Committee for Risk Assessment

RAC

Annex 1

Background document

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

succinic anhydride

EC Number: 203-570-0
CAS Number: 180-30-5

CLH-O-0000001412-86-123/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

16 September 2016

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Succinic anhydride

EC Number: 203-570-0

CAS Number: 108-30-5

Index Number: 607-103-00-5

Contact details for dossier submitter:

Environment Agency Austria, Spittelauer Lände 5, A-1090 Vienna

on behalf of the Austrian Competent Authority (Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management, Stubenring 1, 1010 Vienna, Austria)

Version number: 02 **Date:** **23.09.2015**

CONTENTS

Part A.

Part B.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Succinic anhydride
EC number:	203-570-0
CAS number:	108-30-5
Annex VI Index number:	607-103-00-5
Degree of purity:	confidential information (Annex I)
Impurities:	confidential information (Annex I)

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	Acute Tox. 4*; H302 Eye Irrit. 2, H319 STOT Single Exp. 3, H335	--
Current proposal for consideration by RAC	Removal of asterisk (*) from Acute Tox 4, H302 Resp. Sens. 1; H334 Skin Sens. 1; H317 Eye Dam. 1; H318 Skin Corr. 1, H314	--
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 4; H302 STOT SE 3, H335 Resp. Sens. 1, H334 Skin Sens. 1, H317 Eye Dam. 1, H318: Skin Corr. 1, H314	--

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	Not assessed in this dossier.
2.2.	Flammable gases	None	--	None	Not assessed in this dossier.
2.3.	Flammable aerosols	None	--	None	Not assessed in this dossier.
2.4.	Oxidising gases	None	--	None	Not assessed in this dossier.
2.5.	Gases under pressure	None	--	None	Not assessed in this dossier.
2.6.	Flammable liquids	None	--	None	Not assessed in this dossier.
2.7.	Flammable solids	None	--	None	Not assessed in this dossier.
2.8.	Self-reactive substances and mixtures	None	--	None	Not assessed in this dossier.
2.9.	Pyrophoric liquids	None	--	None	Not assessed in this dossier.
2.10.	Pyrophoric solids	None	--	None	Not assessed in this dossier.
2.11.	Self-heating substances and mixtures	None	--	None	Not assessed in this dossier.
2.12.	Substances and mixtures which in contact with water emit flammable gases	None	--	None	Not assessed in this dossier.
2.13.	Oxidising liquids	None	--	None	Not assessed in this dossier.
2.14.	Oxidising solids	None	--	None	Not assessed in this dossier.
2.15.	Organic peroxides	None	--	None	Not assessed in this dossier.
2.16.	Substance and mixtures corrosive to metals	None	--	None	Not assessed in this dossier.
3.1.	Acute toxicity - oral	Acute Tox 4, H302	--	Acute Tox 4*, H302	--
	Acute toxicity - dermal	None	--	None	Not assessed in this dossier.
	Acute toxicity - inhalation	None	--	None	Not assessed in this dossier.
3.2.	Skin corrosion / irritation	Skin Corr. 1, H314	--	--	--
3.3.	Serious eye damage / eye irritation	Eye Dam 1, H318	--	Eye Irrit. 2, H319	--

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
				C ≥ 1%	
3.4.	Respiratory sensitisation	Resp. Sens. 1, H334	--	--	--
3.4.	Skin sensitisation	Skin Sens. 1, H317	--	--	--
3.5.	Germ cell mutagenicity	None	--	None	Not assessed in this dossier.
3.6.	Carcinogenicity	None	--	None	Not assessed in this dossier.
3.7.	Reproductive toxicity	None	--	None	Not assessed in this dossier.
3.8.	Specific target organ toxicity –single exposure	**STOT SE 3; H335** **C ≥ 1%**		STOT SE 3; H335 C ≥ 1%	**Not assessed in this dossier.**
3.9.	Specific target organ toxicity – repeated exposure	None		None	Not assessed in this dossier.
3.10.	Aspiration hazard	None	--	None	Not assessed in this dossier.
4.1.	Hazardous to the aquatic environment	None	--	None	Not assessed in this dossier.
5.1.	Hazardous to the ozone layer	None	--	None	Not assessed in this dossier.

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

Labelling:

Hazard pictograms:
GHS07
GHS05
GHS08

Signal word:
Danger

Hazard statements:
H302: Harmful if swallowed
H314: Causes server skin burns and eye damage
H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled
H317: May cause an allergic skin reaction
H335: May cause respiratory irritation

Precautionary statements:
No statement codes are proposed since precautionary statements are not included in Annex VI of Regulation EC no. 1272/2008.

Proposed notes assigned to an entry:

--

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Succinic anhydride (Index No. 607-103-00-5) was classified as Xi, R36/37 (Irritating to eyes; Irritating to respiratory system) (concentration limit ≥1%) in Commission Directive 91/325/EEC of 1st March 1991 adapting to technical progress for the twelfth time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to classification, packaging and labelling of dangerous substances (91/325/EEC).

2.2 Short summary of the scientific justification for the CLH proposal

Succinic anhydride was proposed for substance evaluation based on article 45(5) of the REACH Regulation. The evaluation was targeted to all sections of the chemical safety assessment given in the IUCLID dossier and chemical safety report of the lead registrant (full registration, joint submission). Based on the in-depth evaluation of the hazard data it is proposed that the current harmonised classification entry for human health should further include classification for skin and respiratory sensitising properties (Skin Sens. 1, Resp Sens. 1). Moreover, the harmonised classification for the eye irritation properties should be revised. A classification for Eye Dam 1 is deemed warranted. Beside, results of skin irritation/corrosion studies demonstrate that succinic anhydride should be classified as Skin Corr. 1B substance. The asterisk (*) indicating minimum CLP classification for Acute oral Toxicity 4 (H302) is no longer necessary since the data confirms the classification.

Based on thorough evaluation of available data a revision and an extension of the current harmonised classification entry is deemed necessary and an adaption is proposed.

2.3 Current harmonised classification and labelling

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

Table 4: Current Annex VI Table 3.1 – Harmonised classification and labelling of hazardous substances

Classification		Labelling		Specific Conc. Limits, M-factors
Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code	
Acute Tox 4* Eye Irrit. 2 STOT SE 3	H302 H319 H335	GHS07 Wng	---	STOT SE 2; H335: C ≥ 1% Eye Irrit. 2; H319: C ≥ 1%

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

Table 5: Current Annex VI, Table 3.2 – Harmonised classification and labelling of hazardous substances from Annex I to Directive 67/548/EEC

Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration limits
607-103-00-5	succinic anhydride	203-570-0	108-30-5	Xi; R36/37	Xi R: 36/37 S: (2-)25	Xi; R36/37: C ≥ 1%

2.4 Current self-classification and labelling

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

Self-classification notifications for succinic anhydride are summarized in the C&L Inventory (http://echa.europa.eu/regulations/clp/cl-inventory-database).

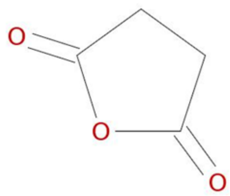
There are 26 aggregated notifications present in the inventory and the total number of notifiers is 1065. 16 notifications classified the substance according to the current harmonised classification (accessed on 07th of October 2014), without any additional classification.

Beside the current harmonised classification, further classification for the respiratory sensitisation potential is indicated in the C&L Inventory (Resp. Sens. 1 (H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled), Skin Sens. 1 (H317: May cause an allergic skin reaction).

RAC general comment

Succinic anhydride has an existing entry in Annex VI to CLP, where it is classified as Acute Tox 4* (H302); Eye Irrit. 2 (H319: C≥ 1%); STOT SE 3 (H335: C≥ 1%).

The proposal is based on a substance evaluation recently performed under REACH. The present opinion only addresses the endpoints (acute oral toxicity, eye irritation/damage, skin corrosion/irritation, skin sensitisation, respiratory sensitisation and specific target organ toxicity - single exposure) that were evaluated by the dossier submitter (DS) in their proposal or addressed in the public consultation (acute oral toxicity, eye irritation/damage, skin corrosion/irritation, skin sensitisation, respiratory sensitisation and specific target organ toxicity - single exposure).



Succinic anhydride is a reactive compound that hydrolyses in water (reported half life is 5 min according to the CLH report). This reaction is exothermic and the tissue at site of contact can be damaged. The reactivity is lower in non-polar solvents such as oil. Therefore, on the one hand, the choice of solvent will influence what is actually tested (succinic anhydride and/or succinic acid) and on the other hand, hydrolysis can be expected to partially occur in real life for local effects where succinic anhydride will be in an environment (skin – sweat; eye – tear; respiratory tract - humidity) where it will hydrolyse and form succinic acid. The acid form of succinic anhydride (e.g. succinic acid) has a high water solubility (62.9 g/L at 20 °C). The DS has therefore used data for succinic acid to support the proposed classification for eye damage.

The DS has also used read across to the hazardous properties of **maleic anhydride**, a close structural homologue, to fill the data gap for respiratory sensitisation and to support classification for skin corrosion. However the DS did not address the STOT RE endpoint which, as a result, was not addressed by RAC, even though maleic anhydride was proposed to be classified as STOT RE 1.

Maleic anhydride is a much more reactive anhydride (reported half-life in water is 0.3 min according to the CLH report). The higher reactivity of maleic anhydride as compared to the succinic anhydride is also reflected in the greater severity of eye damage effects. Thus there are quantitative differences in the reactivity between succinic and maleic anhydride. However although the reactivity of succinic anhydride might be lower than that of maleic anhydride, the LLNA data show that it is of biological relevance. From a mechanistic point of view this is not surprising since the acid anhydride structure is considered to be a strongly acylating.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Pursuant to Article 45(4) of the REACH Regulation the MSCA of Austria has initiated substance evaluation for succinic anhydride. In the course of the evaluation, the evaluating

MSCA noted that the current harmonised classification entry is incomplete. A review of available data revealed that the classification listed in Annex VI of Regulation EC No. 1272/2008 is not in line with the classification provided in the registration and with the classification provided by notifiers in the C&L Inventory.

According to article 36(1) of the CLP Regulation substances that fulfil the criteria for respiratory sensitization (Cat. 1) (Annex I, section 3.4) shall normally be subject to harmonised classification. The current harmonised classification of succinic anhydride needs to be amended.

Due to new evaluation and interpretation of existing human health hazard data a change of the existing entry is proposed. Furthermore, new human health data became available.

CLP classification criteria have been modified/amended (e.g., for acute toxicity), which has been taken into consideration in the current proposal for modification of the harmonised classification. Besides, in the C&L inventory classification and labelling entries are not consistent.

The submitted data also demonstrate that succinic anhydride possesses skin sensitisation properties and therefore a classification for Skin Sens. 1 is warranted. Furthermore, the hazard data provided in the registration dossier by the lead registrant (full registration, joint submission) indicate that succinic anhydride should be classified as Eye Dam. 1 instead of Eye Irrit. 2.

Test results of skin corrosion/irritation tests demonstrate that succinic anhydride needs a further classification for its skin corrosive properties (Skin Corr. 1B, H314: Cause severe skin burns and eye damage). The current Annex VI entry for succinic anhydride includes also Acute Tox 4* with the hazard statement H302 (Harmful if swallowed) as a minimum classification as indicated by the reference * in Table 3.1. Evaluation of experimental data of oral toxicity data shows that the indication of the minimum classification [(*)] is no longer necessary.

Based on thorough evaluation of available hazard data an extension and revision of the current harmonised classification is proposed.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

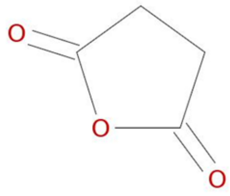
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 6: Substance identity

EC number:	203-570-0
EC name:	Succinic anhydride
CAS number (EC inventory):	108-30-5
CAS number:	108-30-5
CAS name:	Butanedioic anhydride
IUPAC name:	Dihydrofuran-2,5-dione
CA index name:	2,5-Furandione, dihydro-
CLP Annex VI Index number:	607-103-00-5
Molecular formula:	$C_4H_4O_3$
Molecular weight range:	100.0728

Structural formula:



1.2 Composition of the substance

Data on the composition of the substances are considered as confidential (Annex I – confidential Annex).

1.2.1 Composition of test material

The composition of the test material is indicated in the individual test description and is considered as relevant for the harmonized classification for succinic anhydride.

1.3 Physico-chemical properties

Table 7: Summary of physico-chemical properties

Property	Value	Reference[1]	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Solid, colourless needles	REACH registration (2013)	--
Melting/freezing point	119.0 °C	REACH registration (2013)	measured data, OECD TG 102
Boiling point	263.5°C	REACH registration (2013)	measured data, OECD TG 103
Relative density	1.234 at 20°C.	REACH registration (2013)	Source: The Merck Index, Eighth edition
Vapour pressure	0.2 Pa at 25°C .	EpiSuite v.4.1	measured data;
Surface tension	Study technically not feasible	REACH registration (2013)	--
Water solubility	Substance hydrolyses fast. Water solubility of hydrolysis product succinic acid: 62.9 g/L at 20°C	REACH registration (2013)	measured data, OECD TG 105
Partition coefficient n-octanol/water	Substance as such hydrolyses in n-octanol/water. Log POW of hydrolysis product succinic acid: -0,59 Theoretical Log POW of anhydride (substance as such)using KOWWIN (v1.68), EPISUITE 4.10: Log POW: 0.8102	REACH registration (2013)	measured data, OECD TG 117
Flash point	Study technically not feasible	REACH registration (2013)	--
Flammability	Not flammable	REACH registration (2013)	EU Method A.10
Explosive properties	Study technically not feasible	REACH registration (2013)	--
Self-ignition temperature	Data waiving	REACH registration (2013)	--
Oxidising properties	Data waiving	REACH registration (2013)	--
Granulometry	The median particle size D50 of the test items deduced from the particle distributions is 1197µm. D10 = 377 µm. D90 = 2309 µm.	REACH registration (2013)	measured data, OECD TG 110

Property	**Value**	**Reference[1]**	**Comment (e.g. measured or estimated)**
Stability in organic solvents and identity of relevant degradation products	Data waiving	REACH registration (2013)	--
Dissociation constant (pKa)	Succinic acid: 4.67 and 5.64 at 25°C	REACH registration (2013)	OECD TG 112
Viscosity	Data waiving	REACH registration (2013)	

[1] REACH registration refers to full registration and joint submission; registration was updated in the year 2013.

Additional information on pyhsico-chemical properties:

The knowledge on phys-chem parameters and the behavior of succinic anhydride under certain conditions are of specific importance for the interpretation of toxicological test results.

Succinic anhydride hydrolyses fast and to a full extent in water (in the range of minutes) to its corresponding acid form. Thus, it is expected that the anhydride is present as acid in aqueous media. The acid form reveals high water solubility.

Regarding non-protic/non-aqueous media the anhydride is expected to be stable and not to undergo hydrolysis. It is dissolved depending on the solubility in these media. Referring to the calculated log POWs of 0.81 for succinic anhydride, succinic anhydride is predicted to be more soluble in n-octanol than in water. The POW value is a theoretical value, as the anhydride is hydrolysed in water and might even form esters with n-octanol. Nevertheless, the value support the finding, that the anhydride also reveals high solubilities in polar, organic media. The solubility decrease with the reduction of the polarity of the solvent. Nevertheless, succinic anhydride is still expected to be soluble in a non-polar media like oil (molecules revealing high molecular weights and low content of polar elements) as vehicle and is not expected to be hydrolyzed to the corresponding acid form.

As mentioned the anhydride form is converted in aqueous media to the corresponding acid. Therefore, water solubility and pKa values are indicated in the table above for the acid. For further details on solubility and behavior of succinic anhydride and the structural similar maleic anhydride in different media are provided in the non-confidential Annex IV.

2 MANUFACTURE AND USES

2.1 Manufacture

Succinic anhydride has been fully registered as a joint submission in a tonnage band of 1,000 – 10,000 tonnes per year (ECHA dissemination website, accessed on 18th of August 2014).

2.2 Identified uses

Succinic anhydride is used as monomer for production of resins. The substance is registered for industrial and for professional use, no consumer uses have been identified. Following product categories are listed in the registrations: PC 1: Adhesives, sealants, PC 9a: Coatings and paints, thinners, paint removes, PC 32: Polymer preparations and compounds, PC 9b:

Fillers, putties, plasters, modelling clay, PC 19: Intermediate (ECHA dissemination website, accessed on 15th of September 2014).

On overview of registered uses is given in the following table:

Table 8: Registered uses (ECHA dissemination site, 08th of September 2014)

Process category (PROC)	**Chemical product category (PC)**	**Environmental release cate-gory (ERC)/ Sector of end use (SU)**
	Manufacture	
PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing)	--	ERC 1: Manufacture of substances
Uses at Industrial Sites: Industrial use as monomer for production of resins		
PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing)	PC 1: Adhesives, sealants PC 9a: Coatings and paints, thinners, paint removes PC 32: Polymer preparations and compounds PC 9b: Fillers, putties, plasters, modelling clay	ERC 6c: Industrial use of monomers for manufacture of thermoplastics SU 8: Manufacture of bulk, large scale chemicals (including petroleum products) SU 0: Other: SU3: Industrial use
Uses at Industrial Sites: Industrial use as intermediate for production of substances or other intermediates		
PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 8b: Transfer of substance or preparation (charging/discharging)	PC 19: Intermediate	ERC 6a: Industrial use resulting in manufacture of another substance (use of intermediates) SU 8: Manufacture of bulk, large scale chemicals (including petroleum products)

Process category (PROC)	Chemical product category (PC)	Environmental release cate-gory (ERC)/ Sector of end use (SU)
from/to vessels/large containers at dedicated facilities PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing)		
Uses by Professional Workers: Laboratory Use		
PROC 15: Use as laboratory reagent	PC 21: Laboratory chemicals	SU 0: Other SU22: Professional use

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Not evaluated in this dossier.

4.2 Acute toxicity

Table 9: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
OECD TG 401 (Acute Oral Toxicity), equivalent or similar Test species: rat (Sprague-Dawley), male/female Route: oral/gavage Concentration dose: Preliminary study: 50, 139, 387, 1078 and 3000 mg/kg bw Number of animals: 4 per dose (2 males/2 females) Principal study: 1214, 1500, 1854, 2291, 2832, and 3500 mg/kg/bw Number of animals: 10 per dose (5 males/5 females) Test material: succinic anhydride Vehicle: corn oil Test substance is not expected to be hydrolysed to acid form in vehicle.	LD50: 2157.2 mg/kg bw (male) LD50: 1510.5 mg/kg bw (female) LD50: 1794.9 mg/kg bw (male/female)	Klimisch 1: reliable without restriction (indicated in REACH registration) Key study	Reagan, E.L. (1982)

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

The study of Reagan et al. (1982) was declared in the REACH registration (full registration, joint submission) as reliable without restriction and has been carried out according to the OECD TG 401 (Acute oral toxicity). A standard protocol for the determination of acute median lethal oral dose has been followed.

For the dose range finding study animals were assigned to groups of two males and two females at five dose levels and for the principal study five males and five females at six dose levels.

After an acclimation of 7 days, animals were fasted overnight prior to receiving a single oral dose of the test substance. The test article was administered at a constant concentration and the volume of dosing solution did not exceed 5 mL per animal, where possible.

Animals in the principal study were observed at least 14 days or even longer until all signs of reversible toxicity subsided. Animals were observed three times on the day of dosing and twice daily for the remaining study. All visible toxic effects were recorded. Body weights were recorded at the beginning of the study and on days 8 and 15. All test animals were subject to gross necropsy after death or termination of the study.

The preliminary study gave mortality results only at the highest dose level of 3000 mg/kg bw (4/4). In the main study lower dose levels were applied to establish a LD50 value. Following doses have been applied to five male and female animals per dose group: 1214, 1500, 1854, 2291, 2832, and 3500 mg/kg/bw.

In males a decreased activity and death was seen at dose levels of 1500 mg/kg and higher and soft stools were reported for male rats dosed at 2291 mg/kg. Ataxia was observed for males at the two highest dose levels. Decreased activity and death was observed at all dose levels in females and soft stools were observed in females dosed at 1214 mg/kg. Ataxia was observed at doses of 1500 mg/kg and higher.

Black pylorus in stomach and intestines containing a blood-like substance were seen in males at doses of 2291 mg/kg and above. At the highest dose, green areas on the lungs were seen in males at necropsy. In females, black stomach pyloric and intestines containing a blood-like substance were seen at doses of 1854 mg/kg and higher. At necropsy, green areas on the lungs were seen in females dosed at 2291 and 3500 mg/kg.

The data demonstrate that female rats are more sensitive to adverse acute toxic effects of succinic anhydride application.

Following mortality rates have been observed:

Table 10: Mortality in oral acute toxicity study (Reagan, 1982).

Dose Level (mg/kg) Males	Cumulative mortality	Dose Level (mg/kg) Females	Cumulative mortality
1214	0/5	1214	1/5
1500	1/5	1500	3/5
1854	2/5	1854	3/5
2291	3/5	2291	5/5
2831	3/5	2832	5/5
3500	5/5	3500	5/5

LD50 values of 2157.2 mg/kg bw and 1510.5 mg/kg bw for male and females, respectively, have been deduced. The LD50 value for males and females is 1794.9 mg/kg bw.

4.2.1.2 Acute toxicity: inhalation

Not evaluated in the present dossier.

4.2.1.3 Acute toxicity: dermal

Not evaluated in the present dossier.

4.2.1.4 Acute toxicity: other routes

Not evaluated in the present dossier.

4.2.2 Human information

No relevant information available.

4.2.3 Summary and discussion of acute toxicity

Following administration of succinic anhydride (vehicle: oil) by gavage to male and female Sprague-Dawley rats a LD50 value of 2157.2 and 1510.5 mg/kg bw, respectively was deduced. The substance is not expected to be hydrolysed prior to administration.

At the three highest dose levels in females (2291, 2832 and 3500 mg/kg bw) and the highest dose level in males (3500 mg/kg bw) all test animals died by day 2 of the study. Clinical signs included a decreased activity, ataxia and soft stools. Gross necropsy revealed blackening of the pyloric region of the stomach and a blood-like, viscous substance in the intestines. A LD50 value for males and females of 1794.9 mg/kg bw can be deduced.

4.2.4 Comparison with criteria

According to the CLP criteria, classification as Acute Toxicity 4 needs to be assigned if the acute toxicity value expressed as LD50 value or as acute toxicity estimates is between 300 and 2000 mg/kg bw. The LD50 deduced from the existing studies is 1794.9 mg/kg bw und thus a classification for Acute oral Toxicity 4 is deemed appropriate.

Currently succinic anhydride is harmonised classified as Acute Tox 4* (H302).

A removal of the asterix is suggested. The asterisk (*) indicating a minimum CLP classification for Acute oral Tox 4 is no longer necessary since the data confirm the classification.

4.2.5 Conclusions on classification and labelling

Based on the available data removal of the asterisk (*) from the current harmonised classification Acute Tox 4 (H302: Harmful if swallowed) is proposed.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity: oral

One rat gavage acute toxicity study (OECD TG 401, GLP compliant, corn oil as vehicle) is available (Reagan *et al.*, 1982). Decreased activity and death was recorded from the lowest dose. Other adverse effects observed were: soft stools, ataxia, black pylorus in stomach and intestine, and green areas in the lung. The DS proposed to remove the current minimum classification for Acute Tox. 4, H302 on the basis that the recorded combined female and male LD_{50}-value, of 1795 mg/kg bw, is within the limit (300 < ATE ≤ 2000 mg/kg bw), which according to the CLP Regulation justifies classification as Acute Tox. 4, H302.

Comments received during public consultation

One Member State Competent Authority (MSCA) commented during the PC. The MSCA supported the proposed classification but commented that according to the CLP Regulation the ATE value used for classification should be based on the lowest observed LD_{50}-value and consequently the LD_{50}-value for females (1510 mg/kg bw) should be used rather than the combined value for female and male rats (1795 mg/kg bw) that was used by the DS. The DS concurred with the commenting MS.

Assessment and comparison with the classification criteria

Succinic anhydride will hydrolyse in water or protic solvents to form succinic acid. With the vehicle used in the available oral acute toxicity study, corn oil, no hydrolysis of succinic anhydride is expected to occur in the dosing solution.

According to the CLP Regulation, the lowest calculated LD_{50}-value should be taken into account for classification. In the present study, the lowest calculated LD_{50} value is that observed with female rats (female: 1510 mg/kg bw; males: 2157 mg/kg bw; female and male combined: 1795 mg/kg bw). The lowest LD_{50}-value (1510 mg/kg bw) is within the limits, 300 < ATE ≤ 2000 mg/kg bw/day, which according to the CLP Regulation justifies classification as Acute Tox. 4, H302. The RAC concludes, as proposed by DS, that it is justified to remove the minimum classification and to classify succinic anhydride as **Acute Tox. 4;H302**.

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

Not evaluated in the present dossier.

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

Summary of the Dossier Submitter's proposal

Succinic anhydride has currently a harmonised classification as STOT SE 3, H335 ("May cause respiratory irritation"). The DS did not evaluate STOT SE. However this endpoint was open for commenting during the public consultation of the CLH report and therefore this endpoint should be addressed in this Opinion.

Comments received during public consultation

One MSCA commented on this hazard class. The MSCA remarked that the data justifying STOT SE 3, H335 was not presented in the CLH report and thus the source of STOT SE 3 classification is unknown. The MS also commented (with reference to section 3.8.2.5 of the Guidance of the application of the CLP criteria) that it should be assessed whether the current classification in STOT SE 3, H335 should remain considering the proposed new classification of succinic acid as Skin Corr. 1.

The DS responded that no information on the discussion leading to the current STOT SE 3 classification (12^{th} ATP to Directive 67/548/EEC) was available and that, according to the registrant, no studies for this endpoint were available. A literature search by the DS gave no result. The DS concluded that it can be assumed that succinic anhydride in contact with mucous membranes of the respiratory tract hydrolyses to the corresponding acid resulting in irritation/corrosion of the respiratory tract, and with reference to section 3.8.2.5 of the Guidance of the application of the CLP criteria they supported the commenting MSCA proposal to remove the current STOT SE 3 classification. Due to the corrosive properties of succinic anhydride and with reference to section 1.2.6 of Annex II to the CLP Regulation, the DS also proposed that labelling with the hazard statement EUH071 (corrosive to the respiratory tract) was justified.

Assessment and comparison with the classification criteria

The only target organ after single exposure of relevance for classification is the respiratory system.

RAC notes that paragraph 6 of section 3.8.2.5 in the Guidance of the application of the CLP criteria gives the following regarding classification in STOT SE 3 for compounds with corrosive properties (see also section on skin corrosion/irritation):

"*It is a reasonable assumption that corrosive substances may also cause respiratory tract irritation when inhaled at exposure concentrations below those causing frank respiratory tract corrosion. If there is evidence that from animal studies or from human experience to support this then Category 3 may be appropriate. In general a classification for corrosivity is considered to implicitly cover the potential to cause RTI*[1] *and so the additional Category 3 is considered to be superfluous, although it can be assigned at the discretion of the classifier. The Category 3 classification would occur only when more severe effects in the respiratory system are not observed."*

Moreover, in paragraph 7 of the same section of the Guidance of the application of the CLP criteria it is stated that Category 3 effects should be confined to changes in the upper respiratory tract.

RAC notes that there is no information available in the CLH report on the rationale for the current classification of succinic anhydride as STOT SE 3. However, with reference to the the Guidance of the application of the CLP criteria (section 3.8.2.5) on classification for STOT SE, and taking into account the corrosive properties of succinic anhydride as well

[1] 1 RTI = Respiratory tract irritation

as the fact that succinic anhydride has sensitising properties that will cause respiratory sensitisation (i.e. a more severe effect on the respiratory system compared to the current classification for irritation of the respiratory tract), RAC concludes that the current classification as STOT SE 3 could be considered superfluous if classifying for respiratory sensitisation.

In addition, considering the corrosive properties of succinic anhydride and the absence of acute inhalation toxicity data, labelling with EUH071 ("Corrosive to the respiratory tract") is required according to section 1.2.5 in Annex II of the CLP Regulation.

The RAC concludes that the current classification as STOT SE 3 **could be deleted** and that labelling with **EUH071 (corrosive to the respiratory tract)** is warranted.

4.4 Irritation/Corrosivity

4.4.1 Skin irritation/corrosivity

Table 11: Summary table of relevant skin irritation/corrosion studies

Method	Results	Remarks	Reference
OECD TG 431 (In Vitro Skin Corrosion: Human Skin Model Test) GLP study 12 well plate, EpiDerm™ Test material: Succinic anhydride Concentration: 25 mg (with 25 µl Milli-Q water to moisten the tissue) Vehicle: no vehicle Exposure duration: 3 minutes and 1 hour	Succinic anhydride is corrosive in the *in vitro* skin corrosion test. Mean relative tissue viability for succinic anhydride was below 15% after the 1-hour treatment	Key study, Klimisch Score: 1 Test compound: Succinic anhydride	Buskens C.A.F (2014)
OECD TG 439 (In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method) GLP study 6 well plate, EpiSkin-SM™ Test material: Succinic anhydride Concentration: 10.6 to 11.8 mg (with 5 µl water to moisten the tissue) Negative control: 25µl PBS (Phosphate buffered saline) Positive control: 5% Sodium dodecyl sulphate 6 well plate Vehicle: no vehicle Exposure duration: 15 minutes	Succinic anhydride is not irritating in the in vitro skin irritation test.	Klimisch Score: 1 Test compound: Succinic anhydride	Verbaan I.A.J. (2014)

Tissue studied: skin Test animal: Male New Zealand White rabbit - Coverage: occlusive (shaved) Vehicle: no vehicle Number of animals: 6 rabbits New Zealand White Vienna rabbits were used to determine the skin irritation potential of Maleic anhydride. Test substance (0.5 g) was applied to two intact skin locations on the backs of six rabbits for four hours	Corrosive Erythema score: 4 of max. 4 (animal: 1 - 6) (Time point: 24/48/72 hrs) (not reversible) (all 6 animals between 3.3 and 4 (site 1 and 2); Edema score: 3.6 of max. 4 (animal: 1 - 6) (Time point: 24/48/72 hrs) (not reversible) (all animals between 2 and 4 (site 1 and 2);	Supporting study 2 (reliable with restrictions) (as indicated in the REACH registration) read across Test compound: maleic anhydride	Chevron Chemical Company (1976)
OECD TG 402 (Acute Dermal Toxicity) GLP study Test animal: Sprague-Dawley rat Number of animals: 5 female and 5 male rats Coverage: semi-occlusive Dose: 2000 mg/kg bw Cellulose patch with test substance was soaked with corn oil to get optimal contact with the skin. Vehicle: no vehicle Duration of exposure 24 hrs	LD50: > 2000 mg/kg bw (male/female) Observation of skin condition: 3/5 males and all females were affected. 1d after administration until maximum of 7 days. local effects: erythema at the application site eschar formation at the application site	Klimisch Score: 1 (reliable without restriction) Supporting study Test compound: succinic anhydride	Wolf, T. (2010)

4.4.1.1 Non-human information

The skin corrosion test (EpiDerm^{TM}) has been carried out according to the OECD TG 431 (In Vitro Skin Corrosion: Human Skin Model Test) and GLP criteria. The test is regarded as reliable without restrictions (Klimisch Score 1). The in vitro test has been carried out with a reconstructed human epidermis (Rhe) model. The tests consist of application of succinic anhydride on the reconstructed human epidermis (Rhe) for 3 minutes and 1 hour. Cytotoxicity (indicator for corrosive effects) is expressed as the reduction of mitochondrial dehydrogenase activity measured by formazan production from 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) at the end of the treatment.

The absolute mean OD540 (optical density at 540 nm) of the negative control (50 µl Milli-Q water) tissues was within the laboratory historical control data range. The positive control (50 µl KOH) had a mean relative tissue viability of 9% after 3 minutes exposure. The maximum inter-tissue variability in viability between two tissues treated identically was less than 23% and the maximum difference in percentage between the mean viability of two tissues and one of the two tissues was less than 13%, indicating that the test system functioned properly.

According to the test guideline TG 431 the basis for the prediction that the test substance is corrosive, is that an reduction of viability is seen after 3 min or/and 60 min. Skin corrosion is expressed as the remaining cell viability after exposure to the test substance. The relative mean tissue viability obtained after 3-minute and 1-hour treatments with succinic anhydride compared to the negative control tissues was 96% and 12%, respectively. The mean relative tissue viability for succinic anhydride was below 15% after the 1-hour treatment, which is indicative for the skin corrosive properties.

The skin irritation test (EpiSkin-SM^{TM}) has been carried out according to the OECD TG 439 (In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method) and GLP criteria. The test is regarded as reliable without restrictions (Klimisch Score 1). The in vitro test has been carried out with a reconstructed human epidermis (Rhe) model. The tests consist of application of succinic anhydride on the reconstructed human epidermis (Rhe) for 15 minutes. Cytotoxicity (indicator for corrosive effects) is expressed as the reduction of mitochondrial dehydrogenase activity measured by formazan production from 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) at the end of the treatment.

The positive control had a mean cell viability of 8% after 15 minutes exposure. The absolute mean OD570 (optical density at 570 nm) of the negative control tissues was within the laboratory historical control data range. The standard deviation value of the percentage viability of three tissues treated identically was less than 9%, indicating that the test system functioned properly.

The relative mean tissue viability obtained after 15 minutes treatment with succinic anhydride compared to the negative control (25µl PBS (Phosphate buffered saline)) tissues was 102%. Since the mean relative tissue viability for succinic anhydride was above 50% after 15 minutes treatment it is considered to be non-irritant according to the TG 439.

Further evidence, that succinic anhydride has skin corrosive properties come from results obtained with maleic anhydride (Chevron Chemical Company, 1976), which is structural similar to succinic anhydride (see non-confidential Annex III: Read across justification).
Maleic anhydride has been tested in an in vivo skin corrosion tests carried out with Vienna white rabbits. Test substance (0.5 g) was applied to two intact skin locations on the back of six rabbits for four hours. No test vehicles were used. Irritation was scored at 4, 24, 48 and 72 hours and at 7 days using a modified system of the Draize scoring system. A severe skin irritation was present throughout the seven-day observation period. The data demonstrate that maleic anhydride has skin corrosive potential (for more details on study outcome see confidential Annex II). Maleic anhydride is harmonised for its skin corrosive potential as skin corrosive 1B (H314) (Index Nr. 607-096-00-9).

Furthermore, an acute toxicity study (Wolf, 2010) carried out with Sprague Dawley rats demonstrates that succinic anhydride has skin corrosive properties. The study is CLP conform (reliable without restriction) and performed according to the OECD guideline 402 (Acute Dermal Toxicity).

Succinic anhydride (2000 mg/kg bw) was administered once for 24 hrs topically on an area (10% of total body arrea) of app. 6.5 cm x 8 cm to the dorsal thoracic region of 5 female and 5 male Sprague Dawley rats. The test substance was applied with a cellulose patch soaked with corn oil. Test sites were covered by a semi-occlusive dressing. The animals were investigated up to 14 days after investigation (body weights, clinical observations) and were sacrificed and necropsied 14 days post administration. Three of five males and all females showed skin changes indicating a local irritant effect of the test substance. Erythema and Eschar formation were observed on day 1 after administration until a maximum of 7 days. No other test substance related effects were observed and no mortality occurred. The cellulose patch with the test substance was soaked with corn oil.

4.4.1.2 Human information

--

4.4.1.3 Summary and discussion of skin irritation

An in vitro corrosion test (EpiDerm™) and moreover (although not necessarily required according to OECD, 2014) an in vitro irritation test (EpiSkin-SM™) have been submitted (see Table 11). The in vitro corrosion test demonstrates corrosive potential, whereas the skin irritation test is negative and thus the observation is an example, for which the approach to determine first the corrosive and in the following (if the test outcome is negative) the skin irritating potential as proposed by OECD 2014 of the IATA (integrated approach on testing and assessment) becomes evident.

It is stated in the guidance (OECD, 2014) - that based on differences in the incubation times in the two-test systems (in the present case: 15 min - skin irritation assay and 3 and 60 min – in vitro skin corrosion) it cannot be excluded that in some situations a skin corrosive chemical is correctly identified as corrosive in the in vitro RhE-based skin corrosion test methods but identified as being non-irritant in the in vitro RhE-based skin irritation test methods. Thus, it might be reasonable assumed that the negative result in the in vitro irritation assay carried out with EpiSkin™ assay is based on the 15 min exposure time. In fact, the probability of a skin corrosive chemical being correctly identified as corrosive in an in vitro RhE-based skin corrosion test method but identified as being non-irritant in an in vitro RhE-based skin irritation test method increases as the exposure time in the in vitro RhE-based skin irritation test method decreases, being higher for EpiSkin™ Skin Irritation Test (SIT) (15 min) and LabCyte EPI-MODEL24 SIT (15 min), smaller for SkinEthic™ RHE SIT (42 min) and minor, if at all existing, for EpiDerm™ SIT (60 min). Therefore, also positive results for skin irritation can be expected if also EpiDerm™ would have been used for the skin irritation test (instead of EpiSkin-SM™). There is evidence that succinic anhydride possess irritating/corrosive properties based on following observations:

1) Transformation step from the anhydride to its corresponding acid is a step in which exposed cellular structures (e.g., skin, eye) can be damaged. The hydrolisation of anhydrides is a critical step, which might possess irritating potential.

2) Cyclic anhydrides (structural analogues to succinic anhydrides) possess moderate to severe skin irritation potential (WHO, 2009). Therefore a skin irritation effect of succinic anhydride cannot be excluded by readacross to the hydrolysis product succinic acid.

3) The most similar structural analogue maleic anhydride (CAS No 108-31-6, EC No 203-571-6) is harmonized classified as Skin Corr. 1B (H314) compound (Regulation (EC) No 1272/2008, Table 3.1).

4) The acute dermal toxicity study indicates transient irritation potential of succinic anhydride (eschar and erythema formation, which lasts for 7 days) (Wolf T., 2010).

Based on the aforementioned reasons, the outcome of the corrosion test has more relevance to determine the skin corrosive potential.

The accuracy (corrosive vs non-corrosive) of the in vitro corrosion test TG 431 is ≥ 87%. It has been demonstrated that > 80% of chemicals used for validation have been correctly classified 1B and 1C.

In both tests a low amount (5-25µl) has been used to moisten the tissue. In the skin corrosion assay 25µl water to moisten the tissue before applying 25 mg Succinic anhydride was used (test was carried out in a 12 well plate). In the skin irritation assay 5µl water to moisten the tissue before applying 10 mg Succinic anhydride was used (test was carried out in a 6 well plate). Thus, it can be assumed that the hydrolysis of succinic anhydride to succinic acid in the skin irritation assay was hampered due to the low amount of water added. The test conditions are expected to be applicable for an estimation of real exposure situations.

Besides, it is also indicated in literature that the transformation step from the anhydride to its corresponding acid is a step in which exposed cellular structures (e.g., skin, eye) can be damaged. The hydrolisation of anhydrides is a critical step, which might possess irritating potential. Thus it is important, that the test substance is applied under re-creation of realistic exposure conditions. A low amount of water might be present at real exposure conditions due to e.g. formation of sweat, presence of air humidity.

Furthermore, the outcome of the study in which maleic anhydride (see Annex III: read across justification) has been applied demonstrates that the read across substance has an severe impact on the skin conditions. Maleic anhydride is harmonised classified for as Skin Corr. 1B (H314) (Index Nr. 607-096-00-9).

Moreover, the acute dermal toxicity study carried out with succinic acid itselfs indicates eschar and erythema formation, which lasts for 7 days due to application of the test substance at the dorsal thoracic region of rats.

4.4.1.4 Comparison with criteria

According to the CLP Regulation *in vitro* alternatives that have been validated and accepted may be used to help make classification decisions (CLP Regulation; 3.2.2). Thus, to determine the irritative/corrosive potential, the outcome of the skin corrosion test (Buskens, 2014) carried out according to TG 431 is considered for classification.

The applied OECD TG 431 (In Vitro Skin Corrosion: Human Skin Model Test; key study) allows distinguishing between 1A vs. 1B-1C. The test however does not allow to distinguish between Skin Corr. Cat. 1B and 1C. As described in the OECD TG 431 the prediction models for the EpiDerm^{TM}is that if the viability measured after 3 min exposure is ≥ 50% and after 60 min exposure < 15% the substance needs to be sub-categorised as skin corrosive 1B or 1C.

Since it is stated in the Guidance on the Application of the CLP criteria[2] that if a substance demonstrated corrosive properties in an OECD in vitro test and sub-classification is not possible a classification for Skin Corr. 1 should be chosen. Therefore, succinic anhydride needs to be classified as Skin Corr. 1 without any subcategorization.

4.4.1.5 Conclusions on classification and labelling

The positive results of the Rhe-based in vitro skin corrosion test demonstrate corrosive potential of succinic anhydride. The skin corrosive properties of succinic anhydride is substantiated by the in vivo acute dermal toxicity test and also by test results obtained with maleic anhydride (read across). Based on the available data, succinic anhydride needs to be classified as Skin Corr. 1 (H314: Causes severe skin burns and eye damage) according to the CLP Regulation.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposal to classify succinic anhydride as Skin Corr. 1, H314 was mainly based on the result from an EpiDerm™ *in vitro* skin corrosion test (Buskens 2014, OECD 431 and GLP compliant). Twenty five µl of distilled water were used to moisten the tissue before applying 25 mg of solid succinic anhydride onto the surface of the epidermis. A relative tissue viability of 96 % and 12 % after 3 and 60 min treatment, respectively, was recorded in this study. According to the criteria in the prediction model for this assay a substance needs to be sub-categorised as being a corrosive 1B/1C substance if the cell viability measured after 3 minutes exposure is ≥ 50% and the viability after 60 min of exposure is < 15 % (OECD TG 431). The DS concludes that in line with the guidance provided in section 3.2.2.4 of the Guidance on the application of the CLP criteria (version 4.1), succinic anhydride should be classified as Skin Corr. 1, since the available data from the *in vitro* assay cannot be used for subcategorisation.

According to the DS, the skin corrosive properties of succinic anhydride are corroborated by the result from the *in vivo* acute dermal toxicity test using succinic anhydride and also by read-across of this hazardous property from maleic anhydride, a close structural analogue to succinic anhydride that has a harmonised classification as Skin Corr. 1B (for more details on the DS read-across justification, see Annex III of the Background document.).

In the acute rat dermal toxicity study (Wolf 2010, OECD TG 402, GLP compliant), 2000 mg/kg bw succinic anhydride were applied topically for 24 h using a cellulose patch (6.5 x 8 cm) soaked with corn oil. Test sites were covered by a semi-occlusive dressing. Observations done in the 14 day period following application revealed that 3 out of 5 males and all 5 females showed skin changes, indicating a local irritant effect of the test substance. Erythema and eschar formation were observed on day 1 after administration

[2] Guidance on the Application of the CLP Criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures

until a maximum of 7 days. No other test substance related effects were observed and no mortality occurred.

In the skin irritation/corrosion toxicity test (Chevron chemical company, 1976; performed prior to introducing TGs and GLP), the structural analogue maleic anhydride was applied directly (no test vehicle, using occlusive coverage) on two intact skin locations on the back of 6 Vienna white rabbits for 4 h. A modified system of Draize scoring system was used for scoring irritation at 4, 24, 48 and 72 h and after 7 days. Mean scores for erythema (4, maximal score being 4) and oedema (3.6 of max 4) were recorded for observations taken at 24, 48 and 72 h. The effects had not reversed after 7 days.

Comments received during public consultation

One comment (supporting the proposed classification) was received for this endpoint.

Assessment and comparison with the classification criteria

No *in vivo* dermal irritation/corrosion study is available for succinic anhydride.

RAC notes that the way succinic anhydride has been applied in the *in vitro* test systems, i.e. directly on the tissue using water only for moistening of the tissue, mimics the expected human exposure conditions.

Section 3.2.2.2.4 in Annex I of the CLP Regulation states that "*in vitro* alternatives that have been validated and accepted shall be used to make classification decisions". Section 3.2.2.1.2.4 of the Guidance of the application of the CLP criteria (version 4.1) gives information on available *in vitro* tests that have been validated for classification and the document also contains some broad guidance on how to use these test results for classification of skin corrosion or skin irritation. Thus, for the two reconstructed human epidermis (RhE) *in vitro* tests used when evaluating the corrosive/irritating properties of succinic anhydride, the guidance document states that EpiDerm™ (a test method for corrosivity) does not allow for subcategorisation for skin corrosivity and that EpiSkin – SM™ (a test method for skin irritation) can only be used to distinguish irritants from non-irritants. RAC notes that according to the OECD TG 431 the EpiDerm™ test cannot distinguish between Cat 1B and 1C substances and combined 1B/1C substances are over-predicted by the test as being Cat 1A. In addition, when 80 chemicals were tested, all corrosive substances were correctly classified as being corrosive and 74 % of the non-corrosive substances were correctly classified as non-corrosive. Consequently, 26 % of the used non-corrosive substances were overclassified as being corrosive (mainly as 1B/1C) substances (OECD TG 431).

The result from the EpiDerm™ test (96 % viability after 3 min of incubation and 12 % viability after 60 min incubation) fulfils, according to the criteria in the prediction model for EpiDerm™ as specified in the OECD TG 431, the requirement (i.e. the viability measured after 3 min is ≥ 50% and the viability measured after 60 min is <1 5%) for a skin corrosive 1B/1C substance.

The CLH report also contains results from an *in vitro* skin irritation test, EpiSkin-SM™ (Verbaan 2014, OECD TG 439 and GLP compliant). Five µl of distilled water were used to moisten the tissue before 10 mg of solid succinic anhydride were applied to the surface of the epidermis. The result from this study (cell viability of 102 %, as compared to control after 15 min exposure and 42 h post-treatment incubation) fulfils the requirement (cell viability ≥ 50 %) as specified in OECD TG 439 for a non-irritating substance. RAC notes the inconsistency between the readout from the two RhE-based *in vitro* studies (i.e. non-irritating in EpiSkin-SM™ vs. corrosive in the EpiDerm™). However, as stated in the Integrated Approach on Testing and Assessment (IATA) for skin corrosion and irritation (OECD 2014), this inconsistency can be explained by the use of different exposure times (15 min in the skin irritating test and ≤ 60 min in the corrosivity test) and therefore it cannot be excluded that in some situations a skin corrosive chemical is correctly identified as corrosive in the RhE-based skin corrosion test but identified as being non-irritant in the *in vitro* RhE-based skin irritation test method. RAC also notes that the IATA document states that these two methods should be applied sequentially, the order being decided based on the predicted corrosive/irritating properties of the substance. No information on why both tests were performed is available in the CLH report. Keeping the guidance from the IATA in mind and also with by using additional supportive information (result from the acute dermal toxicity test on succinic anhydride, the reactive properties of anhydrides and the fact that maleic anhydride, a very close analogue to succinic anhydride, is a corrosive substance), RAC agrees with the DS that more weight should be given to the result of the EpiDerm™ test than to the result of the EpiSkin test in the evaluation of the skin corrosive properties of succinic anhydride.

According to the Guidance of the application of the CLP criteria, version 4.1 (sections 3.2.2.1.2.4 and 3.2.2.6), positive results from an *in vitro* corrosion test, such as EpiDerm™ test, can be used for classification for corrosivity but the EpiDerm™ test does not allow subcategorisation within the corrosive category. Thus, on its own, the result from the EpiDerm™ test justifies classification of succinic anhydride as Skin Corr. 1, H314.

At a first glance the result (irritation) in the rat acute dermal toxicity test (Wolf 2010) seems to contradict the result from the EpiDerm™ test (corrosivity). However there are several factors that need to be taken into consideration when using the information from this rat acute dermal toxicity test for assessing skin corrosive/irritating properties:

- In this study, succinic anhydride was applied with/in corn oil, preventing hydrolysis in the vehicle but perhaps also decreasing the contact of succinic anhydride with the skin.
- The dose (as expressed in mg/cm^2) used in this study was lower, 10 mg/cm^2 (if assuming a bodyweight of ~250 g for the rat) than the one (80 mg/cm^2) required in the validated test method for acute dermal irritation/corrosion (OECD TG 404).
- The used exposure time (24 h) is longer than the 4 h used in a TG 404 study.
- In comparison to the skin of rabbit, the rat skin is less sensitive (Guidance on IR/CSA, version 4.1, section R7.2.6.2) and rabbit is the preferred test species for *in vivo* testing for skin corrosive properties according to OECD TG 404.
- In addition, there are differences in the level of examination in an acute dermal toxicity study and a skin corrosion *in vivo* test.
- Further guidance on the use of data from an acute dermal toxicity study for assessing skin corrosive/irritating properties is provided in the Guidance on the application of the CLP criteria (section 3.2.2.6 in version 4.1), as well as in the Guidance on IR/CSA, version 4.1, section R.7.2.6.2. Both these documents

highlight the uncertainties described above and indicate that in a case like the present one data from the acute dermal oxicity study in rat should be used when a WoE determination is needed.

Taking all these factors into account, RAC is of the opinion that the result (irritation; transient erythema and eschar formation in 8/10 animals) from the rat acute dermal toxicity study (i.e. a test method that has not been validated for assessing skin corrosion/irritation) can be viewed as representing the effects of a less potent corrosive substance. Thus the result from the rat acute dermal toxicity study (Wolf 2010) should not be viewed as contradicting the result from the EpiDerm™ study, showing a corrosive effect.

The RAC concludes, in agreement with the DS proposal, that based on available data classification of succinic anhydride as **Skin Corr. 1; H314** is justified.

4.4.2 Eye irritation/Eye damage

Table 12: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Test species: rabbit (normal, albino) Tissue studied: eye Test material: succinic anhydride, succinic acid, maleic anhydride. Guided by the results different solution have been tested. 15% solution of succinic anhydride, succinic acid and maleic anhydride 5% solution of succinic anhydride, succinic acid and maleic anhydride 1% solution of maleic anhydride 1% maleic anhydride solution has been also tested since the eye damaging effects were highly severe with 5% and 10% solution. Vehicle: propylene glycol or water (not specified). Test material is expected to be dissolved in vehicle. The severity of eye burns from a large number of chemicals has been graded (Grade 1-10: not corrosive – highly corrosive)	Succinic anhydride and succinic acid scored as Grade 8: (15% solution gives over five points 5% solution gives injury of up to 5 points.) Maleic anhydride scored as Grade 10 (1% solution yields a score over 5) A score of 5.0 is representative for severe injury; corresponds to necrosis, visible only after staining and covering about three-fourths of the surface of the cornea; or a more severe necrosis covering a smaller area	2 (reliable with restrictions) key study Guideline similar study reported in sufficient detail to enable confident assessment for the method. Published in a peer-reviewed journal. Test material: Succinic anhydride; succinic acid (read across, analogue approach) maleic anhydride (read across, analogue approach WoE) See non-confidential Annex III	Carpenter CP, Smyth HF Jr (1946)

Method	Results	Remarks	Reference
OECD TG 405 (Acute Eye Irritation / Corrosion) Tissue studied: eye Test species: rabbit (New Zealand White) Vehicle: unchanged (no vehicle) Number of animal: 1 animal (right eye) Obervation period: 1, 24, 48, 72 hrs post application 6, 8, 10, 13, 15 and 21 days after the installation 24 hrs after application of the test substances both eyes were rinsed with warm water.	Category 1 (irreversible effects on the eye) ***Cornea score:*** 4 of max. 4 (animal #1) (Time point: 24-72 h and day 21) (not reversible (reactions persisted to termination on day 21) (reactions persisted to termination on day 21) ***Iris score:*** 2 (animal #1) (Time point: 24-72 h) (corneal reactions persisted to termination on day 21, it is assumed that iridial reactions would also persist) (It was not possible to provide a score for iridial change recorded due to extensive corneal opacity precluding assessment or ophthalmological examination of the iris, it is assumed that a maximum score of 2 would have been assigned.) ***Conjunctivae score:*** 3 of max. 3 (animal #1) (Time point: 24-72 h and to day 21) (not fully reversible within: 21 days) (Although reactions showed some amelioration over three week observation period, some conjunctivitis remained at termination) ***Chemosis score:*** 3.7 of max. 4 (animal #1) (Time point: 24-72 h and up to day 21) (fully reversible within: 15 days) (marked chemosis persisted to 72 h after instillation but reactions lessened over the first week and the conjunctival swelling had overtly resolved by day 15)	1 (reliable without restriction) key study read-across from supporting substance: succinic acid	Bernat, E. (1999)

4.4.2.1 Non-human information

The first key study is a non-GLP and non-TG conform study (Carpenter and Smyth, 1946).

In the study the grade of severity of eye burns from a large number of chemicals (n=180) has been examined and the injury has been translated into a numerical score.

Depending on the severity of the eye damage a score of maximum 20 points is given to the test compound. Thereafter, the scores are translated into different grades (1-10). Grade 1 does not indicate any damage (undiluted chemical gives zero to one point), whereas grade 10 (1% solution gives injury of over 5%) stands for severe eye damage.

Solid test materials (e.g., succinic anhydride) were dissolved prior to application preferable with propylene glycol. Concentrations of 15% and 5% have been used to determine the skin corrosion properties. The solutions have been applied to Albino rabbit eyes (5 eyes) to the centre of the cornea while the lids are retracted.

The outcome of the test indicates that succinic anhydride and succinic acid have the same grading (injury grade 8 out of 10), which indicates a corrosive potential of the test substances. Grade 8 is defined for a 5% solution giving injury of up to 5.0 points, and 15% solution scoring over 5.0 points. A score of 5.0 corresponds to necrosis, visible only after staining and covering about three-fourths of the surface of the cornea; or a more severe necrosis covering a smaller area.

In the study of Carpenter and Smyth (1946) the effects of succinic anhydride and succinic acid were graded similar (severe eye damage); the substances were put in the injury grade 8 (out of 10), which is indicative for a high eye damaging potential. It has to be remarked that, under aqueous conditions succinic anhydride hydrolyses to succinic acid. Therefore, the study outcome is somehow not unexpected.

Thus, the study by Bernat (1990) carried out with succinic acid is also considered as key study for further evaluation of the adverse effects on the eye. The study has been conducted under GLP conditions and is a TG conform study (OECD TG 405). Approximate equivalent of 0.1 ml succinic acid has been applied to one eye of one rabbit. No additional animals were tested, since severe eye lesions have been observed. Severe irreversible corneal alterations were observed (score 4) until 21 days post application with the majority of cornea affected. The iris could not be examined due to corneal alterations. The redness decreased with the time continuously, however conjunctivitis was still present until day 21 post application.

The outcome of the study of Bernat et al. (1999) demonstrates that succinic acid causes irreversible damage to the eye (details on study outcome see Annex II – confidential annex).

Application of maleic anhydride to rabbit’s eye has provoked more severe eye damage in the study of Carpenter et al. (1946) than the application of succinic anhydride or succinic acid. Already a low dose (1% solution) provokes a clear eye damaging effect and a score of over 5.0 (which is indicative for necrosis).

4.4.2.2 Human information

--

4.4.2.3 Summary and discussion of eye irritation

In conclusion, based on the evidence coming from a comparative study that succinc anhydride and succinic acid possess adverse effects on the eye in the same order of magnitude (grade 8 out of 10) (Carpenter, 1946) and due to the fact that the anhydride form is rapidly hydrolysed to the succinic acid form in aqueous solution, the study carried out with the acid form (Bernat, 1999) is valid for evaluation. A read across justification is provided in Annex III.

The study carried out with succinic acid (Bernat, 1999) is a GLP and guideline conform study and unambiguously demonstrates that succinic acid has to be classified for its severe damage to eyes. Furthermore, also the structural similar compound maleic anhydride warrants a classification regarding its adverse effects on the eye (Eye Dam. 1). Succinic anhydride is listed in Annex VI of the Regulation (EC) No. 1272/2008 as Eye Irrit. 2. The data presented in the REACH registration (full registration, joint submission) demonstrate that a classification according to the Regulation (EC) No. 1272/2008 to classify the substance as Eye Dam. 1 is warranted. Therefore, a revision of the current Annex VI entry is proposed.

4.4.2.4 Comparison with criteria

The study of Carpenter (1949) indicates that application of succinic anhydride to rabbits eye leads to severe eye damage (grade 8 out of 10 (highest score)). Since the study outcome demonstrates that succinic anhydride and succinic acid have the same potency regarding the adverse effects on the eye and the fact that succinic anhydride hydrolyses rapidly under aqueous conditions to succinic acid, the guideline conform study carried out with succinic acid (Bernat, 1999) can also be taken into consideration for classification. Since the study demonstrates that the adverse effects on the cornea, iris, conjunctiva are not fully reversed within an observation period of 21 days in one test animal the criteria to categorise the substance into the Category 1 (irreversible effects) are meet (details see Table 12).

4.4.2.5 Conclusions on classification and labelling

Based on the available data succinic anhydride needs to be classified for its eye damaging properties as Eye Dam 1 (H318: Causes serious eye damage). The current harmonised classification of succinic anhydride as Eye Irrit. 2; H319: C ≥ 1% needs to be revised.

RAC evaluation of serious eye damage/irritation
Summary of the Dossier Submitter's proposal Two animal studies are available for the assessment of this endpoint. A study by Carpenter and Smith (1946) is presented as a key study, albeit having a Klimisch score of 2 and being performed prior to introducing TGs and the GLP system. The results presented are part of a more comprehensive study where succinic anhydride is one of many chemicals tested (180 chemicals including succinic acid and maleic anhydride) for eye corrosivity in albino rabbits. Based on all data, the tested chemicals were graded between 1 (not corrosive) and 10 (highly corrosive). Test solutions of 0.005 mL of undiluted test compound (based on information from the

dossier disseminated on the ECHA website for this substance) and 15 %, 5 % and 1 % were applied to the centre of the cornea and 18-24 h later the eye was examined in strong diffuse daylight, then stained with fluorescein and the injury was scored (a score of 5.0 corresponds to necrosis, visible only after staining and covering about three-fourths of the surface of the cornea; or a more severe necrosis covering a smaller area of the cornea).

Succinic anhydride and succinic acid were both assigned grade 8 (on the basis that when applied undiluted and as 15 % solutions they yielded scores of over 5.0 (A 5 % solution was not over 5.0) i.e. both substances caused severe eye damage.

Maleic anhydride was assigned a grade 10 in this study (1 % solution yields a score over 5).

The second study presented was an acute eye irritation/corrosion study (OECD TG 405, GLP compliant) using succinic acid (Bernat 1999). It is considered a key study for reading across to succinic anhydride and its use is justified by the previous study of Carpenter and Smith (1946) where succinic acid and the anhydride produced similar eye damaging results. In the study by Bernat, severe irreversible corneal alterations were observed (score 4) until 21 days post application, with the majority of the cornea being affected. The iris could not be examined due to corneal alterations. The redness decreased continuously over time. However, conjunctivitis was still present until day 21 post application.

Based on available data succinic anhydride has eye damaging properties that, according to the DS, justifies classification as Eye Dam 1, H318.

Comments received during public consultation

One MSCA and one individual commented on this hazard class.

Both comments concerned the fact that succinic anhydride has been classified as a skin corrosive substance and according to the CLP Regulation classification for eye damage is then considered to be implicit. In addition, the MSCA view was that in light of succinic anhydride’s skin corrosive properties it was superfluous to include a read-across to succinic acid and that enough supportive evidence is given by the study of Carpenter and Smith (1946).

The DS responded that they were aware of the wording in the Guidance of the application of the CLP criteria but since data were available it should be included and assessed in the CLH report. In addition since succinic anhydride hydrolyses under aqueous conditions to succinic acid, the available data for succinic acid were included by the DS to support the classification of succinic anhydride as Eye Dam. 1.

Assessment and comparison with the classification criteria

RAC notes that succinic anhydride has skin corrosive properties that according to the CLP Regulation (section 3.3.2.2.2 of Annex I) shall be considered as also leading to serious eye damage (Category 1). RAC is of the opinion that since animal data are available on eye irritation/corrosion in the CLH report this data should be evaluated and taken into account in the WoE analysis of the eye damaging properties of succinic anhydride.

The only available study using succinic anhydride is the study by Carpenter (1946; non-

GLP and not conforming to TG 404) that also provides data for succinic acid and maleic anhydride. The results as such indicate that succinic anhydride causes severe injuries (necrosis of the cornea) to the eye 24 h after exposure. However, no information on reversibility is included and therefore it is difficult to fully interpret the result for classification purposes. In addition, there are some limitations in the reporting of this study and it is not clear if the undiluted substance perhaps was applied as a solid substance. In addition, when succinic anhydride was applied as a solution, it appears that water (or propylene glycol) was used as vehicle when preparing the test solutions. Considering that succinic anhydride will hydrolyse in an aqueous media it is likely that the results from the experiments on succinic anhydride more likely reflect the eye damaging properties of succinic acid. This conclusion is supported by the fact that similar eye damaging score was seen independently if succinic anhydride or succinic acid were tested.

In the eyes, the tear fluid provides conditions that will favour hydrolysis of succinic anhydride to succinic acid. As proposed by the DS, RAC considers that it is scientifically justified that data on succinic acid's eye damaging properties (as revealed in the study by Bernat 1990) are taken into account when evaluating the eye damaging properties of succinic anhydride. However, it cannot be ruled out that succinic anhydride is more potent than succinic acid.

The RAC concludes in agreement with the DS proposal that although the risk for severe eye damage is implicit for corrosive substances (and consequently testing for eye irritation/corrosion should be avoided), in this case the available animal data also justify a classification as **Eye Dam. 1; H318**. However, in light of the classification for skin corrosion and its assigned hazard statement H314 (causes severe skin burns and eye damage) a separate labelling with H318 (causes serious eye damage) is not needed.

4.4.3 Respiratory tract irritation

Not evaluated in the dossier.

4.5 Corrosivity

See Chapter 4.4.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 13: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
OECD TG 429 (Skin Sensitisation: Local Lymph Node Assay) Test species: mouse (CBA) female (5 animals per group-including spare animals) Concentration: 10, 25, and 31.3% w/w Vehicle: N,N- Di-methylformamide (DMF) Prior to the main experiment solubility testing with different vehicles were carried out. Test material: Succinic anhydride Test material is dissolved and not hydrolysed to succinic acid.	Sensitising properties A stimulation index of 9.2, 11.6, and 11.0 was calculated for the low, mid and high dose groups, respectively. The negative and positive control groups had a stimulation index of 1 and 7.3, respectively.	Klimisch 1: reliable without restriction Key study	Weber E (2010)

4.6.1.1 Non-human information

The study of Weber et al. (2010) has been carried out under GLP conditions and according to the OECD TG 429 (Skin Sensitisation: Local Lymph Node Assay).

It is stated in the study report that recommended vehicles for succinic anhydride are acetone/olive oil (4:1 v/v), di-methylformamide, methyl ethyl ketone, propylene glycol and dimethylsulfoxide and that according to the guidelines the vehicle should be selected on the basis of maximising the test concentrations and solubility whilst producing a solution/suspension suitable for application of the test substance.

The outcome of solubility testing of the test substance with the guideline-recommended vehicles showed that the highest concentrations suitable for application of the test substance can be achieved with DMF (31.3% w/w). A range finding study was conducted prior to the main experiment. According to the guidelines the test substance should be tested at 3-5 consecutive concentrations from within the concentration series 100%, 50%, 25%, 10%, 5%

and 2,5%. The highest concentration should be the highest achievable concentration, which does not induce systemic toxicity and excessive local irritation. Therefore in the range finding study two animals were treated with 25 µl of the test substance on the dorsum of each ear on three consecutive days at concentrations of 31.3% (w/w) and 25% (w/w). Ear thickness and body weight were measured on day 1 before the first administration and on day 4 about 24 hrs after the last administration. None of the animals showed overt systemic toxicity, excessive local skin irritation at the application sites or an important increase in ear thickness in the range finding study. Therefore, 31-.3% was chosen as the highest test substance concentration.

In the main experiment succinic anhydride dissolved in N,N- Dimethylformamide (DMF) was applied epicutaneously to the dorsal surface of both ears to four female CBA/Ca mice per dose group, in doses of 10% (w/w), 25% (w/w), 31.3% (w/w), once a day for three consecutive days. The volume applied was 25 µl per ear. Positive and negative controls were used. 3H-methyl thymidine was intravenously administered into the vein tail of all mice five days after application. Five hours later the animals were sacrificed and incorporation of 3H-methyl thymidine in cells of the auricular lymph node was determined. Disintegrations per minute (dpm) were 43520, 54949, and 52036 for the low, mid and high dose groups, respectively. The negative and positive control groups had a dpm of 4733 and 34509, respectively. One animal in the low dose group died. The animal showed no adverse effects and the gross necropsy was without any observation. Thus, the death is not associated with test substance administration. No adverse effects have been noted in any animal.

Body weight gain was within the range expected from animals of the same strain, sex and age. Furthermore, no skin irritation effects at the application sites were observed in the test substance groups and the negative control group throughout the whole study. Slight erythema was noted in all animals of the positive control group on days 3-4, indicating slight local skin irritation.

The stimulation indexes (disintegration per minute (dpm) test group/ dpm negative group) were 9.2 (10% w/w), 11.6 (25% w/w) and 11.0 (31.3% w/w). No linearity between dose and response has been observed. However, the stimulation indexes of all test substance concentrations were greater than 3, which demonstrates that succinic anhydride possess sensitising properties. An EC 3 value (amount of test chemical required to elicit a stimulation index of 3) could not be derived adequately The standard linear interpolation method requires a response on either side of the classification threshold of a 3.0 stimulation index. In the study of Weber et al. (2010) all stimulation indexes values exceed 3 but are not linear. A derivation of an EC3 values may be associated with great uncertainty.

Succinic anhydride is an acylating agent which reacts with N-terminal amino acids. The anhydride structure is an alert for skin sensitising properties (OECD, 2011, Aptula AO et al. 2006, Roberts DW et al., 2007a, Roberts DW et al., 2007b, QSAR toolbox v.3.3.5). Also, the structural most similar anhydride form maleic anhydride (CAS: 108-31-6) is harmonised classified as Skin Sens. 1.

4.6.1.2 Human information

No relevant information available.

4.6.1.3 Summary and discussion of skin sensitisation

4.6.1.4 Comparison with criteria

The results of the local lymph node assay demonstrate the sensitising properties of succinic anhydride. A classification as Skin Sens. 1 (H317: May cause an allergic skin reaction) is deemed necessary since positive data from appropriate animal study are available (CLP regulation 3.4.2.2.3/ 3.4.2.2.2).

The criteria for classification of skin sensitizers based on LLNA study is EC3 value ≤ 2% for sub-category 1A and EC3 value > 2% for sub-category 1B.

An EC 3 value (amount of test chemical required to elicit a stimulation index of 3) could not be derived adequately The standard linear interpolation method requires a response on either side of the classification threshold of a 3.0 stimulation index. In the study of Weber et al. (2010) all stimulation indexes values exceed 3 but are not linear. A derivation of an EC3 values may be associated with great uncertainty.

Therefore a classification as Skin Sens. 1 (H317: May cause an allergic skin reaction) without sub-classification is proposed.

4.6.1.5 Conclusions on classification and labelling

Based on the data of Weber et al. (2010) succinic anhydride has to be classified according to Regulation (EC) No 1272/2008 as Skin Sens 1 (H 317: May cause an allergic skin reaction).

In the CSR of the lead registrant (full registration, joint submission) succinic anhydride is self-classified accordingly.

4.6.2 Respiratory sensitisation

Table 14: Summary table of relevant respiratory sensitisation studies/information

Method	Results	Remarks	Reference
OECD toolbox v.3.3.3 QSAR predictions	Cyclic anhydride structure is an alert for respiratory sensitisation. Anhydrides are binding to the N-terminal of amino aicds. Details on the chemical mechanism related to the sensitising mechanism of cyclic anhydrides are described in Annex III (read-across justification). Allergic effects are likely since anhydride specific IgE and IgG antibodies are formed and anhydride challenges to sensitised animals causes obstructive bronchial reactions.	It is highlighted in the ECHA guidance that cyclic anhydrides structures are an alert for respiratory sensitisation. Therefore, succinic anhydride should be considered for classification.	ECHA Guidance R.7a[3] Jarvis et al. (2005)
Test species: Sprague-Dawley rat Induction: inhalation Challenge: inhalation Rats were exposed to a maleic anhydride aerosol 6 hours/day for five days. Following a 3-week rest period, the animals were challenged for 6 hours. One group was not challenged (i.e., non-exposed/non-challenged control).	The maleic anhydride-exposed/maleic anhydride-challenged animals had small, but statistically significant ($p < 0.05$), increases in maleic anhydride-specific serum IgG antibody compared to the controls (challenged and non-challenged; females higher than males). Two rats of the MA-exposed/non-challenged group had more than 10 lung foci (i.e., positive	Key study experimental result Read across source substance: Maleic anhydride (structural most similar anhydride form)	Amoco Corporation (1991)

[3] Guidance on Information Requirements and Chemical Safety Assessment. Chapter R. 7a: Endpoint specific guidance, Version 4.0, July 2015

Method	Results	Remarks	Reference
	response); however, mean values for lung foci, weight, and volume were not significantly different from control values. Microscopic lung lesions were minimal.		
OECD TG 429 (Skin Sensitisation: Local Lymph Node Assay) Test species: mouse (CBA) female (5 animals per group-including spare animals) Concentration: 10, 25, and 31.3% w/w Vehicle: N,N- Di-methylformamide (DMF) Prior to the main experiment solubility testing with different vehicles were carried out. Test material: Succinic anhydride Test material is expected to be dissolved and not hydrolysed to succinic acid.	Sensitising properties A stimulation index of 9.2, 11.6, and 11.0 was calculated for the low, mid and high dose groups, respectively. The negative and positive control groups had a stimulation index of 1 and 7.3, respectively.	Klimisch 1: reliable without restriction Test compound: succinic anhydride Supporting study	Weber E (2010)
Human			
Case study Four cases of asthma of workers working with maleic anhydride were reported.	In 3 out of 4 cases inhalation tests with MA provoked a late asthmatic reaction and increase in airway responsiveness to inhaled histamine. Only one of these three had specific IgE antibodies in serum.	Only abstract available. Proceedings of British Thoracic Society Supporting study	Graneek et al., (1986)
Inhalation challenge test Nine workers who were admitted to hospital for investigation of occupational asthma	Two workers who suffered from work-related asthmatic symptoms associated with maleic anhydride,	Focus of the investigation: late asthmatic reactions and changes in histamine responsiveness	Graneek et al., (1988)

Method	Results	Remarks	Reference
Work related exposure to toluene diisocyanate (TDI), maleic anhydride (MA), timellitic anhydride (TMA), carmine, or colophony (pine wood resin).	was investigated. The study subjects were exposed to 5-minute inhalation to maleic anhydride dust Both subjects showed immediate and late asthmatic responses to maleic anhydride challenge.	provoked by occupational agents. Exposure history not described.	
Case report 34yr old worker developed symptoms of cough, rhinitis, breathlessness, and whizzing. Symptoms were present within minutes of exposure to dust during the loading of phthalic anhydride and maleic anyhdride to reactor. The symptoms were only present during loading. In May 1990 he developed acute asthma attack. Manufacture of polyester resins and alkyd resins. Bronchial provocation test	Outcome: Worker had positive challenge test to maleic anhydride but reacted negatively to maleic anhydride	Exposure: Production of polyester was carried out about three times a week. Four workers were concerned with bathing the powdered chemicals into the reactor. After approximately 2 yrs. worker was transferred to alkyd resin production wich was carried out daily. The batching process was similar, but the proportion of maleic anhydride was less.	Lee et al., (1991)
13 Patients, with work related respiratory symptoms related to acid anhydride exposure and inhaydride specific IgE. One of the woker had symptoms related to MA exposure in vitro RAST (radioallergosorbent) inhibition study detected	specific IgE antibodies to a maleic anhydride-human serum albumin conjugate from worker that was occupationally exposed by inhalation to maleic anhydride.	Supporting study Mechanistic study	Topping et al., (1986)
Cohort study 506 workers	3.2% were sensitised with an immediate skin prick test reaction to acid anhydride human serum	Supporting study No clear prevalence of sensitised workers attributed to maleic	Barker et al., (1998)

Method	Results	Remarks	Reference
Skin prick test Aim of the study: Clarification of risk factors for sensitisation and respiratory symptoms Co-exposure: Phthalic anhydride, maleic and trimellitic anhydride	albumin (AA-HAS) Sensitisation to acid anhydrides was associated with work related respiratory symptoms and with smoking at the time of exposure to acid anhydride.	anhydride exposure is presented in the paper, and the workers were not only exposed to maleic anhydride but also to phthalic and trimellitic anhydride.	

4.6.2.1 Non-human information

Beside the structural alert that cyclic anhydride possess respiratory sensitisation potential (ECHA Guidance[4], OECD QSAR toolbox v.3.3.5), the hazard identification of succinic anhydride related to respiratory sensitisation is based on the read-across approach to maleic anhydride (analogue approach), for which experimental data and human data are available. Furthermore, a local lymph node assay carried out with succinic anhydride has been considered in a weight of evidence approach.

The cyclic anhydride structure is listed in ECHA guidance[2] to be an example of structural alerts for respiratory sensitisation based on QSAR predictions. A query of the OECD toolbox reveals that succinic anhydride is considered to have sensitising properties (for details on mechanistic feature, see Annex IV). Although there is currently no testing strategy available for respiratory sensitisation and thus the performance of tests for respiratory sensitisation is currently not required under REACH, the substance can be considered for classification as respiratory sensitizer following the flow chart for integrated evaluation strategy (ECHA Guidance[2], page 282, Figure R.7.3.2), which highlights that if there are any structural alerts such as acid anhydride the chemical can be considered for classification.

Furthermore a read-across from maleic anhydride has been applied to further substantiate the respiratory sensitising property of succinic acid. Maleic anhydride is the structural most similar anhydride structure to succinic anhydride and is harmonised classified for Resp. Sens. Cat. 1. A justification for the read-across approach is provided in Annex III (justification for read-across).

Maleic anhydride has been tested for potential respiratory sensitization in Sprague Dawley rats. The animals were exposed to a particulate aerosol target concentration of 0 or 500 µg/m^3 maleic anhydride, 6 hours/day for five days. After three weeks, the animals were challenged with 500 µg/m^3 for 6 hours. The analytical time weighted averaged concentration of maleic anhydride was 500 and 317 µg/m^3, for the induction and challenge phases, respectively. Maleic anhydride exposed and challenged rats had a slight, but significant, increase in maleic anhydride-specific serum IgG antibody levels compared to non-exposed control animals. Other endpoints of acid anhydride respiratory sensitization reactions in the rat model such as

[4] Guidance on Information Requirements and Chemical Safety Assessment. Chapter R. 7a: Endpoint specific guidance, Version 4.0, July 2015

increased haemorrhagic lung foci, increased lung weight and volume, and extensive lung pathology did not occur (Amoco Cooperation, 1991).

Succinic anhydride has been tested in the local lymph node assay (LLNA) test (Weber et al., 2010). The study demonstrates that succinic anhydride has skin sensitising properties (for details see Chapter 4.6.1.1.). Although the LLNA test was developed and validated for identification of contact allergens, there is evidence that chemical respiratory allergens will also elicit positive responses in this assay (Kimber, 1995). Chemicals known to cause respiratory allergy and occupational asthama have been shown to test positive in the LLNA. Among such chemicals are acid anhydrides (such as trimellitic anhydtide and phtalic anhydride). In the ECHA guidance[2] it is stated that the current view is that most, if not all, chemical respiratory allergens are able to elicit positive responses in the LLNA (or in other skin sensitisation test). Maleic anhydride is harmonised classified as Resp. Sens. 1 (H 334: may cause allergy or asthma symptoms or breathing difficulties if inhaled). The read across approach to maleic anhydride is plausible, beside the structural similarity, the toxicity pattern of the two compounds is identical (for details see non-confidential Annex III) and both possess the structural alert (anhydride group) for its sensitising properties. The sensitising properties of succinic anhydride have been demonstrated in the LLNA test described under section 4.6.1.1.

4.6.2.2 Human information

In this chapter human studies are summarised related to exposure of workers to maleic anhydride - the structural most similar anhydride form to succinic anhydride. Maleic anhydride is also mono-cyclic and has identical toxicological pattern (see justification for read-across Annex III) as the target substance succinic anhydride.

Graneek et al. (1986) reported on four cases of asthma in workers exposed to maleic anhydride. In three, inhalation tests with maleic anhydride provoked a late asthmatic reaction and an increase in airway responsiveness to inhaled histamine. One patient had maleic anhydride-specific IgE antibodies present in the serum; these were in low titer and it was hypothesised that there may have been a cross reaction to IgE specific for trimellitic anhydride, to which this individual was also exposed. The fourth worker, although negative in inhalation testing, had specific serum IgE antibodies present. Electrophoresis of human serum albumin ± maleic anhydride suggests conjugation, and the conjugate identified specific IgE in patient four.

In the study of Graneek et al. (1988) airway responsiveness of two workers who suffered from work-related asthmatic symptoms associated with maleic anhydride, was investigated by bronchial challenge tests. Both subjects were declared as atopic, however clinical or exposure histories were not described. The study subjects were exposed to 5-minute inhalation to maleic anhydride dust (produced by tipping a powder containing 0.2 or 1% maleic anhydride in lactose). A control was also conducted involving exposure to lactose powder. Both subjects showed immediate and late asthmatic responses to maleic anhydride challenge, observed as reductions in forced expiratory volume and an increased responsiveness to histamine at 3 and 24 hours post-challenge.

In a case report study by Lee et al. (1991) a 34-year old man developed a cough, rhinitis, breathlessness and wheezing approximately one month after beginning working in a factory producing alkyd-polyester. The symptoms occurred within minutes of exposure to dust during the loading of chemicals into a reactor. After removal from exposure, a complete relief was observed. New exposure led to an acute asthmatic attack again. Breathing zone sampling (duration of sampling not stated) indicated airborne dust concentrations of maleic anhydride

0.8 mg/m3 (0.2 ppm) for inhalable particles and 0.2 mg/m3 (0.05 ppm) for respirable particles; equivalent concentrations for phthalic anhydride were 1.4 and 0.3 mg/m3 (0.23 and 0.05 ppm), respectively. Bronchial challenge tests were performed with phthalic anhydride and maleic anhydride. A control challenge was conducted using lactose. Maleic anhydride provoked immediate and last asthmatic responses; the immediate response was accompanied by rhinitis and lacrimation. Phthalic anhydride elicited no response. The worker also had non-specific airway hyperresponsiveness, assessed by histamine challenge (it was not stated if this hyperresponsiveness was observed in conjunction with anhydride challenge).In the study of Topping et al. (1986) an in vitro RAST (radioallergosorbent) inhibition study detected specific IgE antibodies to a maleic anhydride-human serum albumin conjugate from a worker that was occupationally exposed by inhalation to maleic anhydride.

The cohort study of Barker et al. (1998) aims to clarify risk factors for sensitisation and respiratory symptoms among workers exposed to different acid anhydrides. From the cohort (out of 506 workers from 79% information was obtained) 3.2% were sensitised with an immediate skin prick test reaction to acid anhydride human serum albumin (AA-HAS) conjugate and 8.8% work related respiratory symptoms. Sensitisation to acid anhydrides was associated with work related respiratory symptoms and with smoking at the time of exposure to acid anhydride. In summary, the intensity of exposure and cigarette smoking may be risk factors for sensitisation to acid anhydrides. But, no clear prevalence of sensitised workers attributed to maleic anhydride exposure is presented in the paper, and the workers were not only exposed to maleic anhydride but also to phthalic and trimellitic anhydride.

In this context it needs to mentioned, that in the year 2009 the WHO published a concise international chemical assessment document on the human health aspect of cyclic acid anhydrides (Kim et al., 2009). It is summarized, that in humans cyclic acid anhydrides can cause irritation and sensitization after direct contact with the skin and the mucous membranes or after exposure by inhalation. The irritative symptoms (itching, lacrimation, sneezing, rhinorrhoea, cough, and dyspnoea) begin immediately following exposure to high concentrations of dusts or vapours. The most common allergic diseases are rhinoconjunctivitis and asthma, both immediate-type IgE-mediated allergies. Also, late-type respiratory symptoms with specific IgG antibodies have been described. Less frequent consequences are the severe disease called pulmonary disease–anaemia syndrome, contact eczema, contact urticaria, allergic laryngitis, and allergic alveolitis. Allergic reactions of the skin and conjunctiva and allergic respiratory manifestations are well known effects of occupational exposure to cyclic acid anhydrides. Respiratory diseases include occupational allergic rhinoconjunctivitis and occupational asthma. Urticaria and allergic rhinoconjunctivitis often precede asthma. Cases of haemorrhagic alveolitis, haemorrhagic anaemia, allergic alveolitis, and allergic laryngitis have also been reported in association with exposure to anhydrides. The proof of IgE mediation in immediate type asthma or rhinitis due to acid anhydrides is convincing. There have been several human case reports published, which demonstrate the respiratory sensitisation hazard of acid anhydrides. Experiments with sensitized animals have demonstrated the formation of anhydride-specific IgE and IgG (Kim et al., 2009). Allergic reactions of the conjunctiva and respiratory tract have been reported in humans after exposure to the cyclic anhydrides.

4.6.2.3 Summary and discussion of respiratory sensitisation

The evaluation of the respiratory sensitising potential of succinic anhydride is based on read across to maleic anhydride (analogue approach) and on QSAR based estimations. The experimental data and also evidence from human case reports and epidemiological studies indicate that the maleic anhydride has respiratory sensitising properties and maleic anhydride is harmonised classified as Resp. Sens. 1 (H 334: may cause allergy or asthma symptoms or breathing difficulties if inhaled).

The justification for the read across approach to maleic anhydride is in detailed described in Annex III. Succinic anhydride and maleic anhydride are structural similar, the toxicity pattern of the two compounds is comparable and both possess the same structural alert – anhydride structure – responsible for the sensitising properties (OECD, 2011, OECD QSAR toolbox v.3.3.5., ECHA guidance[3]). Anhydride is an alert for sensitisation properties, since anhydrides have the potential to bind covalent to proteins.

There is a growing body of evidence that effective sensitisation of the respiratory tract by chemicals defined as respiratory allergens can and does occur in response to dermal contact (Kimber et al., 2002). Succinic anhydride has been tested in the local lymph node assay (LLNA) test (Weber et al., 2010) (for details see chapter 4.6.1.1..). The study demonstrates that succinic anhydride has skin sensitising properties. Although the LLNA test was developed and validated for identification of contact allergens, there is evidence that chemical respiratory allergens will also elicit positive responses in this assay (Kimber, 1995).

Furthermore, cyclic anhydrides are listed as examples of structural alerts for respiratory sensitisation in the ECHA guidance (ECHA guidance , p. 273) and based on QSAR predictions succinic anhydride is identified to be a sensitiser (OECD, 2011, OECD, QSAR toolbox v.3.3.5).

In the chemical safety report of the registrant(s) (full registration, joint submission) succinic anhydride is self-classified for Resp. Sens 1 and the risk characterisation of succinic anhydride has been carried out accordingly. This approach was accepted by the evaluating MS in the frame of substance evaluation, since RMM have been set in a precautionary manner. No further testing regarding respiratory sensitisation properties of succinic anhydride is foreseen since substance is subject for classification for respiratory sensitisation and no validated methods are currently in place to determine respiratory hazard (for details see: ECHA Guidance Chapter R.7a[5])

Based on the weight of evidence approach succinic anhydride is proposed to be classified for Resp. Sens.1 (H334: may cause allergy or asthma symptoms or breathing difficulties if inhaled). Data do not provide enough information to subcategorise the substance

4.6.2.4 Comparison with criteria

Cyclic anhydrides are listed as examples of structural alerts for respiratory sensitisation in the ECHA guidance (ECHA guidance[6], p. 273). There are several human and animal studies demonstrating the sensitising effects of maleic anhydride, which is used as a source substance for the read across approach (Annex III: Read Across justification).

The criteria to categorise succinic anhydride as Resp. Sens 1 are met based on the applied read across approach. But, the available human data do not allow to conclude on frequency of occurrence in humans or a probability of occurrence of a high sensitisation rate in humans. Also the severity of the allergic reactions is not or insufficient described in the human studies to make a conclusion on sub-categorisation.

5

6 Guidance on Information Requirements and Chemical Safety Assessment. Chapter R. 7a: Endpoint specific guidance, Version 4.0, July 2015

4.6.2.5 Conclusions on classification and labelling

The substance fulfils the criteria to be classified as Resp. Sens 1 (H 334: May cause allergy or asthma symptoms or breathing difficulties if inhaled) based on the criteria settled down in the Regulation (EC) No 1272/2008.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

No human or animal data on succinic anhydride is presented for this endpoint in the CLH report. The DS's proposal to classify succinic anhydride for respiratory sensitisation, category 1, is based on a weight of evidence analysis of available data.

The following factors were taken into account by the DS in their WoE analysis:

- Read-across of this hazardous property from the structural analogue maleic anhydride, which has a harmonised classification in Resp. Sens. 1. A justification for the read-across approach is provided in Annex III of the background document. Human and animal data on maleic anhydride was included in the CLH report (see Table 14 in the background document). According to the DS, the severity of the allergic reactions is not sufficiently described in the case reports to allow a conclusion regarding frequency of human sensitisation, making it impossible to conclude on sub-categorisation for maleic anhydride. To which extent the data on maleic anhydride that is presented by the DS represents the data set that was the basis for classifying maleic anhydride for respiratory sensitisation with Xn; R42 under the Dangerous Substances Directive is not clarified in the CLH report.
- The chemical structure "Cyclic anhydride" is considered to be a structural alert for respiratory sensitisation (REACH guidance on IR/CSA, Table R.7.3-3, and OECD QSAR toolbox v.3.3.5). Searching the OECD toolbox for succinic anhydride reveals that this compound is considered to have respiratory sensitising properties. The mechanism is ring opening acylation at a carbonyl group (i.e. the polarized C=O bond gives the carbon atom some degree of positive charge, and this charge attracts negatively charged nucleophiles (protein molecules) and encourages reactions). For further details, see Annex III to the Background Document. The DS also concluded that the substance can be considered for classification as a respiratory sensitiser by following the flow chart for integrated evaluation strategy (REACH guidance on IR/CSA, figure R.7.3-4), which highlights that if there are any structural alerts such as acid anhydride, the chemical can be considered for classification.
- Succinic anhydride is a skin sensitiser. The DS argued that although the LLNA test was developed and validated for identification of contact allergens, there is evidence that low molecular weight chemical respiratory allergens will also elicit positive responses in this assay (Kimber, 1995). Chemicals known to cause respiratory allergy and occupational asthma have been shown to test positive in the LLNA. Among such chemicals are acid anhydrides (such as trimellitic anhydride and phthalic anhydride).

Comments received during public consultation

Two MSCAs supported the classification proposal. A third MSCA found that from a scientific point of view, the presented data suggested that succinic anhydride causes respiratory sensitisation. However, the MSCA remarked that there was no available human data, which according to the MSCA, was required according to the criteria provided in section 3.4.2.1. of Annex I to the CLP Regulation and in the guidance provided in section 3.4.2.1.3 of the Guidance of the application of the CLP criteria (version 4.1) in order to classify a substance for respiratory sensitisation. The commenting individual also highlighted the fact that no human data were available. The DS acknowledged that the evaluation of this endpoint is difficult since there are no validated testing methods for respiratory sensitisation and consequently testing is not necessary under REACH. The DS's view is that the available data and the WoE approach taken, fulfill the formal criteria for classification since section 1.1.1 of Annex I to the CLP Regulation has to be taken into account when applying the criteria for classification as Resp. Sens.

The commenting individual also added that small changes in the structure of a substance, such as the presence of a double bond in maleic anhydride, could impact the reactivity and consequently also the potential for respiratory sensitisation. In his opinion, the read-across from maleic anhydride was not sufficiently justified partly because toxicokinetic information that would be useful when assessing the validity of the read-across approach was missing.

No toxicokinetic data are available for either succinic anhydride or maleic anhydride and would, according to the DS, be of limited value since respiratory sensitisation is mainly a local effect.

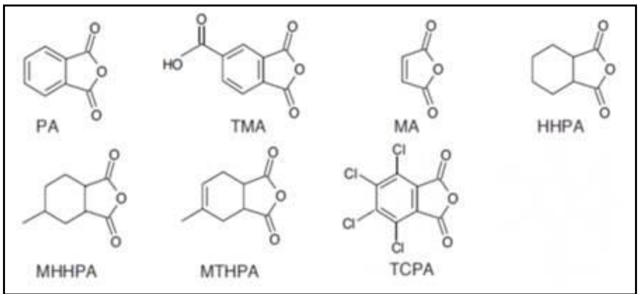
The only difference in structure between succinic anhydride and maleic anhydride is that the former lacks a double bond in its cyclic structure. According to the DS, the positive result from the succinic anhydride LLNA study provides evidence that despite lacking a double bond, the reactivity of succinic anhydride is biologically relevant since a protein binding mechanism is given for succinic anhydride.

Assessment and comparison with the classification criteria

RAC notes that currently there is no formally recognised and validated animal or *in vitro* test methods for evaluation of respiratory sensitisation. According to the criteria for respiratory sensitisation in section 3.4.2.1 of Annex I to the CLP Regulation, classification for respiratory sensitisation is normally based on human data and supportive evidence (such as measurements of immunoglobulin E (IgE) and other specific immunologic parameters in mice, and specific pulmonary response in guinea pigs) may come from animal studies. No human data is available for succinic anhydride. Thus the criteria for respiratory sensitisation cannot be applied directly. However, RAC supports, from a scientific perspective, the WoE approach taken by the DS when classifying succinic anhydride even though human data are missing for succinic anhydride. Annex I, parts 2 to 5 in the CLP Regulation, set forth the criteria for classifying substances under the CLP Regulation. However, if these criteria cannot be applied directly, as is the case for succinic anhydride, the CLP Regulation requires that a WoE approach that takes all available data into account should be used (Article 9(3) and Annex I, Section 1.1.1.3). Further general guidance related to how to use read-across and (Q)SAR in a WoE assessment is provided in section 1.4 of the Guidance on the application of the CLP criteria.

RAC notes that a number of cyclic acid anhydrides (not including succinic anhydride, see Figure 1) that all contain a cyclic anhydride structure but otherwise differ in structure are known human respiratory sensitisers. They have harmonised classifications as Resp. Sens. 1 and in addition they all also have harmonised classification as Skin Sens. 1 and Eye Dam. 1. The allergic hypersensitivity (rhinoconjunctivitis and asthma) by cyclic anhydrides is caused by induction of an IgE mediated specific immune response (immediate type). In humans (as well as in animals), specific antibodies of the IgE type have been found in the blood from workers exposed to these anhydrides. These antibodies are involved in the allergic processes and their presence points to allergic sensitisation. Sensitisation is a crucial and necessary step in the development of allergies. There are indications that cyclic anhydrides might also induce other types of immune responses, involving induction of specific IgG antibodies, and delayed-type of responsiveness (Health council of the Netherlands, 2010). Consequently, the "cyclic anhydride" structure has been included as a structural alert for respiratory sensitisation in structure activity tools such as the OECD QSAR toolbox. Succinic anhydride has a chemical structure that is equivalent to the "cyclic anhydride" structure and is therefore considered to be a putative respiratory sensitiser by this QSAR model.

Fig 1. Structural formulas of some cyclic acid anhydrides. All have harmonised classification in Resp. Sens. 1: There are human case reports of occupational rhinitis and asthma allergy for all these anhydrides (adapted from Keskinen *et al.*,2004).



Abbreviations: PA, phthalic anhydride; TMA, trimellitic anhydride; MA, maleic anhydride; HHPA, hexahydrophthalic anhydride; MHHPA, 4-methyl hexahydrophthalic anhydride; MTHPA, methyl tetrahydrophthalic anhydride; TCPA, tetrachlorophthalic anhydride.

From a mechanistic point of view there seems to be a general agreement within the scientific community that, for low molecular weight compounds, the initial step in the process of respiratory as well as skin sensitisation is that the compound of interest has an intrinsic reactivity such that it can react with functional groups in macromolecules (i.e. proteins) to form "non-self" antigens. The fact that compounds containing a cyclic anhydride structure have the capacity to form such structures is evident, since they have skin sensitising properties (harmonised classified in Skin Sens. 1).

Although succinic anhydride has a very similar structure to its structural homologue maleic anhydride, the much shorter hydrolysis half-life in water, as well as the more severe eye damaging score in the study by Carpenter and Smith (1946) suggest that maleic anhydride has a higher reactivity as compared to succinic anhydride. Based on this difference in reactivity, it can be questioned whether it is appropriate to use read-across to the hazardous property of maleic anhydride to fill the data gap for respiratory sensitisation. However, the studies by Walinder *et al.* (1995) and Zhang *et al.* (1998) show that there was no correlation between the hydrolysis rate constant and the IgE (in rat) or of IgG_1 (guinea pig) serum titers that were recorded 28/21 days after an intradermal injection of various cyclic anhydrides.

Structure activity relationship of the sensitising property of an equimolar <u>intradermal</u> dose of a number of cyclic acid anhydrides (including maleic anhydride and succinic anhydride as well as most of the anhydrides presented in Figure 1) was investigated by analysing immunoglobulin (Ig) titers (21/28 days post dosing) in guinea pigs and in the Norwegian brown rat (see in-depth analysis of RAC). Overall the magnitude of the induced titers varied between the different anhydrides and even small structural changes, as replacing a hydrogen atom with a methyl group, affected the immunogenic response. Succinic anhydride failed to induce an immune response in rats, as measured by Ig-titers 28 days after an intradermal dose of 20 µmol succinic anhydride. When SA (succinic anhydride) was substituted with methyl groups (DMSA) and even more so when substituents were ethyl groups (DESA), an increase in antibody titer was recorded. The titers increased even more when DESA was ring closed to the more rigid cis-HHPA. Further methylation to 4-MHHPA caused no additional increase in the titers. However even higher titers were observed after immunisation with the corresponding aromatic anhydrides PA and 4-MPA. Replacing a hydrogen atom with a methyl group in maleic anhydride decreased the reactivity (Zhang *et al.*, 1998). Furthermore, the immunogenic response was also dependent on the way the compound was presented. When rats were immunised with either SA- or MA-rat serum albumin conjugates (synthesised *in vitro* by mixing anhydrides and protein), similar levels of specific IgE and of specific IgG titers were recorded whereas the titers differed markedly when MA or SA was injected intradermally in its "free form" (no IgG or IgE was detected for SA whereas a clear increase in both antibody titers was detected after immunisation with maleic anhydride). RAC notes the different results in these two experiments and interprets the discrepancy as using a preformed SA- or MA- protein conjugate for immunisation that only investigates the "non-self" recognition of the conjugate whereas the result from the experiment using "free anhydride" also takes into account possible differences in reactivity in the proceeding step of adduct-formation. RAC is of the opinion that the result from the experiment using SA-protein conjugates should be viewed as representing an expected response if the induction dose of free anhydride had been higher than the standardised dose used for all anhydrides in the study by Zhang *et al.* (1998). In addition, the positive result from the LLNA study clearly shows that succinic anhydride as such is reactive enough to produce a biologic relevant immunologic response. Thus, although the reactivity of succinic anhydride is lower than that of maleic anhydride, the experiments from Zhang (1998) show that, at least in rats, dermal exposure to succinic anhydride protein conjugate increases the serum titers for specific IgE antibodies, which is a key component for hypersensitivity reactions such as IgE-mediated rhinitis/conjunctivitis/asthma. However, although the presence of specific IgE are indicative of a possibility for IgE-mediated respiratory hyperreactivity, they do not prove that succinic anhydride inhalation exposure can cause hypersensitivity reactions.

In summary, RAC has considered

- that allergic respiratory manifestations are well known effects of occupational exposure to cyclic acid anhydrides and thus many cyclic acid anhydrides have harmonised classification as Resp. Sens. 1.
- the known reactivity of cyclic acid anhydrides.
- the QSAR-predictions of respiratory sensitisation of succinic anhydride.
- the reactivity of succinic anhydride and the *in vitro* formation of protein conjugates.
- the demonstration of IgE in sera of rats exposed intradermally to succinic anhydride protein conjugates.
- the positive LLNA results in mice.

Based on a WoE analysis by taking the available data into consideration, RAC is of the opinion that it is justified to classify succinic anhydride as **Resp Sens. 1**. Although succinic anhydride might have a lower potency to induce respiratory sensitisation as compared to its structural homologue, maleic anhydride (as well as possibly compared to other cyclic anhydrides), the available data clearly show that succinic anhydride has the potential to cause respiratory sensitisation.

Supplemental information - In depth analyses by RAC

The structure activity relationship of organic cyclic anhydrides as antigens in animal models has been investigated in a number of articles from the same research group (Welinder *et al.*, 1995; Zhang *et al.*, 1997 and 1998). The results from these mainly dermal studies were also included in the joint report ("136. Cyclic anhydrides") from The Nordic expert group for criteria documentation of health risks from chemicals and the Dutch expert committee on occupational standards (Keskinen *et al.*, 2004). However, these results were not included in the proposal from the DS and they are therefore described in detail below.

In the study by Welinder *et al.* (1995; non-guideline and non-GLP, and considered to be reliable with restrictions because of somewhat unclear reporting) the relationship between chemical structure and immunogenicity was investigated for 13 dicarboxylic acid anhydrides (see Figure 2), including succinic anhydride. Guinea pigs (9/anhydride) were immunised intradermally using an equimolar dose (30 µmol dissolved/dispersed in olive oil; i.e. injection at two sites/animal using 0.05 ml of 0.3 M solution/site). According to the authors of the paper, this dose had been found to be optimal in a preliminary investigation with cis-HPPA, one of the more potent anhydrides (no data included). Blood was drawn 21 days post-dosing and analysed for presence of IgG, specific IgG_1 and specific IgG_2 antibodies. Specific IgE was assessed by the passive cutaneous anaphylaxis test.

Fig. 2. Chemical structures of the organic acid anhydrides used in the studies by Welinder *et al.*, (1995) and Zhang *et al.*, (1998) (from Zhang *et al.*, 1998).

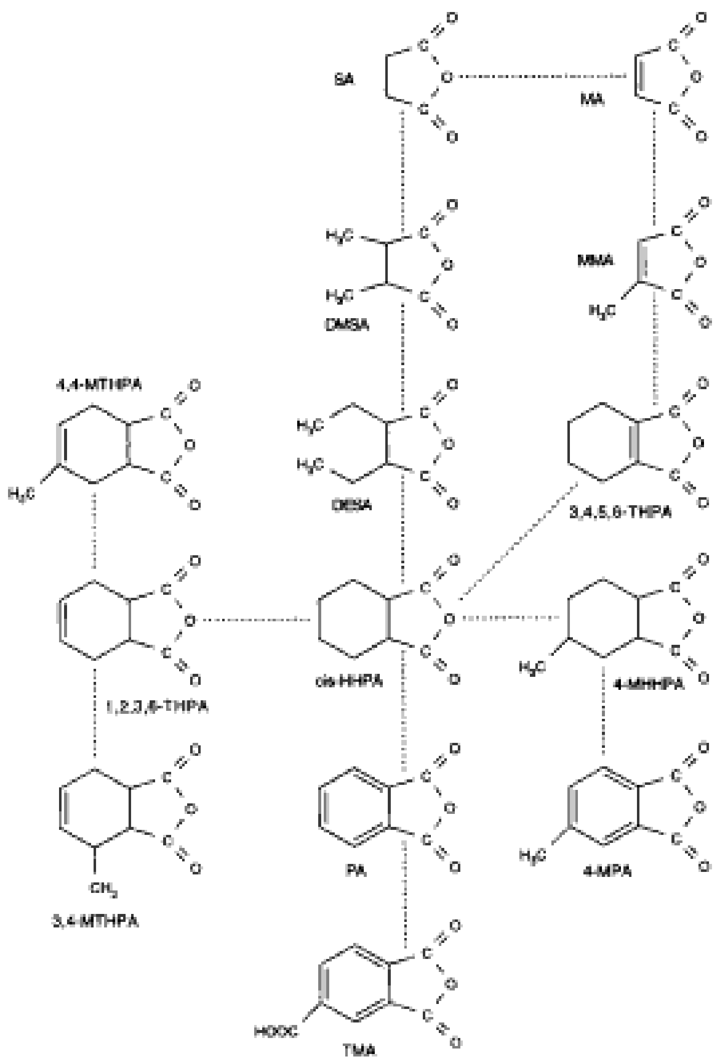


Table 1. Titers of specific total IgG antibodies and subclasses of IgG antibodies (IgG_1 and IgG_2) by enzyme-linked immunoabsorbent assay (ELISA) of sera from guinea pigs (SA, *n* = 7; $THPAA_{3456}$, *n* = 3, others, *n* = 9) after intradermal immunisation with different organic acid anhydrides.

Anhydride#	IgG* titer		IgG_1* titer		IgG_2* titer	
	Median	Range	Median	Range	Median	Range
SA[1]	100	50-800	300	100-3200	200	50-1600
MA	1600	800-3200	51200	12800-102400	6400	1600-12800
MMA	1600	400-3200	6400	3200-25600	1600	800-6400
cis-HHPA	1600	800-6400	12800	3200-25600	800	200-1600
trans-HHPA	400	100-1600	1600	200-12800	1600	100-3200
4-MHHPA	1600	800-6400	12800	6400-102400	3200	1600-6400
1,2,3,6-THPA	200	100-200	6400	1600-12800	200	100-400
3,4,5,6-THPA	200	100-200	800	400-800	400	100-400

3,4-MTHPA	3200	400-12800	25600	1600-51200	3200	400-6400
4,4-MTHPA	6400	3200-12800	102400	19200-204800	12800	3200-25600
PA (exp 1)	3200	1600-6400	51200	51200-102400	3200	1600-6400
4-MPA	1600	200-3200	51200	3200-102400	1600	200-3200
TMA	1600	1600-3200	12800	6400-25600	1600	800-1600

* The titer is expressed as the highest dilution giving an absorbance value above 3 standard deviations of the controls (1:3200 = 3200). 1) note that for SA and 3,4,5,6 THHPA the values presented only represent the median and range value from animals with positive titers (7/9 and 3/9, respectively).

#SA, succinic anhydride, MA, maleic anhydride; MMA, methylmaleic anhydride; *cis*-HHPA, *cis*-hexahydrophthalic anhydride; *trans*-HHPA, *trans*-hexahydrophthalic anhydride; 4-MHHPA, 4-methyl hexahydrophthalic anhydride; 1,2,3,6-THPA, 1,2,3,6-tetrahydrophthalic anhydride; 3,4,5,6-THPA, 3,4,5,6-tetrahydrophthalic anhydride; 3,4-MTHP, 3,4-methyltetrahydrophthalic anhydride; 4,4-MTHP, 4,4-methyltetrahydrophthalic anhydride; PA, phthalic anhydride; 4-MPA, 4-methylphthalic anhydride; TMA, trimellitic anhydride.

When compared to the other organic anhydrides (30 µmol of each), SA sensitised fewer animals (7 out of 9, as compared to 9/9 for all other anhydrides except $THPA_{3456}$ that sensitised 3/9 animals). The median value of total IgG, IgG_1 and IgG_2 titer in the SA sensitised (7/9, see Table 1) was clearly far lower as compared to most other anhydrides (roughly 16x lower when compared to the recorded median IgG value for maleic anhydride).

No positive readout was recorded for succinic anhydride, cis/trans HHPA or THPA in the passive cutaneous anaphylaxis test (a test method that is considered to have low sensitivity, data not shown). This suggest that these anhydrides had not induced formation of IgE antibodies. However, it is important to keep in mind that in guinea pigs it is mainly the IgG1 and not IgE (as in humans) that are the anaphylactic antibodies.

The result also indicates a considerable variation in the sensitising potential between different organic acid anhydrides. Notably introducing a double bond between C1 and C2 in succinic anhydride, i.e. forming maleic anhydride, markedly increased the formation of antibodies, However introducing the corresponding double bond in HHPA (i.e. forming 3,4,5,6-THPA) markedly decreased the IgG_1 levels (a 16-fold reduction). If the double bond instead was positioned between C4 and C5 (1,2,3,6-THPA) the effect was less pronounced (only a two-fold reduction). Similarly when a hydrogen atom in the anhydride was substituted with a methyl group (compare 1,2,3,6-THPA with MTHPA) it increased the Ig_1 levels whereas substitution of methyl groups in HHPA (4-MHHPA) and PA (MPA) had no influence on IgG_1 levels.

The authors indicate that the interpretation of the result is not straightforward and that several factors such as immunisation regime (including use of a standardised dose), the solubility of the anhydrides, and the conjugates used in the ELISA assays may be important for the outcome of the tests.

In the study by Zhang (1998; non-guideline and non-GLP, and considered to be reliable with restrictions due to somewhat unclear reporting), the sensitising properties of 14 organic acid anhydrides (see Figure 2) were evaluated. Rats (Norwegian Brown; 7/each anhydride) were dosed intradermally, using an equimolar dose (injection at two sites/animal using 0.05 ml of a 0.2 M solution/site; each anhydride was dissolved in dioxane (final concentration of 3 %) and further in liquid paraffin). Serum samples were collected 28 days after dosing and analysed by ELISA (using anhydride-RSA conjugate for coating) for presence of specific IgE and specific IgG antibodies.

Table 2. Titers of specific IgE and IgG measured by enzyme-linked immunosorbent assay of

sera from rats (n = 7) after intradermal immunisation of different organic acid anhydrides.

Anhydride#	IgE titer*		IgG titer*	
	Median	Range	Median	Range
SA	< 50	< 50	< 50	< 50
DMSA	200	100-400	800	400-1600
DESA	800	400-800	1600	800-1600
MA	800	800-3200	1600	1600-3200
MMA	200	100-200	400	200-800
cis-HHPA	1600	1600-3200	3200	1600-3200
4-MHHPA	1600	800-1600	3200	1600-3200
1,2,3,6-THPA	800	200-1600	800	200-1600
3,4,5,6-THPA	< 50-50	< 50	< 50-50	< 50
3,4-MTHPA	800	400-800	1600	800-1600
4,4-MTHPA	800	800-3200	1600	800-3200
PA	3200	1600-3200	3200	1600-3200
4-MPA	3200	3200-6400	6400	3200-6400
TMA	1600	800-6400	1600	800-3200

*The titer is expressed as the highest dilution to give an absorbance value > 3 S.D: of controls (1:3200 = 3200). #SA, succinic anhydride; DMSA, dimethylsuccinic anhydride; DESA, dimethylsuccinic anhydride; MA, maleic anhydride; MMA, 4-methylmaleic anhydride; cis-HHPA, cis-hexahydrophthalic anhydride; 4-MHHPA, 4-methyl hexahydrophthalic anhydride; 1,2,3,6-THPA, 1,2,3,6-tetrahydrophthalic anhydride; 3,4,5,6-THPA, 3,4,5,6-tetra hydrophthalic anhydride; 3,4-MTHP, 3,4-methyltetrahydrophthalic anhydride; 4,4-MTHP, 4,4-methyltetrahydrophthalic anhydride; PA, phthalic anhydride; 4-MPA 4-methylphthalic anhydride; TMA, trimellitic anhydride.

IgE and IgG antibodies were detected in serum obtained after intradermal immunisation of rats with various free OAA. However, the magnitude of the induced titers varied. SA (20 µmol) and 3,4,5,6-THPA failed to give detectable IgE antibody production, while the highest IgE titers were shown by cis-HHPA, 4-MHHPA, TMA and especially PA and 4-MPA. A close correlation was observed between IgE and IgG titers.

To compare the sensitising properties of "free anhydride" to that of a preformed anhydride – protein conjugate, rats (7/each anhydride-potein conjugate) were dosed intradermally (1.4 mg of anhydride-RSA conjugate in 0.15 M NaCl, total dosing volume 0.14 ml) with either succinic anhydride-RSA (Rat Serum Albumin), Maleic anhydride- RSA or *cis*-HHPA-RSA conjugate.

The result (see Table 3) show that rats immunised with SA or MA bound to the RSA carrier induced similar titers.

Table 3. Titers of specific IgE or IgG by enzyme-linked immonoabsorbant assay of sera from rats after intradermal immunisation with 1.4 mg of different protein conjugates between organic acid anhydrides and rat serum albumin (RSA) in saline.

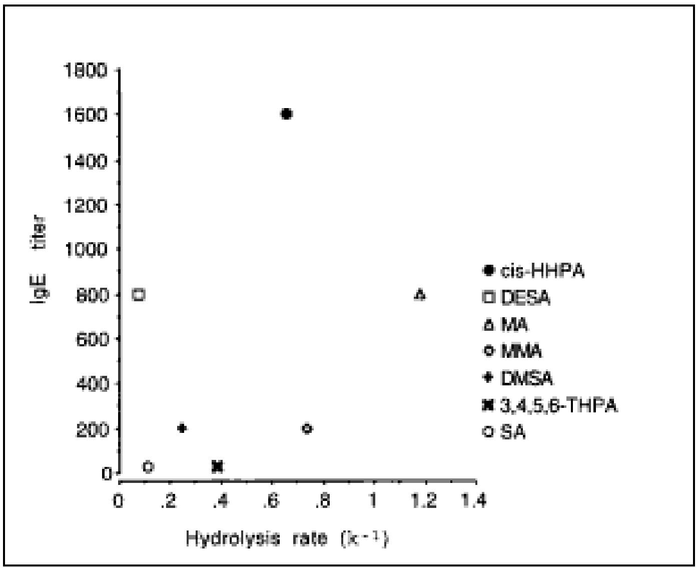
Antigen#	IgE titer*		IgG titer*	
	Median	Range	Median	Range
SA-RSA	200	50-800	400	200-1600
MA-RSA	200	100-400	400	200-1600
cis-HHPA-RSA	800	400-800	800	800-1600

*The titer is expressed as the highest dilution to give an absorbance value > 3 S.D: of controls (1:400 = 400). #SA-RSA, Succinic anhydride-rat serum albumin conjugate; MA-RSA, Maleic anhydride-rat serum albumin conjugate; *cis*-HHPA-RSA, cis-hexahydroophthalic anhydride-rat serum albumin conjugate.

Immunisation with serum-albumin conjugates mainly reflects the "non-self" recognition of the conjugate rather than the amount of free anhydride which reacts with proteins after injection. Thus the result from the carrier bound SA can be viewed as representing a possible response from a situation where the dose used for immunisation of "free" anhydride had been higher.

Cyclic acid anhydrides induce antibody formation by conjugation *in vivo*; the formed conjugates are recognised as nonself proteins by the immune system. Thus, the antibody formation may be influenced by the chemical reactivity of the anhydrides. However, no correlation was seen between the hydrolysis rate constant and the antibody titers in this study or in the previous study in guinea pigs (see Figure 3). Thus, DESA and SA have similar hydrolysis rate constants but differed markedly (~800x) in the IgE titer. Maleic anhydride and DESA induced similar IgE titers, but the hydrolysis rate constant for DESA is ~1/10 of the one for maleic anhydride.

Fig. 3. Correlation between induced IgE titers and the hydrolysis rate constants (from Zhang *et al.*, 1998).



4.7 Repeated dose toxicity

Not evaluated in this dossier.

4.8 Germ cell mutagenicity

Not evaluated in this dossier.

4.9 Carcinogenicity

Not evaluated in this dossier.

4.10 Toxicity for reproduction

Not evaluated in this dossier.

4.11 Other effects

Not evaluated in this dossier.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in this dossier.

6 OTHER INFORMATION

Not evaluated in this dossier.

7 REFERENCES

Amoco Corporation (1991). Respiratory sensitization study of maleic anhydride: a research project. TSCAT, 86-920000056, OTS 0533622; cited in OECD SIDS for CAS. Nos. 108-31-8/ 110-16-7, 2004. Testing laboratory: IITS Research Institute. Owner company: Amoco Corporation. Study number: 1277C. Report date: 1991-09-30.

Aptula A.O., Roberts D.W., Mechanistic Applicability Domains for Nonanimal-Based Prediction of Toxicological End Points: General Principles and Application to Reactive Toxicity, Chem. Res. Toxicol. 2006, 19(8), 1097-1105.

Barker R. D. et al. (1998). Risk factors for sensitisation and respiratory symptoms among workers exposed to acid anhydrides: a cohort study. Occup Environ Med 1998; 55:684–691.

Bernat, E. (1999). "Bernsteinsäure": Acute eye irritation/corrosion study with rabbits. Testing laboratory: Osterreichisches Forschungszentrum Siebersdorf Ges. m. b. H. Report no.: OEFZ--L-0250. Owner company: DSM Fine Chemicals Austria, Linz, St-Peter-Strasse. Study number: DSM94. Report date: 1999-12-01.

Buskens C.A.F (2014). In vitro skin corrosion test with succinic anhydride using a human skin model. WIL Research Europe B.V. pp. 26 (OECD TG 431)

Carpenter CP, Smyth HF Jr (1946). Chemical burns of the rabbit cornea. Am J Ophthalmol 29: 1363-72.

Graneek, B. J. et al (1986). Occupational exposure caused by maleic anhydride: bronchial provocation testing and immunologic data. Thorax 41: 25. (only abstract available).

Graneek, B. J. et al (1988). Late asthmatic reactions and changes in histamine responsiveness provoked by occupational agents. Clin. Respir. Physiol. 23: 577-581

Jarvis J, Seed MJ, Elton R, Sawyer L, Agius R. Relationship between chemical structure and the occupational asthma hazard of low molecular weight organic compounds. Occup Environ Med. 2005 Apr;62(4):243-50.

Kim, J. H., Gibb, H. J., Iannucci, A (2009). Concise International Chemical Assessment Document 75. Cyclic acid anhydrides: Human Health Aspects. World Health Organisation

Kimber I. 1995. Contact and respiratory sensitization by chemical allergens: uneasy relationships. Am. J. Contact Derm. 6, 34

Kimber I and Dearman RJ. 2002. Chemical respiratory allergy: role of IgE antibody and relevance of route of exposure. Toxicology 181-182, 311-315.

Lee, H. S. et al (1991). Occupational asthma due to maleic anhydride. Br. J. Industr. Med. 48: 283-285.

OECD, 2011. Report of the expert consultation on scientific and regulatory evaluation of organic chemistry mechanism-based structural alerts for the identification of protein-binding chemicals. Series on Testing and Assessment No. 139. ENV/JM/MONO(2011)9

OECD – 2014. New guidance document on an integrated approach on testing and assessment (IATA) for skin corrosion and irritation. Series on Testing and Assessment No. 203. ENV/JM/MONO(2014)/19

Reagan, E. L. (1982). Acute oral LD50 assay in rats (OECD) with cover letter dated 040882. US EPA, Office of Toxic Substances, Doc ID 88-8100369. Testing laboratory: Food and Drug Research Laboratories, Inc. Report no.: 6771G. Owner company: Buffalo Colour Corp. Report date: 1981-04-14.

Roberts D.W., Aptula A.O., Patlewicz G., Electrophilic Chemistry Related to Skin Sensitization. Reaction Mechanistic Applicability Domain Classification for a Published Data Set of 106 Chemicals Tested in the Mouse Local Lymph Node Assay, Chem. Res. Toxicol. 2007, 20(1), 44-60.

Roberts D.W., Patlewicz G., Kern P.S., Gerberick F., Kimber I., Dearman R.J., Ryan C.A., Basketter D.A., Aptula A.O., Mechanistic Applicability Domain Classification of a Local Lymph Node Assay Dataset for Skin Sensitization, Chem. Res. Toxicol. 2007, 20(7), 1019-1030.

Topping M. D. et al. (1986). Specificity of the human IgE response to inhaled acid anhydrides. J. Allergy and Clinical Immunol. 77: 834-843, 1986.

Verbaan I.A.J. (2014) In vitro skin irritation test with succinic anhydride using a human skin modell. WIL Research Europe B.V. pp. 30 (OECD TG 439)

Weber E (2010). Succinic anhydride: Skin sensitization study (Local lymph node assay). Testing laboratory: Seibersdorf Labor GmbH. Report no.: SL-LT-038/10. Owner company: DSM Fine Chemicals GmbH. Study number: DSM590. Report date: 2010-03-08.

Winter, C. A., and Tullius, E. J. (1950). The irritating effects of maleic acid and of maleic anhydride upon the eyes of rabbits. Am. J. Ophthalm. 33: 387-388, 1950;

Wolf, T. (2010). "Succinic anhydride": Acute Dermal Toxicity Study in Rats. Testing laboratory: Seibersdorf Labor GmbH - Toxicology, A-2444 Seibersdorf, Austria. Report no.: SL-LT-086/10. Owner company: DSM Fine Chemicals GmbH. Study number: DSM620. Report date: 2010-04-28.

Additional references

Additional references not included in the CLH report

Health Council of the Netherlands (2010). Cyclic acid anhydrides: Health-based recommended occupational exposure limit. The Hague: Health Council of the Netherlands, 2010; publication no. 2010/02OSH (available at https://www.gezondheidsraad.nl/sites/default/files/201002OSH.pdf)

Keskinen, H. (2004). The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals and The Dutch Expert Committee on Occupational Standards : 136. Cyclic acid anhydrides (available at http://hdl.handle.net/2077/4327).

Zhang, X-D., Welinder, H., Jönsson, BAG. and Skerfving, S. (1998). Antibody responses of rats after immunization with organic acid anhydrides as a model of predictive testing. Scand J Work Environ Health, 24(3):220–227.

Welinder, H., Zhang, X-D., Gustavsson, C., Björk, B. and Skerfving, S. (1995). Structure-activity relationships of organic anhydrides as antigens in an animal model. Toxicology, 103: 127-136.

8 ANNEXES

CONFIDENTIAL ANNEXES (I-II)

ANNEX I Information on impurities (confidential)

ANNEX II Details on skin and eye corrosion tests (confidential)

NON-CONFIDENTIAL ANNEXES

ANNEX III Read across justification

In the present CLH report for the classification of succinic anhydride read across using maleic anhydride (CAS: 108-31-6) and succinic acid (CAS: 110-15-6) as source substances has been applied for the endpoints listed in the following table.

Table 1: Endpoints for which read-across has been applied

Endpoint	Source Substance	Study type and reference
Respiratory sensitisation	Maleic anhydride	Key study *Amoco Corporation (1991). Respiratory sensitization study of maleic anhydride: a research project. Report date: 1991-09-30. Supporting studies/Case Reports *Graneek, B. J. et al (1986). Occupational exposure caused by maleic anhydride: bronchial provocation testing and immunologic data. *Graneek, B. J. et al (1988). Late asthmatic reactions and changes in histamine responsiveness provoked by occupational agents. *Lee, H. S. et al (1991). Occupational asthma due to maleic anhydride. *Topping M. D. et al. (1986). Specificity of the human IgE response to inhaled acid anhydrides. *Barker R. D. et al. (1998). Risk factors for sensitisation and respiratory symptoms among workers exposed to acid anhydrides: a cohort study.
Skin Corrosion	Maleic anhydride	Supporting study *Chevron Chemical Company (1976). Skin corrosion potential of maleic anhydride. TSCAT,

		878214793, OTS 0206657
Eye irritation/eye damage	Succinic acid	Key study *Bernat, E. (1999). "Bernsteinsäure": Acute eye irritation/corrosion study with rabbits.

***Evaluation of data used for read across for adequacy**

According to the ECHA Guidance "Guidance on information requirements and chemical safety assessement, Chapter R.6: QSARs and grouping of chemicals, the used data needs to be assessed for its adequacy. Therefore, the available experimental data have been evaluated for adequacy according to Chapter R.4 ("Evaluation of available information").

For a detailed evaluation of the available data for adequacy please refer to the respective endpoint(s) in this document (Chapter 4.6.2, Chapter 4.4.1, Chapter 4.4.2). The laboratory animal studies for the analogue approach are classified with Klimisch score 1 or 2.

Already defined categories among anhydrides:

An analogue approach has been also proposed for maleic anhydride and maleic acid by OECD (2004). The analogue rationale is that maleic anhydride is readily hydrolysed to maleic acid under aqueous conditions. The difference is that maleic anhydride forms haptens by acylating amino acids, resulting in an immunological response (dermal and respiratory sensitisation) (OECD, 2004).

U.S. EPA has defined a category of four cyclic anhydrides members, which are bicyclic (including hexahydrophthalic anhydride, methylhexahydrophthjalic anhydride, tetrahydrophtalic anhydride, methyltetrahydropthalic anhydride) and the tricyclic anhydride nadic methyl anhydride) (U.S EPA, 2009). The category is based on similar chemical sructures, physico-chemical properties, and toxicological properties.

Maleic anhydride and succinic acid used as source substances (analogue approach):

In accordance with ECHA Guidance (Chapter R.6)[7], substances whose physicochemical and/or toxicological and/or ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity, may be considered as a group or "category" of substances. The similarities may be due to a number of factors

- Common functional group
- Common precursor or breakdown products
- Constant pattern in changing potency
- Common constituents or chemical classes

In the case of the present read across maleic anhydride and succinic anhydride have a common functional group and belong to the same chemical class (mono-cyclic anhydrides).

[7] Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.6: QSARs and grouping of chemicals.

A stepwise approach for applying read across is set out in Chapter R.6 section 6.2.3 "Guidance on a stepwise procedure to perform the analogue approach"[5]. The outcome of these step wise approach to perform the read across from maleic anhydride to succinic anhydride for the endpoints respiratory sensitisation and skin corrosion and for the the read across approach from succinic acid to succinic anhydride for eye irritation/damage and is set out in this document.

In the following the read across has been described according to the reporting format for the analogue approach described in the ECHA Guidance (R.6.2.6.1)[5].

1. **Hypothesis for the analogue approach**

1.1 Maleic anhydride used as source substance

Endpoint: Respiratory sensitisation

Maleic anhydride displays a high structural similarity to succinic anhydride (see Figure 1). Both chemicals are monocyclic anhydrides. The only structural difference is that maleic anhydride has a double bound in its ring structure. The read across approach is used for the endpoint respiratory sensitisation (key studies) and in a weight of evidence approach for the endpoint skin corrosion.

Figure 1: Maleic anhydride (source substance) and succinic anhydride (target substance)

The anhydride structure is an alert for respiratory sensitising properties (ECHA Guidance, Chapter R.7a[8]). According to the OECD QSAR toolbox v.3.3.5 and the OECD review (OECD, 2011) the acid anhydrides possess sensitising properties based on following mechanism:

The underlying mode of action is that the polarized C=O bond gives the carbon atom some degree of positive charge, and this charge attracts negatively charged nucleophiles (protein molecules) and encourages reactions (details see Figure 2).

[8] Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.7a: Endpoint specific guidance, Version 4.0, July 2015

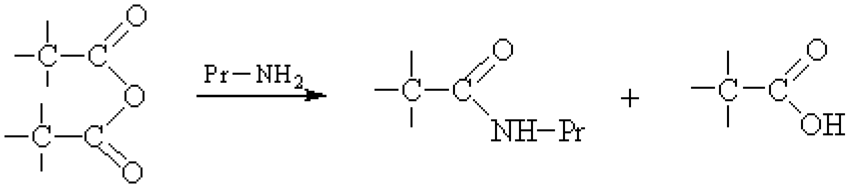


Figure 2: Protein binding mechanism of acid anhydrides

After acyl halides, acid anhydrides are the most reactive carboxylic acid derivatives. Nucleophilic acyl substitution in acid anhydrides involves cleavage of a bond between oxygen and one of the carbonyl groups. This reaction proceeds in two stages via a tetrahedral intermediate. Its formation is rate-determining and is the step that is accelerated by a catalyst. The acid anhydrides are activated toward nucleophilic addition by protonation of one of their carbonyl groups. The protonated form of acid anhydride is present to only a very small extent, but it is quite electrophilic and protein molecule adds to it.

References cited in QSAR toolbox v.3.3.5.: Aptula A.O et al, 2006, Roberts D.W et al., 2007, Roberts D.W et al., 2007

Endpoint: Skin corrosion

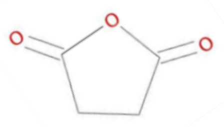
It is stated in the OECD review that dicarboxylic acids are known irritants and the formation of the acid is the basis for skin and eye irritation seen with succinic and other anhydrides (Kim, 2009).

Maleic anhydride has been used as source substance in a weight of evidence analysis to underline that succinic anhydride does possess skin corrosive potential. In vitro (skin corrosion test) data and acute dermal toxicity tests with succinic anhydride itself does demonstrate skin corrosive potential. These data are considered sufficient for classification. However, data from maleic anhydride, structural most similar substance to succinic anhydride are presented in order to demonstrate that structural similar substance does also possess skin corrosive potential.

1.2 Succinic acid used as source chemical

Endpoint: Eye irritation/eye damage

Succinic anhydride is converted under aqueous conditions to its corresponding acid form, succinic acid. Therefore, it is assumed that under certain circumstances (e.g., presence of water) succinic acid can be used as source substance for read across. In the tear fluid of the eye aqueous conditions are present and thus it is assumed that the anhydride is converted to succinic acid, within minutes. The structures of the chemicals are depicted in Figure 3.



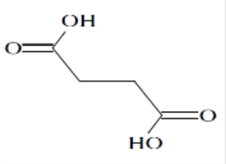


Figure 3: Succinic anhydride (target substance) and succinic acid (source substance).

Succinic acid is used as source substance for eye irritation/eye damaging potential, since it has been shown in a comparative study that succinic acid has similar eye damaging effects as succinic anhydride (Carpenter and Smyth, 1949). In the same study it was indicated that maleic anhydride has more eye damaging effects than succinic anhydride or succinic acid.

2. Description of source chemical(s)

An overview of the source chemicals including its CAS numbers, names and synonyms are given in following table.

Table 2: Substance identity of source substance(s)

Substances	Maleic anhydride	Succinic acid
CAS number	108-31-6	110-15-6
CAS name	2,5-Furandione	Succinic acid
IUPAC name	Furan-2,5-dione	Succinic acid
EC number	203-571-6	203-740-4
EC name	Maleic anhydride	Succinic Acid
Molecular formula	$C_4H_2O_3$	$C_4H_6O_4$

3. Purity/Impurities

The purity of the analogue substances is according to registrants information very high (above 99.5%). Impurities are not likely to influence the overall toxicity.

4. Analogue approach justification

4.1 Maleic anhydride: source substance

The very close structural similarity and the same common functional group of maleic anhydride and succinic anhydride (see Figure 1) and similar properties (see Table 2) supports consideration of these substances as analogues for the purpose of read-across. In particular a read across for respiratory sensitisation is regarded as appropriate since both substances possess the structural alert for respiratory sensitisation (ECHA Guidance IR/CSA[5]).

Furthermore maleic anhydride has been used as source substance for skin corrosive effects to underpin the outcome of an in vitro test with succinic anhydride itself.

a) Structural similarity

The analogues maleic anhydride and succinic anhydride are monocylic anhydrides and belong to the same chemical class. They exhibit same structural alert (anhydride structure). The only difference is that maleic anhydride has a double bound, whereas succinic anhydride has a single bound in the ring structure.

As shown in Figure 2 the analogues possess anhydride structures which encourages reaction with amino group of proteins and consequently fall within a chemical group with sensitising potential (QSAR toolbox v.3.3.5, ECHA Guidance IR/CSA[5]).

b) Chemical property similarity

Succinic anhydride as well as maleic anhydride are low molecular weight, polar compounds and hydrolyse fully and fast to its corresponding acid form (succinic acid, maleic acid) in the range of minutes. The hydrolysis products succinic acid and maleic acid are ionic compounds, able to dissociate in water depending on the pH of the solution. Therefore, water solubility and partition coefficient n-octanol/water (log Kow) do not apply in principle for the anhydrides. Therefore, water solubility and partition coefficient n-octanol/water of the acids is provided instead.

Based on these physico-chemical properties and resulting behaviour of the analogues, it is justified that maleic anhydride is an appropriate reference material for read across.

c) Mammalian toxicological data

As depicted in Table 2 succinic anhydride and maleic anhydride have some similar toxicological patterns in regard to mammalian toxicological endpoints. Both are harmonised classified for acute oral toxicity (Cat. 4). The LD50 value is between 300 mg/kg bw and 2000 mg/kg bw. No dermal acute toxicity has been observed, the LD50 values are above 2000 mg/kg bw. The substances are corrosive to skin and to eye. For maleic anhydride skin corrosiveness was detected in animal tests, whereas for succinic anhydride skin corrosive potential was indicated in in vitro data using human epidermal skin models.

Both substances are skin sensitizers according to the outcome of studies carried out with laboratory animals. For the analogue maleic anhydride there is also evidence from human studies.

The evaluation of the mutagenic and carcinogenic data revealed that the substances do not have mutagenic properties and there are no incidences that the substances are carcinogenic to humans. Furthermore there is no or insufficient indication that the substances have negative effects on reproductive toxicity.

4.2 Succinic acid: source substance:

Succinic anhydride is under aqueous conditions converted to its corresponding acid form, succinic acid. Therefore, it is assumed that under certain circumstances (e.g., presence of water) succinic acid can be used as source substance for read across to evaluate the hazard of succinic anhydride.

a. Break down product of succinic anhydride

Succinic anhydride is converted rapidly to the corresponding acid under aqueous conditions (within range of minutes). It is assumed that in the tear fluid of the eyes, this conversion takes rapidly place (more rapidly than for example on skin tissue) und thus for the endpoint eye irritation and corrosion we consider a read-across appropriate.

b. Comparative study with succinic anhydride and succinic acid

In the study of Carpenter and Smyth (1946) varies different compounds have been tested to determine eye irritating effects, amongst others succinic anhydride and succinic acid. Both compounds have the same severity index for eye damaging effects, which supports that in respect to eye damaging properties these two compounds can be used as analogues.

5. Data matrix

For the succinic anhydride, maleic anhydride and succinic acid data were gathered for the respective endpoints and evaluated for relevance (for details see Table 2: Matrix of data availability).

Data were gathered on standard physico-chemical properties, and toxicological effects. Standard physico–chemical properties include physical state, molecular weight, melting point, boiling point, relative density, aqueous solubility, vapour pressure. These physico-chemical properties can often provide supporting information for the read across.

For the mammalian toxicological endpoints a summary of the evaluation for the endpoints acute toxicity (dermal, oral), irritation/corrosion, skin sensitisation, repeated dose toxicity, genetic toxicity (*in vitro* and *in vivo*), reproductive toxicology is provided.

6. Conclusions per endpoint for C&L

The current harmonised classification entry (CLP Regulation (EG) Nr. 1271/2008, Annex VI (Tabelle 3.1.) as well as the further classification proposed by the DS of the present CLH proposal for succinic anhydride and further CLH proposal for maleic anhydride are depicted in Table 3.

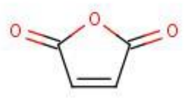
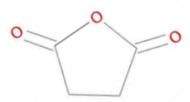
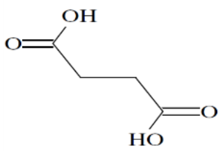
In the present CLH proposal for the endpoint respiratory sensitisation, skin irritation/corrosion and eye irritation/corrosion a read across approach to maleic anhydride or succinic acid is proposed.

In the case of the endpoint skin and eye irritation/corrosion data of succinic anhydride itself are presented in the corresponding chapters, the read-across approach is applied to further substantiate the hazard identification. Whereas, for respiratory sensitisation no data are available with succinic anhydride.

Applying the read-across approach (together with data of the target substance itself) following C&L is proposed:

Resp. Sens. 1, H334
Skin Corr. 1B, H414
Eye damage 1, H318

Table 3: Data matrix for the analogue read-across: Physico-chemical properties and mammalian toxicity

Substances	**Maleic anhydride (MAN)**	**Succinic anhydride (SAN)**	**Succinic acid**
Read across	Source chemical	Target Chemical – same functional group	Source Chemical - hydrolysis product of succinic anhydride
CAS No	108-31-6	203-570-0	110-15-6
Smiles	C1(OC(=O)C=C1)=O	C1(OC(=O)CC1)=O	C(CC(=O)O)C(=O)O
Molecular structure			
Physico-chemical properties			
Molecular weight	98,06 g/mol	100,07 g/mol	118,09 g/mol
State of the substance at 20°C and 101,3 kPa	solid, colourless needles	solid, colourless needles	white, crystalline, colourless, solid
Melting/freezing point	53 - 58°C	119.0°C	185 - 187°C
Boiling point	200°C at 1013.25 hPa	263.5°C	235°C
Relative density	1.48 g/cm^3 at 20 °C	1.23 g/cm^3 at 20°C	1,57 g/cm^3 at 15 °C.
Vapour pressure	15.1Pa at 22 °C	0.2 Pa at 25°C	0.000025 Pa at 25 °C.
Dissociation constant	Maleic acid: 1.92 and 6.23 at 25°C	Succinic acid: 4.67 and 5.64 at 25°C	Succinic acid: 4.67 and 5.64 at 25°C
Water solubility	Substance hydrolyses fast. Water solubility of hydrolysis product maleic acid: 478,8 g/L at 20°C	Substance hydrolyses fast. Water solubility of hydrolysis product succinic acid: 62.9 g/L at 20°C	62.9 g/L at 20°C

Partition coefficient n-octanol/water	Substance as such hydrolyses in n-octanol/water. Log P_{OW} of hydrolysis product maleic acid: -0,48 Theoretical Log KOW of anhydride (substance as such) using KOWWIN (v1.68), EPISUITE 4.10: Log P_{OW}: 1.6187 See section 1 for explanation	Substance as such hydrolyses in n-octanol/water. Log P_{OW} of hydrolysis product succinic acid: -0,59 Theoretical Log KOW of anhydride (substance as such)using KOWWIN (v1.68), EPISUITE 4.10: Log P_{OW}: 0.8102 See section 1 for explanation	Log P_{OW}: -0,59
Half-lives [min]	0.3	4.4	stable
Mammalian Toxicity			
Acute Toxicity - oral	LD_{50}>300 mg/kg bw, <2000 mg/kg bw	LD50>300 mg/kg bw, <2000 mg/kg bw	LD50 > 2000 mg/kg bw
Acute Toxicity - dermal	LD_{50}>2000 mg/kg bw	LD50>2000 mg/kg bw	--
Irritation/Corrosion	Skin corrosive Eye corrosive (experimental data, human data)	Skin corrosive (in vitro test system) Eye corrosive (experimental data, read across)	Not irritating to slight irritating Eye corrosive (laboratory animal)
Skin Sensitisation	Positive (LLNA, Bühler test, human data, etc.)	Positive (LLNA)	Negative (LLNA, GMPT)
Repeated dose	Slight kidney toxicity	--	--
Genetic Toxicity in vitro	Not genotoxic/not mutagenic	Not genotoxic/not mutagenic	No indication for reproductive toxicity
Genetic Toxicity in vivo	Not genotoxic/not mutagenic	Not genotoxic/not mutagenic	--
Reproductive Toxicity - Fertility - Developmental Toxicity	No reproductive toxicity effects (fertility and developmental toxicity)	Insufficient evidence for reproduction toxicity (fertility and developmental toxicity)	No indication for reproductive toxicity

Table 4: Overview of harmonised C&L entry[1] and proposed further classification by DS

Substances	**Maleic anhydride**			**Succinic anhydride**			**Succinic acid**		
C&L	**Classification**		**Labelling**	**Classification**		**Labelling**	**Classification**		**Labelling**
	Hazard Class and Category Code(s)	**Hazard Statement Code(s)**	**Hazard Statement Code(s)**	**Hazard Class and Category Code(s)**	**Hazard Statement Code(s)**	**Hazard Statement Code(s)**	**Hazard Class and Category Code(s)**	**Hazard Statement Code(s)**	**Hazard Statement Code(s)**
Harmonised classification[1]	Acute Tox 4* Skin Corr. 1B Skin Sens. 1 Resp. Sens. 1	H302 H414 H317 H334	H302 H414 H317 H334	Acute Tox. 4* Eye Irrit. 2 STOT SE 3[4]	H302 H319 H335[4]	H302 H319 H335	No harmonised classification and labelling		
Further classifications /subclassifi-cations/ labelling proposed by DS	Eye damage 1 Skin Sens 1A STOT RE 1[2] STOT RE 2[3]	H318 H317 H372[2] H373[3]	H318 H317 H372[2] H373[3] EU H071	Resp. Sens. 1 Skin Sens. 1 Eye damage 1 Skin Corr. 1	H334 H317 H318 H414	H334 H317 H318 H414	**C&L inventory main selfclassifi-cation(s)** Eye Dam. 1 Eye Irrit. 2 Skin Irrit 2 STOT SE 3[4]	H318 H319 H315 H335[4]	H318 H319 H315 H335

[1]CLP Regulation (EG) Nr. 1271/2008, Annex VI (Tabelle 3.1.)
[2]H372: Causes damage to the respiratory tract through prolonged or repeated exposure
[3]H373: May cause damage to kidney through prolonged or repeated exposure
[4]H335: May cause respiratory irritation

References to Annex III:

Aptula A.O., Roberts D.W., Mechanistic Applicability Domains for Nonanimal-Based Prediction of Toxicological End Points: General Principles and Application to Reactive Toxicity, Chem. Res. Toxicol. 2006, 19(8), 1097-1105.

Carpenter CP, Smyth HF Jr (1946). Chemical burns of the rabbit cornea. Am J Ophthalmol 29: 1363-72.

Kim, J. H., Gibb, H. J., Iannucci, A (2009). Concise International Chemical Assessment Document 75. Cyclic acid anhydrides: Human Health Aspects. Owner company: World Health Organisation

OECD, 2011. Report of the expert consultation on scientific and regulatory evaluation of organic chemistry mechanism-based structural alerts for the identification of protein-binding chemicals. Series on Testing and Assessment No. 139. ENV/JM/MONO(2011)9

Roberts D.W., Aptula A.O., Patlewicz G., Electrophilic Chemistry Related to Skin Sensitization. Reaction Mechanistic Applicability Domain Classification for a Published Data Set of 106 Chemicals Tested in the Mouse Local Lymph Node Assay, Chem. Res. Toxicol. 2007, 20(1), 44-60.

Roberts D.W., Patlewicz G., Kern P.S., Gerberick F., Kimber I., Dearman R.J., Ryan C.A., Basketter D.A., Aptula A.O., Mechanistic Applicability Domain Classification of a Local Lymph Node Assay Dataset for Skin Sensitization, Chem. Res. Toxicol. 2007, 20(7), 1019-1030.

ANNEX IV Solubility and behavior of maleic and succinic anhydride in different media

Hydrolysis and water solubility of anhydrides

Hydrolysis

Based on the low molecular weights and the high proportions of polar groups, maleic anhydride and succinic anhydride are soluble in polar media. Both anhydrides do not persist in water as protic media, are hydrolysed rapidly and form the corresponding acids- maleic and succinic acid. Based on the rate of polar elements per molecular weight and the protic/ionic nature of the acids, the hydrolysis products reveal even higher affinities for water than the anhydrides.

Half-lives in the range of a few minutes at 25°C and neutral pH are reported for hydrolysis of cyclic anhydrides (see table 1 below).

Using EPISUITE (v.4.1, model HYDROWIN v2.00) for the prediction of hydrolysis rates numerous studies are listed and several half-lives for various anhydrides at 25°C and neutral pH are indicated (Bunton et al, 1963, Bunton & Fendler, 1965, Hawkins, 1975) (summarised in Table below). A half-life of 4.4 min is reported for succinic anhydride. The structurally most similar anhydrides also reveal similar half lives in the range of a few minutes.

Table 1: Reported half-lives of structural similar anhydrides

Anhydride	Half-life
Acetic anhydride	4.3 min
Glutaric anhydride	4.4 min
Phthalic anhydride	1.5 min
Succinic anhydride	4.4 min

Based on registration data provided by the registrants, half-life of succinic anhydride was measured to be 5 min during a method validation study (Leslie and Mosel, 2010). This is in accordance with the measured value provided by EPISUITE 4.1.

Referring to registration data of maleic anhydride, a half-life of 0.3 min is reported (Bunton, C. A. et al. 1963). Although this is significantly faster than the hydrolysis of succinic anhydride, both anhydrides are considered to be transformed fast in the range of minutes. Explanation for differences and structural parameters for the hydrolysis of cyclic anhydrides are provided by Eberson and Landström (1972). The higher hydrolysis rate of maleic anhydride is explained as a result of ring strain, or as being due to activation of one carbonyl group for nucleophilic attack by electronic relay through the double bond. The authors expect ring to be the predominant factor based on their observations. The measured half-lives of the anhydrides provided by the registrants (0.3 min for maleic anhydride and 5 min for succinic anhydride) are also supported by the measured rate constants indicated for these substances in the same study.

Water solubilities

As the anhydrides are not stable and degrade fast in aqueous media, water solubilities for the substances as such cannot be derived. Therefore, the water solubilities of the acids are often reported instead or results refer to measurements under non-equilibrium conditions, when hydrolysis is still ongoing. Furthermore, as water solubilities of acids are also pH-dependent, various different values are found in the literature.

The water solubilities of maleic anhydride and succinic anhydride are sometimes described qualitatively to be moderate or even low for succinic anhydride. These estimations are referred to full miscibility. 478,8 g/L for maleic acid and 62,9 g/L for succinic acid might be considered to be moderate or low in comparison to full miscibility. Nevertheless referring to physiological and environmental relevant concentrations, the water solubilities of the acids are high in comparison to other organic compounds.

In conclusion, maleic anhydride and succinic anhydride are considered to be hydrolysed fast and fully in the range of minutes in aqueous media. The formed acids reveal high water solubilities. Referring to the hydrolytic half-levels of other anhydrides, the same order of magnitude is observed.

Solubilities and stability in other media than water

The following solubilities for maleic anhydride in various solvents are found:

Table 2: Solubility of maleic anhydride in solvents*

Solvent	Solubility at 25°C [g/kg]
Acetone	2270
Ethyl acetate	1120
Chloroforme	525
Benzene	500
Toluene	234
o-xylene	194
Carbon tetra chloride	6
Ligroin	2,5
Dioxane	soluble
Ethanol	soluble with ester formation

* O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001., p. 1020
http://pubchem.ncbi.nlm.nih.gov/compound/maleic_anhydride#section=Flash-Point

Table 3: Solubility of succinc anhydride in solvents*

Solvent	Solubility at 25°C [g/L]
Ethanol	25,6
Ether	6,4
Chloroforme	8,7

*Furia, T.E. (ed.). CRC Handbook of Food Additives. 2nd ed. Cleveland: The Chemical Rubber Co., 1972., p. 233 (available from:
http://pubchem.ncbi.nlm.nih.gov/compound/7922#section=Flash-PointI)

Regarding non-protic/non-aqueous media the anhydrides are expected to be stable and not to hydrolyse. They are dissolved depending on the solubility in these media. Protic media like water or alcohols react or can react with the anhydrides.

Using QSAR-model KOWWIN Program (v1.68) (EPISUITE 4.10), a log KOW of 1.6187 (KOW ≈ 41.6) is predicted for maleic anhydride and a log KOW of 0.8102 (KOW ≈ 6.5) for succinic anhydride.
Taking the definition of KOW into account, this means that maleic anhydride is considered to be 41.6 times more soluble in n-octanol than in water, whereas succinic anhydride is predicted to be

only 6.5 times more soluble in n-octanol than in water. Nevertheless, it needs to be considered that the QSAR-predicted estimates for (log) KOW exist only in theory for both anhydrides, as they are not stable in water and might potentially also react with n-octanol (protic media forming esters).

Nevertheless, referring to these theoretical QSAR-estimates, it is also predicted that maleic anhydride has a higher affinity for/solubility in the same non-polar media than succinic anhydride (solvent: n-octanol in this case), as demonstrated in the measured values provided in the tables given above (table 2 and table 3).
The solubilities decrease if the polarity of the solvent is lowered. Whereas, maleic anhydride still reveals comparatively high solubilities in non-polar media, the solubility of succinic anhydride is significantly lower in the same solvent. Therefore, maleic anhydride might be still dissolved fully in a non-polar media like oil (molecules revealing high molecular weights and low content of polar elements) as vehicle, whereas more polar vehicles might be necessary for ensuring full solvation of succinic anhydride like propylene glycol or dimethylformamide as used in the studies performed (for details of vehicles used and the behaviour of the anhydride see respective chapters).

In conclusion, maleic anhydride and succinic anhydride are considered to reveal significant solubilities in other solvents than water. As a general rule, substances reveal highest solubilities in media revealing same or similar polarities than the substance itself. Referring to the indicated solvents, maleic anhydride is demonstrated to be more soluble than succinic anhydride in the same solvent. It can be reasonable considered that the anhydrides are dissolved sufficiently in solvents used for the toxicity studies (e.g., oil) and are stable in non-protic media.

References:

Bunton, C. A., Fuller, N. A., Perry, S. G., and Shiner, V. J (1963). The hydrolysis of carboxylic anhydrides. Part III. Reactions in initially neutral solutions. J. Chem. Soc. 542, pp. 2918-2926.

Bunton, C. A., Fendler J. H., Fuller N. A., Perry S. and Rocek J., The hydrolysis of carboxylic anhydrides. Part VI. Acid hydrolysis of cyclic anhydrides. J. Chem. Soc., 1965, 6174-6180

Eberson, L.; Landström, L., Studies on Cyclic Anhydrides. IV. Rate Constants for the Hydrolysis of Some Cyclic Anhydrides Exhibiting Ring Strain. acta.chem.scand.27-1159, 1972, pp 1159-1161.

Hawkins M. D., Hydrolysis of phthalic and 3,6-dimethylphthalic anhydrides, J. Chem. Soc., Perkin Trans. 2, 1975, 282-284

Leslie, S and Moseley, R. H. (2010). Succinic Anhydride: Development and Validation of an Analytical Method, and Evaluation of Hydrolysis. Testing laboratory: Covance Laboratories Limited. Report no.: 8225318. Owner company: DSM Fine Chemicals GmbH. Report date: 2010-08-27.

OECD, 2004. Maleics: Maleic Anhydride and Maleic Acid. SIAM 18, 20-23 April (available at: http://webnet.oecd.org/hpv/ui/ChemGroup.aspx)

U.S. EPA (2009) Harzard characterization Document. Screening level hazard characterisation. Cyclic anhydrides categories.