Committee for Risk Assessment

RAC

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

Adopted

16 September 2016

16 December 2016

CLH-O-0000001412-86-123/F

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

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

Chemical name: **succinic anhydride**

EC Number: **203-570-0**

CAS Number: **108-30-5**

The proposal was submitted by **Austria** and received by RAC on **30 September 2015.**

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

PROCESS FOR ADOPTION OF THE OPINION

Austria has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **9 December 2015**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **25 January 2016**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Anne-Lee Gustafson**

Co-Rapporteur, appointed by RAC: **Bert-Ove Lund**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **16 September 2016** by **consensus**.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	607-103-00-5	succinic anhydride	203-570-0	108-30-5	Acute Tox. 4* Eye Irrit. 2 STOT SE 3	H302 H319 H335	GHS07 Wng	H302 H319 H335		* Eye Irrit. 2; H319: C ≥ 1% STOT SE 3; H335: C ≥ 1%	
Dossier submitters proposal	607-103-00-5	succinic anhydride	203-570-0	108-30-5	**Retain** STOT SE 3 **Add** Resp. Sens. 1 Skin Sens. 1 Skin Corr.1 **Modify** Acute Tox. 4 Eye Dam. 1	**Retain** H335 **Add** H334 H317 H314 **Modify** H318	**Retain** GHS07 Wng	**Retain** H335 H302 **Add** H334 H317 H314 **Remove** H319		**Retain** STOT SE 3; H335: C ≥ 1 % **Remove** Eye Irrit. 2; H319: C ≥ 1% *	
RAC opinion	607-103-00-5	succinic anhydride	203-570-0	108-30-5	**Add** Resp. Sens. 1 Skin Sens. 1 Skin Corr.1 **Modify** Acute Tox. 4 Eye Dam. 1 **Remove** STOT SE 3	**Retain** H302 **Add** H334 H317 H314 H318 **Remove** H335	**Add** GHS08 GHS05 Dgr **Remove** GHS07 Wng	**Retain** H302 **Add** H334 H317 H314 **Remove** H335	**Add** EUH071	**Remove** STOT SE 3; H335: C ≥ 1 % Eye Irrit. 2; H319: C ≥ 1% *	
Resulting Annex VI entry if agreed by COM	607-103-00-5	succinic anhydride	203-570-0	108-30-5	Acute Tox. 4 Skin Corr.1 Eye Dam. 1 Resp. Sens. 1 Skin Sens. 1	H302 H314 H318 H334 H317	GHS08 GHS05 Dgr	H302 H314 H334 H317	EUH071		

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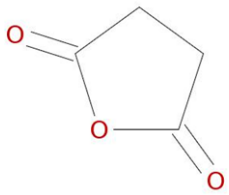
GROUNDS FOR ADOPTION OF THE OPINION

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.

HUMAN HEALTH HAZARD EVALUAION

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**.

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 (12th 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 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.

[1] 1 RTI = Respiratory tract irritation

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 removed** and that labelling with **EUH071 (corrosive to the respiratory tract)** is warranted.

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 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.

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.

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.

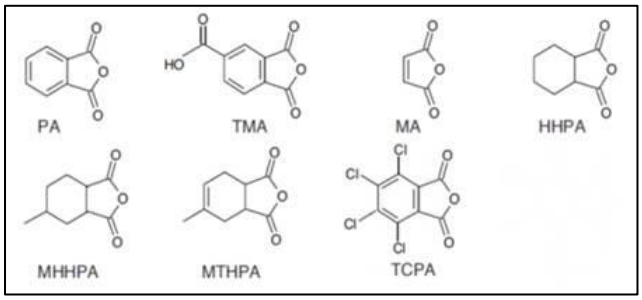
Assessment and comparison with the classification criteria

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 intradermal 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.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposal to classify succinic anhydride as Skin Sens. 1; H317 is based on the result from a local lymph node assay (Weber 2010, OECD 429 and GLP compliant).

Comments received during public consultation

Three MSCA commented on this hazard class during the public consultation. All supported the proposed classification of succinic anhydride as Skin Sens. 1.

Assessment and comparison with the classification criteria

The skin sensitising properties of succinic anhydride were examined in a standard local lymph node assay (OECD TG 429, GLP compliant) (Weber, 2010). N, N-Di-methylformamide a solvent where hydrolysis of succinic anhydride is expected to be low, was used as vehicle. A stimulation index (SI) of 9.2, 11.6 and 11.0 was recorded in the groups treated with an induction dose of 10 % (w/w), 25 % (w/w) or 31.3 % (w/w), respectively. No skin irritation effects were observed at the application sites in the test substance groups or the negative control group throughout the whole study.

The RAC notes the non-linear dose–response relationship and that all recorded SI values were above 3, therefore it is not possible to adequately calculate the concentration needed to elicit a SI value of 3 (EC3). According to the CLP Regulation (see Table 3.4.3 and 3.4.4 of Annex I), EC3 values are needed when LLNA data is used for sub-categorisation. Consequently the available data are not sufficient for sub-categorisation. The RAC concludes that succinic anhydride is a skin sensitiser, the recorded SI values (9.2 -11.6) in the available LLNA study are clearly above the cut off value, SI ≥ 3, for a significant skin sensitising effect (see Table 3.4.2-e of the Guidance of the application of the CLP criteria). According to the CLP Regulation (Annex I: 3.4.2.2.1.1), skin sensitisers shall be classified in Cat. 1 when data are not sufficient for subcategorisation. Thus, the RAC supports the proposal of the DS that classification in **Skin Sens. 1; H317** is justified for succinic anhydride.

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.

ANNEXES:

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

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