-lactic acid has to be considered as rapidly degradable and not bioaccumulative in the environment. The criteria for short-term (acute) or long-term (chronic) hazard classification are not fulfilled.

Hence, no classification and labelling for the environmental hazards “Hazardous to the aquatic environment” is required for L-(+)-lactic acid.

RAC evaluation of aquatic hazards (acute and chronic)
Summary of the Dossier Submitter’s proposal The DS considered L-(+)-lactic acid as rapidly degradable, not bioaccumulative in the environment and not hazardous to the aquatic environment. ***Stability*** Hydrolysis Experimentally derived data on hydrolysis in water are not available. From the structural formula of L-(+)-lactic acid it is clear that only one hydrolysable group is present: the acid group. For the hydrolysis of the acid group, the dissociation constant (pK) of 3.86 should be taken into account (ref. Doc IIIA7.1.1.1.1). As no further hydrolysable groups are available, a test on hydrolysis in aqueous solutions is scientifically not justified. Photolysis According to Holten (1971), the dissociation constant (pK) of the acid group (the only hydrolysable group) of L-(+)-lactic acid is 3.86 and light is absorbed in the wave-length

range of 210 to 250 nm but not in the range of 290 to 800 nm by pure L-(+)-lactic acid. Therefore, no direct phototransformation is expected.

Biodegradation

Based on two Dutch guidelines (NEN 6633 and NEN 6634, the latter being comparable to OECD TG 301D), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were tested by Hanstveit and Pullens (1993) using the supernatant from settled activated sludge from an oxidation ditch which treats domestic sewage. In this study, the pass level for ready biodegradability (60% COD removal in 28 days) on L-(+)-lactic acid (purity 79.5-80%) was reached but since oxygen depletion was only measured on days 0, 5 and 20, it was not possible to determine the time-point at which 10% of the substance was degraded. The BOD (5 days)/COD ratio at a concentration of 4 mg/L was 0.5 based on a BOD_5 value of 0.45 mg O_2/mg and a COD value of 0.90 mg O_2/mg.

In addition, the results of QSAR calculations (seven models) using the Biowin function of EPIWEP 4.1. indicate that L-(+)-lactic acid is readily biodegradable.

Bioaccumulation

By applying the experimentally derived log K_{OW} of -0.74 a BCF_{Fish} of 0.048 L/kg was calculated according to the TGD on Risk Assessment (part II, chapter 3, EC (2003)). Another indicator for a low bioaccumulation is the surface tension which is 70.7 mN/m of 93% L-(+)-lactic acid at 1 g/L in water.

Aquatic toxicity

There are five acute toxicity studies in fish (*Oncorhynchus mykiss, Lepomis macrochirus, Danio rerio* and *Orechromis mossambicus*) with LC_{50} values between 130 mg a.s./L and 320 mg/L, three tests in aquatic invertebrates (*Daphnia magna*) with EC_{50} = 156-750 mg a.s./L and one study in algae (*Selenastrum capricornutum*) with E_rC_{50} = 3.9 g a.s./L. No long-term tests in fish and invertebrate are available but the algae test can be also considered as a chronic test.

The fish studies on *Oncorhynchus mykiss* and *Lepomis macrochirus* by Forbis *et al.* (1984a and 1984b) performed with the test substance SY-83 containing 76.5-83.5% L-(+)-lactic acid and on *Danio rerio* by Bowmer *et al*. (1998) are not considered reliable. This is because the pH value varied between treatments as a function of the L-(+)-lactic acid concentration and because there was no analytical monitoring of the test substance concentration. However, the results can be used as supporting information. In the fourth mentioned study, performed by Hooftman *et al.* (1992), the pH was also dependend on the L-(+)-lactic concentrations but the test concentrations were analytically verified and a concentration loss of approximately 70% was observed. The results of the semi-static bioassay by Saha *et al*. (2006), were comparable to the ones by Forbis. In this study, however, the medium was replaced every 24 hours.

In the *Daphnia magna* studies the same problem with the pH values occurred as in the fish studies. However, for fish as well as for invertebrates there are also QSAR analyses for L-(+)-lactic acid using the ECOSAR model 1.11 which support a low toxicity on fish (177 g a.s./L) and invertebrates (78.8 g/L). According to the ECOSAR model, algae can be considered as the most sensitive species for L-(+)-lactic acid with an effect concentration of E_rC_{50} = 21.3 g a.s./L.

Comments received during public consultation

No specific comments were received, but one MS indicated general agreement with the DS´s proposal not to classify L-(+)-lactic acid for the environment.

Assessment and comparison with the classification criteria

Degradation

A substance is classified to be rapidly degradable when it is demonstrated to be readily biodegradable in a 28-day test for ready biodegradability, while the pass level of the test (70% DOC removal or 60% theoretical oxygen demand) must be achieved within 10 days from the onset of biodegradation. If this is not possible, then the pass level should be evaluated within a 14 day time window if possible, or after the end of the test. Rapidly degradability is also indicated by a BOD(5 days)/COD ratio ≥0.5. Taking into account a mineralization of 67% within of 20 days in the screening test, and a BOD(5 days)/COD ratio of 0.5, the criteria mentioned above are fulfilled. The results of QSAR estimations further support that L-(+)-lactic acid can be classified as rapidly degradable in the environment.

RAC supports the DS´s conclusion that L-(+)-lactic can be considered as rapidly degradable in the environment.

Bioaccumulation

An experimentally derived BCF is not available and the log K_{OW} of -0.74 for L-(+)-lactic acid is far below the trigger value of log K_{OW} ≥ 4 for classification as bioaccumulative. Hence, RAC agrees with the DS that L-(+)-lactic acid has to be considered to have a low bioaccumulative potential in the environment.

Aquatic toxicity

Short-term (acute) aquatic hazard

For L-(+)-lactic acid acute studies are available for fish, invertebrates and algae. For all three trophic levels the available effect values are $L(E)C_{50}$ > 100 mg/L.

The criterion for classification as Aquatic Acute 1; H400 "Very toxic to aquatic life" is LC_{50} ≤ 1 mg/L. Hence, L-(+)-lactic acid does not fulfil this criterion and no classification as Aquatic Acute 1 is necessary.

Long-term (chronic) aquatic hazard

For long-term aquatic toxicity, suitable chronic data is available only for algae. With a NOErC ≥ 1000 mg/L the effect value is far above the critical trigger value for rapidly degradable substances of NOEC ≤ 1 mg/L for classification.

Because there is not suitable chronic data available for all three trophic levels, according to CLP Annex I, figure 4.1.1 in a second step the surrogate approach has to be applied, in which data on the acute toxicity is combined with information on the fate in the environment. However, the trigger value for classification is a $L(E)_{50}$ ≤ 100 mg/L and as all acute effect values are $L(E)_{50}$ > 100 mg/L no classification is needed.

None of the criteria for long-term (chronic) aquatic hazard classification is fulfilled and

there is no need for long-term (chronic) aquatic hazard classification.

RAC agrees with the DS´s proposal that **no classification for environmental hazards *is warranted***.

6 OTHER INFORMATION

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8 ANNEXES

Confidential Annexes