CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Maleic anhydride

EC Number: **203-571-6**

CAS Number: **108-31-6**

Index Number: **607-096-00-9**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Maleic anhydride
EC number:	203-571-6
CAS number:	108-31-6
Annex VI Index number:	607-096-00-9
Degree of purity:	confidential information
Impurities:	confidential information

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 Skin Corr. 1B, H314 Skin Sens. 1, H317 Resp. Sens. 1, H334	--
Current proposal for consideration by RAC	Removal of asterisk (*) from Acute Tox 4, H302 Eye damage 1, H318 Skin Sens 1A, H317 STOT RE 1, H372 (respiratory system) STOT RE 2, H373 (kidney) Supplementary labelling statement: EU H071	--

Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox 4, H302 Skin Corr. 1B, H314 Skin Sens. 1A, H317 Resp. Sens. 1, H334 Eye damage 1, H318 STOT RE 1, H372 (respiratory system) STOT RE 2, H373 (kidney) Supplementary labelling statement: EU H071	--

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

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. 1B, H314	--	Skin Corr. 1B, H314	Not assessed in this dossier.

3.3.	Serious eye damage / eye irritation	Eye Dam 1, H318	--	None	--
3.4.	Respiratory sensitisation	Resp. Sens. 1, H334	--	Resp. Sens. 1, H334	Not assessed in this dossier.
3.4.	Skin sensitisation	Skin Sens. 1A, H317	--	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	None	--	None	Not assessed in this dossier.
3.9.	Specific target organ toxicity – repeated exposure	STOT RE 1, H372 (respiratory system) STOT RE 2, H373 (kidney)	--	None	--
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:

Pictogram: GHS07
GHS05
GHS08

Signal word: Danger

Hazard statements: H302
H314
H317
H334
H372
H373

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

Supplementary labelling statement: EU H071

Proposed notes assigned to an entry:

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2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Maleic anhydride (Index No.: 607-096-00-9) was classified as Xn, R22 (Harmful if swallowed), C, R34 (Causes burns), R42/43 (May cause sensitization by inhalation; May cause sensitization by skin contact) in Commission Directive 98/73/EC of 18 September 1998 adapting to technical progress for the 24th 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.

2.2 Short summary of the scientific justification for the CLH proposal

Maleic 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 (CSR) 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 eye damage (Eye Dam 1) and repeated dose toxicity (STOT RE 1 (inhalative, respiratory system), STOT RE 2 (oral, kidney)). Furthermore a sub-classification of the skin sensitising properties (Skin Sens. 1A) and an additional supplementary labelling element (EU H071) is proposed. 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 an adaption of the harmonised classification 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

Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling	
				Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)
607-096-00-9	maleic anhydride	203-571-6	108-31-6	Acute Tox 4* Skin Corr. 1B Skin Sens. 1 Resp. Sens. 1	H302 H314 H317 H3334	GHS07 GHS05 GHS08 Dgr	---

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
607-096-00-9	maleic anhydride	203-571-6	108-31-6	Xn; R22 C; R34 R42/43	C R:22-34-42/43 S: (2-)22-26-36/37/39-45

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 maleic anhydride by industry are summarized in the C&L Inventory (http://echa.europa.eu/regulations/clp/cl-inventory-database).

There are 18 aggregated notifications present in the inventory and the total number of notifiers is 1780 (n=1780) (accessed August 2014).

Beside the endpoints covered by the current harmonised classification, further classifications have been included in the notifications, such as Eye Dam 1 (H318) (n=497), Acute Tox 3 (H302) (n=19), Acute Tox 1 (H330) (n=19), STOT SE 1 (H370: respiratory organ) (n=19), STOT SE 2 (H371: liver) (n=19), STOT RE 1 (H372: respiratory organ) (n=19), STOT RE 2 (H373: liver, kidney) (n=19), Flam. Liq. 3 (H226) (n=10), Eye Irrit 2 (H319) (n=1), Aquatic Chronic 3 (H412).

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

According to article 36(3) of the CLP Regulation for a substance that fulfils the criteria for other hazard classes or differentiations than those of CMR or respiratory sensitisation (Cat. 1) and the substance is not an active substance regulated under the Plant Protection Product Directive (PPPD) and Biocidal Product Directive (BPD), a harmonised classification and labelling proposal can be submitted if a justification is provided demonstrating the need for such action at Community level.

Pursuant to Article 45(4) of the REACH Regulation the Competent Authority of Austria has initiated substance evaluation for maleic anhydride. In the course of the evaluation, the evaluating MSCA noted that the current harmonised classification entry is incomplete and needs partially be modified.

Due to new evaluation and interpretation of existing human health hazard data a change of the existing entry is needed. Besides, CLP classification criteria have been modified/amended for some endpoints (e.g. acute toxicity and skin sensitisation), which has been taken into consideration in the current proposal for modification of the harmonised classification.

In the C&L inventory some notifiers have additionally to the present harmonised classification entry, classified maleic anhydride for Eye Dam. 1, STOT RE 1 (respiratory system), STOT RE 2 (kidney), however, the C&L entries are not consistent. The toxicological data provided in the

registration dossier by the lead registrant (full registration, joint submission) indicated that maleic anhydride should be classified as Eye Dam 1, STOT RE 1 (respiratory system) and STOT RE 2 (kidney). Furthermore, sub-categorisation for the skin sensitising properties into the hazard class Skin Sens 1A is warranted based on the available data. A supplementary labelling statement EU H071 (corrosive to the respiratory tract) is proposed. The current Annex VI entry for maleic 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. The outcome of the experimental oral toxicity study demonstrates that the indication of the minimum classification (*) is no longer necessary.

In the present dossier it is proposed, that the current human health classification and labelling of maleic anhydride needs to be revised and amended.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

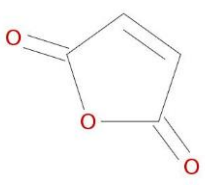
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 6: Substance identity

EC number:	203-571-6
EC name:	maleic anhydride
CAS number (EC inventory):	108-31-6
CAS number:	108-31-6
CAS name:	2,5-Furandione
IUPAC name:	furan-2,5-dione
CLP Annex VI Index number:	607-096-00-9
Molecular formula:	C_4-H_2-O_3
Molecular weight range:	98.0568

Structural formula:



1.2 Composition of the substance

Data on the composition of the substances are considered as confidential.

1.3 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 maleic anhydride.

1.4 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 Form: needles, lumps, pellets Colour: colorless or white	REACH registration (2013), HSDB (accessed August 2014)	--
Melting/freezing point	53 - 58 °C	REACH registration (2013)	--
Boiling point	200°C at 1013.25 hPa	REACH registration (2013)	--
Relative density	1.48 g/cm^3 at 20 °C	REACH registration (2013)	--
Vapour pressure	15.1 Pa (0.114 torr) at 22 °C	REACH registration (2013)	--
Surface tension	Not surface active based on chemical structure.	REACH registration (2013)	--
Water solubility	Substance hydrolyses fast. Water solubility of hydrolysis product maleic acid: 478,8 g/L at 20°C	REACH registration for maleic acid (2013)	--
Partition coefficient n-octanol/water	Substance as such hydrolyses in n-octanol/water. Log POW of hydrolysis product maleic acid: -0,48 Theoretical Log KOW of anhydride (substance as such) using KOWWIN (v1.68), EPISUITE 4.10: Log POW: 1.6187 See section 1 for explanation	EPISUITE 4.10 (accessed on July 2015)	--
Flash point	Not determined. Substance is a solid at 20 °C. The flash point of the molten substance is 100-110 °C (closed cup).	Data waived in REACH registration (2013)	--

Flammability	Non-flammable solid Based on chemical structure pyrophoric properties and flammability in contact with water are not predicted.	Data waived in REACH registration (2013)	--
Explosive properties	Non explosive. There are no chemical groups associated with explosive properties.	Data waived REACH registration (2013)	--
Self-ignition temperature	Not determined. Substance is a solid with a melting point < 160 °C.	Data waived in REACH registration (2013)	--
Oxidising properties	No oxidising properties based on chemical structure.	Data waived in REACH registration (2013)	--

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

Additional information on physico-chemical properties:

The knowledge of phys-chem parameters and the behavior of maleic anhydride under certain

Granulometry	Not determined. Substance is marketed as non-solid or granular form.	Data waived in REACH registration (2013)	--
Stability in organic solvents and identity of relevant degradation products	Not determined.	Data waived in REACH registration (2013)	--
Dissociation constant (pKa)	Maleic acid: 1.92 and 6.23 at 25°C	Ashton H.W. & Partington J.A. (1934)	--
Viscosity	Not determined as the substance is a solid.	Data waived REACH registration (2013)	--

conditions are of specific importance for the interpretation of toxicological test results. Maleic 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 calucated log POW of 1.62 for maleic anhydride, maleic anhydride is predicted to be more soluble in n-octanol than in water. These values are theoretical values, as the anhydride is hydrolyzed in water and might even form esters with n-octanol. Nevertheless, these values support the finding, that the anhydrides also reveal high solubilities in polar, organic media (polar in comparison to other organic media, but less polar than water). The solubility decrease with the reduction of the polarity of the solvent. Maleic anhydride is expected to be still 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. Further details on solubility and behavior of maleic anhydride and the structural similar succinic anhydride in different media are provided in the non-confidential Annex.

2 IDENTITY OF THE SUBSTANCE

2.1 Manufacture

Maleic anhydride has been fully registered as a joint submission in a tonnage band of 100,000 - 1,000,000 tonnes per annum and as an individual submission in a tonnage band of 1,000 - 10,000 tonnes per annum. Furthermore, a registration for intermediate use only has been submitted (ECHA dissemination website, accessed on 18th of August 2014).

2.2 Identified uses

Based on information from REACH registration (full registration, joint submission) maleic anhydride is mainly used for synthesizing e.g. unsaturated polyester resins, coatings, pharmaceutics, pesticides, lubricating-oil additives and foodstuff additives.

Maleic anhydride is registered for industrial use only, no professional and/or consumer uses are registered.

Following uses are indicated in the registration:

Table 8: Manufacture

Use as monomer for the manufacture of unsaturated polyester resins (UPR)	
Process category	PROC 3: Use in closed batch process (synthesis or formulation) PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 15: Use as laboratory reagent
Environmental release category	ERC 6c: Industrial use of monomers for manufacture of thermoplastics ERC 1: Manufacture of substances
Use as intermediate for the manufacture of chemicals	
Process category	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 15: Use as laboratory reagent
Environmental release category	ERC 1: Manufacture of substances ERC 6a: Industrial use resulting in manufacture of another substance (use of intermediates)

Table 9: Uses at Industrial Sites

Use as monomer for the manufacture of unsaturated polyester resins (UPR)	
Process category	PROC 3: Use in closed batch process (synthesis or formulation) PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 15: Use as laboratory reagent
Chemical product category	PC 32: Polymer preparations and compounds PC 19: Intermediate
Environmental release category	ERC 6c: Industrial use of monomers for manufacture of thermoplastics ERC 1: Manufacture of substances
Sector of end use	SU 0: Other: SU3 Industrial uses: Uses of substances as such or in preparation at industrial sites SU 11: Manufacture of rubber products SU 12: Manufacture of plastics products, including compounding and conversion
Use as intermediate for the manufacture of chemicals	
Process category	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 15: Use as laboratory reagent
Chemical product category	PC 19: Intermediate PC 21: Laboratory chemicals
Environmental release category	ERC 1: Manufacture of substances ERC 6a: Industrial use resulting in manufacture of another substance (use of intermediates)
Sector of end use	SU 0: Other: SU3 Industrial uses: Uses of substances as such or in preparation at industrial sites

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 10 summarises the acute oral toxicity studies, which have been submitted within the REACH registration.

Table 10: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
OECD TG 401 (Acute Oral Toxicity), equivalent or similar Test species: rat (Wistar), male/female Route: oral/gavage Test material: maleic anhydride; 99,7% Vehicle: dilution in aqua dest. (under heating treatment - 50°C) Test compound is expected to be hydrolysed and present as maleic acid. Dose: 1000 mg/kg, 1125 mg/kg, 1250 mg/kg, 1990 mg/kg Number of animals: 5 males and 5 females per group	LD_{50}: 1090 mg/kg bw (male/female)	Klimisch 1: reliable without restriction Key study	Mürmann, P. (1984)
OECD TG 401 (Acute Oral Toxicity), equivalent or similar Test species: rat (Wistar), male Route: oral/gavage Test material: maleic anhydride Vehicle: Lutrol ® Test compound is not expected to be hydrolysed in the vehicle.	LD_{50}: 1030 mg/kg bw (male)	Klimisch 2: reliable with restriction Supporting study	Löser E. (1978) cited in OECD SIDS (2004) cited in REACH registration

Dose: 800 mg/kg, 1000 mg/kg, 1010 mg/kg, 1030 mg/kg, 1060 mg/kg and 1080 mg/kg Number of animals: 10 males per group			
OECD TG 401 (Acute Oral Toxicity), equivalent or similar Test species: rat (white rats) male/female Route: oral/gavage Test material: maleic anhydride, purity: 98.5-99.3%	LD_{50}: ca. 825 mg/kg bw (male/female)	Klimisch 3 not reliable	BASF AG (1953)

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Three studies carried out with rats are presented in the REACH registration (full submission, joint registration). Of these, one was declared by the lead registrant (full registration, joint submission) as key study (Mürmann, 1984) and two further studies (Löser (1978) and BASF (1953)) has been presented as supporting studies (Löser, 1978, BASF AG, 1953). The latter one, however, is regarded as not reliable (Klimisch 3).

The study of Mürmann, 1984 (Key study) and the study of Löser (1978) are considered for classification. In the study of Mürmann (1984) the average body weights were 113 g (females) and 134 g (males). The test substance was diluted with aqua dest. and warmed to approximately 50^{o}C. Dose levels were 1000, 1125, 1250, and 1990 mg/kg. Rats were dosed at a constant volume of 10 ml/kg body weight. Animals observed for 14 days post-dosing. Room temperature of 20 ^{o}C (+1 ^{o}C) and relative humidity of 60% (+ 5%) were maintained during the study.

The dosage and the number of death animals of the study are presented in table 11.

Table 11: Number of death animals per dose group

Dose	Number of Deaths (male)	Number of Deaths (female)
1000 mg/kg	1	1
1125 mg/kg	2	4
1250 mg/kg	4	5
1990 mg/kg	5	5

In all dose groups signs of toxicity were observed. The signs of toxicity were seen in a dose dependent manner. All animals in the 1.99 g/kg dose group showed signs of toxicity, nine animals in the 1250 mg/kg dose group, six animals in the 1125 mg/kg dose group and two animals in the 1000 mg/kg dose group. The symptoms occured 15 minutes post applications and included ruffled fur, squtting posture, breathing difficulty, tremors, convulsions and glassy eyes, as well as sedation and ataxia.

An LD_{50} value of 1090 mg/kg has been deduced from the study of Mürmann (1984).

The study has been ranked as Klimisch 1 (reliable without restriction) by the registrants and the ranking is in accordance with the OECD SIDS (OECD SIDS, 2004).

It is acknowledged that maleic anhydride hydrolyses under aqueous test conditions. It can be therefore assumed that maleic acid and its sodium salt were the test materials investigated (OECD SIDS, 2004). However, taken into account that the content of the salvia of the rat is too a high percentage water and that maleic anhydride hydrolysis under physiological conditions (e.g., salvia) to maleic acid the vehicle is considered appropriate to investigate the acute toxic effects of maleic anhydride applied via gavage.

In the supporting study Maleic anhydride was administered via gavage to rats and a LD50 values of 1030 mg/kg bw (Löser, 1978) was deduced. All 10 animals/group from the 1010 mg/kg dose group up to the 1080 mg/kg dose groups showed signs of toxicity. Signs of toxicity included sedation, increased diuresis, diarrhoea and poor general condition. Rats in the 800 mg/kg dose group showed no signs of toxicity and all animals survived.

The dosage and the number of death animals of the study are presented in the table below.

Table 12: Number of death animals per dose group

Dose	Number of Deaths
800 mg/kg	0/10
1000 mg/kg	1/10 by day 4
1010 mg/kg	2/10 by days 2-4
1030 mg/kg	5/10 by days 2-6
1060 mg/kg	9/10 by days 2-7)
1080 mg/kg	10/10 dead (3 hrs to days 2-7)

The test substance was formulated in Lutrol ®. As Lutrol ® has no protic groups, maleic anhydride is not expected to be hydrolysed during preparation step and that the substance is present us such.LD_{50} values are in the same order of magnitude (LD_{50}: 1090 mg/kg bw, 1030 mg/kg bw). Common signs of toxicity include sedation and ataxia, tremor, convulsions, hypothermia, glassy eyes, staggering gait, prone position, difficulty in breathing. Post mortem examination showed hyperaemia and yellow to pale colour of the stomach and duodenum, liver congestion, pale pancreas and kidneys, as well as petechiae on liver tissue.

Numerous further oral acute toxicity studies have been carried out in the past. According to the OECD SIDS further eight oral studies with maleic anhydride under different test conditions, and also with different test vehicles (corn oil, water) were carried out with rats and one study with guinea pig. These studies are considered by the authors of the OECD report as not reliable due to limitations in the study designs and because important criteria of current standard methods are not met (Klimisch 3). Another further seven studies are not assignable according to the authors of OECD report since full study reports are not available. Although these studies have limitations, it is noteworthy, that the deduced LD_{50} values of these studies are in the same order of magnitude (appr. 400 – 1100 mg/kg bw).

4.2.1.2 Acute toxicity: inhalation

Not evaluated in this dossier.

4.2.1.3 Acute toxicity: dermal

Not evaluated in this dossier.

4.2.1.4 Acute toxicity: other routes

Not evaluated in this dossier.

4.2.2 Human information

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4.2.3 Summary and discussion of acute toxicity

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 key study is 1090 mg/kg bw and of the supporting study the LD_{50} value is 1030 mg/kg bw. Therefore, maleic anhydride needs to be classified for its acute toxicity in category 4.

Currently maleic anhydride is harmonised classified as Acute Tox 4* substance (H302). A removal of the asterix is suggested. The asterisk (*) indicating a minimum CLP classification for Acute oral Toxicity 4 is no longer necessary since the data confirm the classification.

4.2.5 Conclusions on classification and labelling

It is proposed to classify maleic anhydride as Acute Tox. 4, H302 (Removal of the asterisk $^{(*)}$).

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

Not evaluated in this dossier.

4.4 Irritation

4.4.1 Skin irritation

Not evaluated in this dossier.

4.4.2 Eye irritation/damage

Table 13: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
OECD TG 405: Acute Eye Irritation/Corrosion New Zealand Albino Rabbits Test substance: maleic anhydride (stored at room temperature 22°C) Vehicle: No vehicle used. Test substance was administered undiluted. Dose: 0.1 g of undiluted test article was placed in the everted lower lid of the right eye. Number of animals: 6 (3 female, 3 male)	Cornea score: 3.8 of max. 4 (Time point: 24/48 hrs) Iris score: 2 of max. 2 (Time point: 1/24/48 hrs) Conjunctivae score: 2.5 of max. 3 (Time point: 24/48 hrs) Chemosis score: 4 of max. 4 (Time point: 24/48 hrs) The study was terminated at day 2 due to severe adverse effects (corrosion).	Key study Reliable without restriction (Klimisch Score: 1)	IIT Research Institute (1981)
Internal standard method (BASF test). Rabbit (Vienna White) Vehicle: oil (unspecified) Test substance is not expected to be hydrolyzed in vehicle. Test substance: Maleic anhydride (purity: 98.5-99.3%) Amount applied: one drop at an interval of 5 minutes (2x), concentration: 10%, 1%, 0.5%	*10% oily solution:* Time point: 1h – 6 days; Symptoms not fully reversible within 6 weeks Symptoms: redness, swelling, corneal opacity; animal 1 died on day 6; animal 2: healing of corneal opacity with scarring *1% oily solution* Time point: 10 min - 1 day; fully reversible within: 3 days; Symptoms: redness (animal 1 and 2), swelling (animal	Reliable with restriction (Klimisch Score: 2) Supporting study experimental result	BASF AG, Department of Toxicology (1953)

and 0.1% Two animals per dose. The findings were recorded until their recovery.	1)) *0.5% oily solution:* Time point: 10 min - 3 h; fully reversible within 4 days; Symptoms: redness, swelling *0.1% oily solution:* Time point: 10 min - 3 h, fully reversible within: 24 h, Symptom: redness		
Rabbit Rabbits were used to determine the eye irritation potential of Maleic anhydride by filling the conjunctival sac of the right eye with either maleic anhydride solution (1%, 5%) or maleic anhydride as powder, and allowing it to remain in contact with the eye for two minutes, then allowing it to drain out. In the case of maleic anhydride solution vehicle not stated in the report and maleic anhydride has been applied as such in form of a powder. Number of animals: 2 per group	Corrosive Severity of effects is dose dependent (1% and 5% solution). 1% solution (n=2) - symptoms: cloudiness of the cornea, hyperemia of the conjunctiva, and edema of the nictitating membrane. Symptoms disappeared after 1 day 5% solution (n=2) - symptoms: qualitative similar but more intense, iris as well as the cornea were involved, the symptoms did not disappear until day 6 or 7 More severe effects for the powder have been observed. Powder (n=2): edema, inflammation, cloudiness of cornea, profuse whitish discharge from the affected eyes, corneal ulcer no reversibility of the symptoms (white, opaque and well vascularized corneas) within 49 days	Klimisch Score 2 (reliable with restrictions) Supporting study experimental result	Winter, C. A., and Tullius, E. J. (1950)

4.4.2.1 Non-human information

Three animal studies have been submitted in the frame of the REACH registration (full registration, joint submission) in which maleic anhydride has been applied to rabbit's eye.

The key study has been carried out according to the OECD TG 405 – Acute Eye Irritation Study (IIT Research Institute, 1981). In the study New Zealand Albino rabbits were used to determine the eye irritation potential. 0.1 g Maleic anhydride (white, crystalline solid) of undiluted test substance was placed in the lower lid of rats (three males and three females). Rats were examined 1, 24 and 48 hours following test administration.

Since no vehicle has been used, it can be assumed that maleic anhydride as such has been tested. In water for example maleic anhydride hydrolyses rapidly to maleic acid (see also chapter 1.4).

According to the study protocol, treated eyes need to be examined 72 hours after dosing. However, the study had to be terminated following 48 hours examination due to signs of severe eye damage and concerns for animal pain and discomfort.

At the 24/48 hour scoring interval, the mean corneal opacity score was 3.8 (maximum score 4), the mean iris lesion score was 2.0 (maximum score 2), the mean conjunctival erythema score was 2.5 (maximum score 3), and mean conjunctival chemosis score was 4.0 (maximum score 4).

No death occurred during the study. Two rabbits were vocal following compound administration and the ocular irritation signs were severe. Furthermore, one animal developed corneal bulging and ocular lesion, which is not addressed by the Draize criteria. The maximum irritation score according to Draize was observed after 48 hours and was 106.7 (out of a maximum of 110). The mean conjunctival erythema score at 24 and 48 hours were 2.5, respectively, the mean conjunctival chemosis score were 4.0, the corneal opacity score 3.8 and the iris lesions score were 2.0. No reversibility took place.

The following gives a detailed overview of the test results.

Table 14: Overview of test results (OECD TG 405; Draize Score) (IIT Research Institute, 1981).

Animal Nr.	Sex	Reading Time	Cornea Opacity	Iris	Conjunctiva			Total irritation score (Draize Score)
					Redness	**Chemosis**	**Discharge**	
1	M	1/24/48	4/4/4	2/2/2	2/2/3	3/4/4	3/3/3	106/108/110
2	M	1/24/48	4/4/4	2/2/2	2/2/3	3/4/4	2/3/3	104/108/110
3	M	1/24/48	4/4/4	2/2/2	2/2/3	4/4/4	3/3/3	108/108/110
4	F	1/24/48	4/4/4	2/2/2	2/2/3	4/4/4	2/3/3	106/108/110
5	F	1/24/48	4/3/3	2/2/2	2/2/3	3/4/4	3/3/3	106 /88 /90
6	F	1/24/48	4/4/4	2/2/2	2/2/3	4/4/4	3/3/3	108/108/110
Mean	**Mean**	**1/24/48**	**4/3.8/3.8**	**2/2/2**	**2/2/3**	**3.5/4/4**	**2.6/3/3**	**106.3/104.7/106.7**

The second study is a non-GLP, non-guideline conform test. Vienna white rabbits were used to determine the eye irritating effects. Different amounts of the test substance have been dropped into

the eye in an interval of 5 minutes (2x). The dose applied were 0.1, 0.5, 1, 10% oily solution of maleic anhydride. In the highest dose group severe symptoms of eye damage have been observed and no reversibility was given within a time period of eight weeks. One out of two animals died due to pneumonia on day 6. In the mid dose groups (0.5, 0.1% oily solution) no abnormalities were detected after 3-4 days of exposure. The symptoms were dose dependent and severe irreversible effects are detected in the 10% oily solution dose group. Detailed test results are depicted in the table below.

Table 15: Test results of the eye irritation test (BASF internal test) (BASF, 1953).

Concentration of test substance	Animals Nr.	Symptoms
10% oily solution	1	After 10 min: swelling After 1 hr : redness, swelling, corneal opacity After 3 hrs: corneal opacity with anaemic regions, blood discharge After 1 day: redness, swelling, corneal opacity, discharge After 4 days: redness, swelling, corneal opacity, stucked eye 6th day: animal died (pneumonia)
	2	After 10 min: swelling, teared eye After 1 hr : redness, corneal opacity After 3 hrs: stucked eye, corneal opacity, blood discharge 6th day: redness, swelling, corneal opacity, discharge After 6 weeks: healing of corneal opacity with scarring After 8 weeks: hair loss (environment of the eye)
1% oily solution	1	After 10 min: redness, swelling, teared eye, corneal opacity After 1 hr : redness, swelling, teared eye After 1 day: swelling, redness, discharge After 3 days: no abnormalities detected
	2	After 10 min: redness, teared eye After 3 hrs: redness, discharge After 3 days: no abnormalities detected
0.5% oily solution	1	After 10 min: redness After 1 hr: redness, swelling After 3 hrs: redness, swelling, discharge After 4 days: no abnormalities detected
	2	After 10 min: redness After 3 hrs: redness, swelling, discharge After 4 days: no abnormalities detected
0.1% oily solution	1	After 10 min, 1 hr, 3 hrs: redness After 24 hrs: no abnormalities detected
	2	

In the third study (Winter, 1950), the conjunctival sac of the right eye was filled with either 1% or 5% solution (vehicle not defined) for two minutes, then allowed to drain. For the 1% solution, there was cloudiness of the cornea, hyperemia of the conjunctiva and edema of the membrane within a few minutes; the eyes appeared to be normal the next morning. For 5% solution, the symptoms were qualitatively similar, but more intense irritation of the iris and cornea was observed and the symptoms were not reversed before day 6 or 7. In another experiment, a small amount of fine powder was placed in the right eye and allowed to be washed away by tears. There were two rabbits for each study (strain not mentioned). For the powder, immediate clouding of the cornea was observed. The treatment seemed painful to the animals. After 23 hours edema, inflammation, discharge from affected eyes and cloudiness of the cornea appeared. After three days, there was corneal ulcer and the affected areas of the cornea were white. The areas on the corneas treated with the powder were white, opaque and well vascularized seven weeks (49 days) after application of the test substance.

4.4.2.2 Human information

4.4.2.3 Summary and discussion of eye irritation/damage

Three individual studies have been submitted within registration. All studies carried out indicate serious eye damage after application of maleic anhydride to the eyes.

The original data of the key study was provided by the industry during substance evaluation. The study is a GLP conform and guideline comparable study (OECD TG 405 study: Acute Eye Irritation/Corrosion) and a classification with the Klimisch score 1 is justified.

The submitted supporting studies (BASF, 1953 and Winter et al., 1950) further demonstrate that maleic anhydride has severe adverse effects after application to rabbit's eyes. The applicant summaries that maleic anhydride is corrosive to the eyes and self-classifies the substance for Eye Dam 1.

4.4.2.4 Comparison with criteria

According to the CLP criteria, classification as Eye Dam 1 needs to be assigned if a substance

(1) produces effects at least in one animal of 3 in cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days;

(2) and/or a substance produces a positive response in 2 of 3 tested animals of corneal opacity ≥ 3 and/or iritis ≥ 1.5 calculated as the mean scores following grading at 24, 48 and 72 hours after application of the test material.

The results of the key study meet the criteria depicted in point two. The corneal opacity score is for all measured time points (1 hour and 48 hours) and test animal ≥ 3 and the iritis score is ≥ 1.5. For details on the study outcome see Table 13 and Table 14. A read out after 72 hours has not been performed since the study had to be terminated after the 48 hours examination due to signs of severe eye damage and concerns for animal pain and discomfort. In the key study undiluted maleic anhydride (white, crystals) were applied to the rabbits eye. The application of crystals added directly to the eye can have an impact on eye irritation and corrosion. However, eye damaging and irreversible effects have also been observed in supporting studies, in which an oily (BASF, 1953) or undefined (Winter, 1950) solution of maleic anhydride has been applied to the rabbit's eye. Therefore, the eye damaging effect can be reasonable attributed to diluted maleic anhydride and not because of the crystallinity state of undiluted maleic anhydride.

Maleic anhydride produced effects in rabbit eye which fulfil the criteria to be classified for Eye Dam. 1. Therefore, classification for Eye Dam 1 (H318: Causes serious eye damage) according to Regulation (EC) No 1272/2008 is proposed.

4.4.2.5 Conclusions on classification and labelling

Maleic anhydride causes severe damage to rabbit eyes. Therefore maleic anhydride needs to be classified as Eye Dam. 1 according to Regulation (EC) No 1272/2008.

According to article 27 of CLP Regulation all hazard statements resulting from the classification shall appear on the label, unless there is evident duplication or redundancy. Therefore, the hazard

statement H318 (Causes serious eye damage) is not proposed, since H314 (Causes severe skin burns and eye damage) is present.

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

4.4.3 Respiratory tract irritation

Data and information in this section is included to justify the addition of EUH071.

4.4.3.1 Non-human information

No data available

4.4.3.2 Human information

Table 16: Relevant human information

Method	Results	Remarks	Reference
Occupational, poisoning incident Number of subjects exposed: 7 male worker Endpoint addressed: respiratory irritation and eye irritation Test material: maleic anhydride	Maleic anhydride exposed workers suffered from symptoms, such as eye burning, wheezing, coughing.	Klimisch score 2: reliable with restrictions supporting study	Union Carbide Corp. (1949)
Occupational study Questionnaire about working conditions and symptoms with exposed, formerly exposed and unexposed workers in powder painting manufactures (incl. medical examination, which included a lung function test) Details on study design: 205 subjects of 32 enterprises participated: 93 exposed and 26 formerly exposed workers in 25 powder paint shops and 86 unexposed workers. They completed a questionnaire about working conditions and symptoms and took part in a medical examination, which included a lung function test. Urine samples, for determination of two OAAs (organic acid anhydrides), and blood samples, for analysis of specific antibodies against the OAAs, were taken. In addition, 33 paint samples were analysed for	The powder painters reported more work-related respiratory symptoms than unexposed subjects did. The prevalence of three or more symptoms was 24% in subjects with low exposure, 44% in highly exposed individuals, 46% in formerly exposed subjects, and 19% in unexposed workers. Asthma symptoms were frequent, 7%, 40%, 15% and 2%, respectively. Regression analyses of the lung volumes did not show any influence of exposure. IgG, but not IgE, against the OAAs and metabolites of OAAs were found in some subjects, but no associations with the exposure could be observed. OAAs were found in only small amounts in the paint	Klimisch score 2: reliable with restrictions weight of evidence Test material (EC name): maleic anhydride	Blomqvist A. et al. (2005)

Method	Results	Remarks	Reference
nine OAAs. Endpoint addressed: respiratory irritation Endpoint addressed: immunotoxicity	samples; maleic anhydride was not found at all. A correlation between report findings and maleic anhydride as causing agent is ambiguous. Conclusions: The exposure to organic acid anhydrides was estimated to be low, and yet, IgG antibodies to OAA were observed in some subjects. The prevalence of work-related symptoms from the eyes and the airways was relatively high among the powder painters, and these symptoms, but not the lung volumes, were clearly related to exposure. The symptoms were probably caused by irritative properties of the powder paint dust.		

In the REACH registration (full registration, joint submission) a case report study (poisoning incident) from the year 1949 (Union Carbide Corp. 1949), in which exposure to maleic anhydride lead to burning eyes, wheezing and coughing, reddened conjunctiva, eyelids evidenced blephritis with reddening and edema, has been described. No information on exposure concentration is indicated. A further report (questionnaires) carried out in 2005 also indicates that workers had adverse effects on the respiratory tract (Blomquist et al., 2005).

4.4.3.3 Summary and discussion of respiratory tract irritation

Human data (e.g. case reports, questionnaires) indicate adverse effects on the respiratory tract, such as serious coughing, reddened mucous membranes of nose and throat, work-related wheeze and breathlessness.

4.4.3.4 Comparison with criteria

Since a mechanism of toxicity is corrosivity (maleic anhydride is harmonised classified as Skin Corr. 1B, H314) malic anhydride needs also be labelled as EU H071 (Corrosive to the respiratory tract) according to the CLP Regulation, Annex II, section 1.2.6.

4.4.3.5 Conclusions on classification and labelling

Corrosive substances may be toxic after inhalation. If maleic anhydride is inhaled, a hazard to the respiratory tract exists, therefore maleic anhydride has to be supplementary labelled with EUH071 (Corrosive to the respiratory tract).

4.5 Corrosivity

The substance has a harmonised classification for its skin corrosive effects (Skin Corr. 1B, H314) effects). Additionally a supplementary labelling EU H071 (corrosive to the respiratory tract) is proposed.

4.6 Sensitisation

4.6.1 Skin sensititsation

Table 17: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Animal data			
Local lymph node assay (LLNA) OECD TG 429 (Skin Sensitisation: Local Lymph Node Assay); equal or similar Test species: mouse (Balb/c) female Vehicle: acetone/olive oil (4:1 v/v) Test compound is not expected to be hydrolysed in vehicle as solvent is not protic. Concentration: 0-2.5% (w/v) Test material: maleic anhydride Number of animals: 4 per group	Sensitising EC3 = 0.16%; w/v = 0.016 M;	Klimisch score 2: reliable with restrictions Key study	Dearman, R.J., et al (2000)
Buehler test OECD TG 406 (Skin Sensitisation), equal or similar Test species: guinea pig (Crl:(HA)BR) Induction: no data Challenge: no data Vehicle: mineral oil Concentration: Induction period: 5% (w/v);	sensitising No with positive reactions: 20 out of 20 animals Test material produced very faint to moderate erythema reactions (eight animals with scores of 0.5 and 12 animals with scores of 1.0 to 2.0). All test animals showed erythema reactions after	Klimisch score 1: reliable without restriction Supporting study	Covance (1999)

Test compound is not expected to be hydrolysed in vehicle as solvent is not protic. Challenge period (21 days after induction application): 0.5% w/v mixture in mineral oil (two weeks following the administration of the third induction. Number of animals: 20	application of 0.5% w/v mixture in mineral oil at the challenge phase.		
Skin painting test Test species: Guinea pig Induction: epicutaneous (5% and 10%) Challenge: epicutaneous (1 and 4% in Aceton 1x) Vehicle: acetone Test compound is expected not to be hydrolysed in vehicle as solvent is not protic. Test material : maleic anhydride Number of animals: 8 per group, 5 control group	Sensitising 0 out of 5 (negative control) 3 out of 8 (test group, dose: 1%); 12 days after induction 7 out of 8 (test group, dose: 5%); 7 days after induction	Klimisch score 2: reliable with restrictions Supporting study	BASF AG, Department of Toxicology (1960)
Test species: Guinea pig (Hartley) (male) Induction: no data Challenge: no data Vehicle: Dowanol DPM (Dipropylenglykolmono-methylether) / Tween 80 (Polyethylene glycol sorbitan monooleate) Test compound is not	not sensitising No. with positive reactions: 0 out of 8 (test group); dose: 5% 7 out of 8 (positive control)	Klimisch score 3: not reliable weight of evidence	Dow Chem Co (1975)

expected to be hydrolysed in vehicle as solvent is not protic. Guinea pigs were exposed to maleic anhydride to determine the skin sensitizing potential of maleic anhydride. Test material: maleic anhydride			
Human data			
Study type: cohort study with workers (n=401) Prick test Test material: acid anhydride	Thirty four out of 401 (8.8%) had work related respiratory symptoms that occurred for the first time while working with acid anhydrides and 12 (3.2%) were sensitised, with an immediate skin prick test reaction to AA-HSA conjugates.	2 (reliable with restrictions) weight of evidence	Barker R.D. et al. (1998)
Prick test, patch test, RASTS and provocation test Case report (occupational) 32-old year old process operator Test material: maleic anhydride	It was concluded that the patient had occupational allergic IgE-mediated rhino conjunctivitis and contact uritcaria from maleic anhydride.	Klimisch score 2: reliable with restrictions weight of evidence	Kanerva L. & Alanko K. (2000)
Survey Type of population: occupational	48 workers (corresponding to 25.26% of the study population) were sensitized due to application of different test substances, including maleic anhydride, with a total of 55 positive patch tests. Dermatitis was present in 22 workers, whereas 44 subjects claimed to have had skin lesions in the past. Authors found 17	Klimisch Score 2: reliable with restrictions weight of evidence	Motolese A. et al. (1993)

	positivities to specific substances: 7 to red iron oxide; 2 to antimony trioxide, manganese dioxide and maleic anhydride; and 1 to red copper oxide, cadmium chloride, vanadium pentoxide and sodium tripolyphosphate.		

4.6.1.1 Non-human information

Four experimental skin sensitisation studies with laboratory rodents have been submitted within the REACH registration (full registration, joint submission), (1) a local lymph node assay (LLNA), (2) a Buehler test, (3) a skin painting test and (4) a further test (study type not defined).

The local lymph node assay was carried out according to the OECD TG 429 (Skin Sensitisation: Local Lymph Node Assay) and was declared in the REACH registration (full registration, joint submission) to be reliable with restriction (Klimisch score 2) (Dearman, 2000).

Four female BAlb/c mice per group were exposed topically on the dorsum of both ears to 25 µl of different maleic anhydride concentrations (0, 0.1, 0.25, 0.5 %w/v) daily for three consecutive days. Five days after the initiation of exposure all mice were injected intravenously via the tail vein with 20 µCi of [3H]methyl thymidine in 250 µl PBS. After five hours the mice were killed and auricular lymph nodes were excised.

The incorporation of 3H-methyl thymidine was measured by ß-scintillation counting as disintegrations per minute (DPM). A stimulation index (SI) relative to the concurrent vehicle-treated control was derived and an EC3 value (estimated concentration of a test substance needed to produce a stimulation index of three) has been assumed. Results of the key study are summarised in Table 18.

Minor drawbacks of the key study have been identified, which are not in accordance with the OECD TG 429: 1) the study has not been carried out under GLP conditions, 2) female BALB/c strains have been used (in the OECD TG CBA/Ca strains are proposed), 3) no positive controls were used, 4) after five days and not as indicated in TG 429 after 6 days [3H]methyl thymidine was injected in the tail vein and five hours later the test animals were sacrificed.

The EC3 value was calculated by interpolating between two points on the SI axis. The estimated concentrations required for a threefold stimulation index (EC3) for maleic anhydride is 0.16% w/v (0.011 M). The stimulation was dose dependent, details are depicted in Table 18.

Table 18: Local lymph Node assay (LLNA) responses to maleic anhydride (Dearman, 2000)

Maleic anhydride concentration (%w/v)	Dpm node-1	SI
0	245	1
0.1	467	1.91
0.25	1191	4.86
0.5	1548	6.32
1	3431	14.00
2.5	3915	15.98

Another guideline comparable (OECD TG 406: Skin sensitisation) study, which is reliable without restriction according to the evaluation published in 2004 by OECD (OECD, 2004; Covance, 1999) was presented within the REACH registration (full registration, joint submission). The test material was administered as a 5% w/v mixture in mineral oil in the induction phase and as a 0.5%w/v mixture in mineral oil for the initial challenge application to 20 guinea pigs (Crl: (HA) BR).

Moderate to strong erythema reactions were elicited from all test animals during the induction phase of the study. Application of the test substance provoked very faint to moderate erythema reactions in all 20 test animals (eight animals with scores of 0.5, and 12 animals with scores of 1.0 to 2.0). Pinpoint areas of subcutaneous haemorrhage lesions were also present in two of these animals. All reactions to maleic anhydride in the test group (scores of 0.5 to 2.0) exceeded the highest reaction in the vehicle control group (score of 0.0).

There are two further studies (non-guideline studies) indicated in the REACH registration (full registration, joint submission). A skin painting study carried out by BASF (1960), which also demonstrates the sensitising potential of maleic anhydride and a study carried out by Dow Chemicals (1975), which is not reliable based on information from REACH registration (joint submission, full registration) evaluation, since it is assumed that the test substance was the hydrolyses product maleic acid and not maleic anhydride.

4.6.1.2 Human information

Human case report and/or cohort studies (Karneva, 2000, Motolese, 1993, Barker, 1998) support the findings of laboratory rodent studies and further demonstrate that maleic anhydride and/or acid anhydrides possess skin sensitising potential.

In the case study of Karneva (2000) a 32-year-old non-atopic process operator, was tested for sensitising properties of various chemicals to which he was supposed to be exposed with the Prick and Patch test. His skin symptoms were itchy wheals, 0.5-3 cm in diameter, over the entire body, including the face. Maleic anhydride – human serum albumin exposure indicated a 14-mm reaction, whereas the organic phthalic acid anhydrides were negative in the prick test. An open test on intact skin with the maleic anhydride-human serum albumin conjugate used for prick testing was negative at the 1st reading (20 min), but whealing reactions were observed after 40 min. His own maleic anhydride (1% aq.) was negative on Open testing (20 min), but when undiluted, provoked strong

whealing (20 min). This was accompanied by severe rhinitis and milder whealing outside the test area, demonstrating stage 3 of the contact urticaria syndrome. Based on the anamnestic data, prick tests, RASTs and provocation tests, it was concluded that the patient had occupational allergic IgE-mediated rhinoconjunctivits and contact urticarial due to maleic anhydride exposure.

In the study of Motolese (1993) 126 enamellers and 64 decorators from 5 factories underwent a dermatological and allergological examination. Out of these in total 190 workers 48 persons were sensitised, with a total of 55 positive patch tests. In total 20 different allergens have been tested. 17 positive patch test findings were attributed to specific substances: 7 to red iron oxide; 2 to antimony trioxide, manganese dioxide and maleic anhydride; and 1 to red copper oxide, cadmium chloride, vanadium pentoxide and sodium iripolyphosphate. 2 out of 190 workers were sensitised due to maleic anhydride exposure (corresponding to 1.05 %).

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 worker 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. Therefore, it is not possible to clarify the skin sensitising and/or respiratory potential of maleic anhydride exposure alone in the presented study.

4.6.1.3 Summary and discussion of skin sensitisation

The outcome of two guideline comparable studies with high reliability (Klimisch Score 1 and 2) demonstrates the high skin sensitising potential of maleic anhydride. Currently maleic anhydride is harmonised classified as skin sens. Cat.1. The LLNA assay demonstrates a EC3 value ≤ 2 % and the Buehler test indicates that ≥ 60 % of test animals responds at > 0,2 % to ≤ 20 % topical induction dose, which indicates that maleic anhydride is a strong to extreme skin sensitizer. Furthermore, human data confirm the results obtained from animal experiments of maleic anhydride being a skin sensitiser.

4.6.1.4 Comparison with criteria

Maleic anhydride is currently classified as Skin Sens. 1. Along with the new criteria laid down in the 2nd ATP of CLP Regulation (286/2011) sensitizers can be classified in sub-categories.

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

In the described LLNA study (Dearman et al., 2000) maleic anhydride was clearly sensitising based on the obtained SI values and an EC3 value of 0.16% w/v (0.011 M) was calculated. The EC3 value is below 2% and therefore maleic anhydride should be classified in sub-category 1A.

The criteria for classification of skin sensitizers to sub-category 1A based on the Buehler assay study is ≥ 15% responding at ≤ 0,1% topical induction dose or ≥ 60% responding at > 0.2 % to < 20% topical induction dose. The criteria for sub-category 1B is ≥ 15% to < 60% responding at > 0.2% to ≤ 20% topical induction or ≥ 15% responding at > 20% topical induction dose.

In the described Buehler study all animals (n=20, 100%) responded to the 0.5% induction dose, therefore the criteria to sub-categorise maleic anhydride into sub-category 1A are met.

Outcome of the guideline conform and/or comparable studies allow drawing conclusion on sub-classification. The studies unambiguously demonstrate, that a sub-classification as Skin Sens. 1A based on the current criteria laid down in the Regulation (EC) No 1272/2008 is warranted.

4.6.1.5 Conclusions on classification and labelling

Maleic anhydride is currently harmonised classified as Skin Sens. 1. According to the criteria laid down in the 2nd ATP of CLP (286/2011), classification as Skin Sens. 1A; H317 is proposed.

4.6.2 Respiratory sensitisation

The information relevant for this endpoint has been assessed and the conclusion was that the current harmonised classification of Maleic anhydride as Resp. Sens. 1 (H334) is justified. No sub-classification is proposed.

4.7 Repeated dose toxicity

Table 19: Summary table of relevant repeated dose toxicity studies (oral, inhalation)

Method	Results	Remarks	Reference
Oral toxicity studies			
OECD TG 408 (Repeated Dose 90-Day Oral Toxicity Study in Rodents), equivalent or similar Test species: rat (Sprague-Dawley) male/female, 15/per sex per dose level Dose: 0, 20, 40 mg/kg bw/day (nominal in diet) Dose: 0, 100, 250 and 600 mg/kg bw/day (nominal in diet)Test material: maleic anhydride Exposure: 90 days (7 days per week); The water and food was available to the animals ad libitum. Test diets were prepared using a 1% premix of maleic anhydride in ground laboratory chow (no vehicle); concentration of maleic anhydride was adjusted each week according to the mean consumption and mean body weight of each treatment group to maintain the designated dose level. Premix was analysed by GC to confirm the concentration and stability of the chemical.	NOAEL: 100 mg/kg bw/day (female) (no effects) NOAEL: 40 mg/kg bw/day (male) (no effects) LOAEL: 250 mg/kg bw/day (female) (renal changes) LOAEL: 100 mg/kg bw/day (male) (renal changes)	Klimisch Score 2: reliable with restrictions Key study	Humiston, C.G.; Frauson, L.A.; Quast, J.F., and Wade, C.E. (1975)
OECD TG 408 (Repeated Dose 90-Day Oral Toxicity Study in Rodents),	LOAEL: 250 mg/kg day (significant changes in organ weights,	Klimsch Score 2: reliable with	Humiston, C. G. and Quest, J. F.

equivalent or similar Test species: rat (Sprague-Dawley) male Dose: 0, 250, and 600 mg/kg bw/day Number of animals: 50 per group Test material: maleic anhydride Exposure: 90 and 183 days Test diets were prepared using a 10 % premix of maleic anhydride in ground laboratory chow (no vehicle); concentration of maleic anhydride was adjusted each week for the first 90 days and monthly thereafter according to the mean consumption and mean body weight of each treatment group to maintain the designated dose level. Premix was analysed by GC to confirm the concentration and stability of the chemical.	histopathological changes, renal changes)	restrictions only 2 dose levels, organ weight determination after 183 days not of all rats Supporting study	(1977)
OECD TG 416 (Two-Generation Reproduction Toxicity), equivalent or similar Test species: rat (Charles River CD rats) male/female Number of animals: 10 males and 20 females oral: gavage 0, 20, 55, and 150 mg/kg bw/day (nominal conc.) During second generation 150 mg/kg/day level was	LOAEL (systemic) (F0/F1): 20 mg/kg bw/day (male/female) (P: Macroscopic and microscopic compound-related changes in the kidneys and bladder; F1: increased absolute kidney weights of adult females NOAEL (fertility) (P/F1): 55 mg/kg/day (male/female) (No effects; highest dose tested without complete maternal	Klimisch score 2: reliable with restrictions Supporting study	Short (1982)

determined due to 100% mortality among the femalses Vehicle: corn oil Test compound is not expected to be hydrolysed in vehicle as solvent is not protic.Exposure: - P/F0: from study initiation to the end of the generation - F1: at 22 days of age and continued throughout the generation (Premating (Premating exposure period: F0 and F1, a minimum of 80 days) and throughout mating, gestation and lactation) (daily, 7 days/week) Test material: maleic anhyride	mortility) LOEL (local) (P): 20 mg/kg bw/day (male/female) (inflammatory changes in stomachs (it was not possible to conclude they were directly related to maleic anhydride)		
OECD TG 409 (Repeated Dose 90-Day Oral Toxicity in Non-Rodents), equivalent or similar Test species: dog (Beagle) male/female Dose: 0, 20, 40, or 60 mg/kg bw/day Number of animals: 4 dogs per sex per dose Test material: maleic anhydride Vehicle: peanut oil Test compound is not expected to be hydrolysed in vehicle as solvent is not protic. Test diets were prepared using a 10 % premix of	NOAEL: 60 mg/kg bw/day (male/female) Transient reduction of food consumption at the beginning of the study , and minor changes of blood parameters in male dogs	Klimisch score 2: reliable with restrictions	Braun, W., Hermann, E., Blau, G. (1975)

maleic anhydride in ground laboratory chow (supplemented with 1% peanut oil); concentration of maleic anhydride was adjusted each week for the first 90 days and monthly thereafter according to the mean consumption and mean body weight of each treatment group to maintain the designated dose level. Premix was analysed by GC to confirm the concentration and stability of the chemical. Exposure: 90 days (7 days per week, ad libitum)			
OECD TG 452 (Chronic Toxicity Studies); equivalent or similar Test species: rat (CDF (F-344) CR1BR) male/female Dose: 0, 10, 32, and 100 mg/kg bw/day Number of animals: 123-126 animals/dose group/sex Test material: maleic anhydride Vehicle: no vehicle Exposure: 2 years (7 days per week)	NOEL: 10 mg/kg/day (male/female) no effects LOEL: 32 mg/kg bw/day (male/female) reduced body weight	Klimisch score 2: reliable with restrictions	Procter & Gamble Company (1983)
Inhalative toxicity studies			
OECD TG 412 (Repeated Dose Inhalation Toxicity: 28/14-Day), equivalent or similar Test species: rat (CD rats) male/female Dose: 0, 0.01, 0.03, 0.1 mg/l	LOAEC (local): 0.01 mg/l air (nominal) (male/female) local effects in the respiratory system) LOAEC (systemic): 0.01 mg/l air (nominal)	Klimisch score 2: reliable with restrictions key study	Goldenthal, E.I.; Jessup, D.C., and Geil, R.G. (1979)

vapour (nominal conc.) 0, 0.012, 0.032, 0.086 mg/l vapour (analytical conc.) analytical concentration determined by GC Details on exposure conditions are provided in the confidential Annex III. Test material: maleic anhydride Exposure: 1 month (6 hrs/day; 5 days per week)	(male/female) changes in the organ weights, reduced body weight)		
Multi-species study Testspecies: rat (CD rats), hamster, monkey (rhesus) Duration: 6 months (inhalation: vapour) Dose: 0, 0.0011, 0.0033, and 0.010 mg/L (0, 0.3, 0.8, and 2.4 ppm) (analytical concentrations of total maleic (i.e ., maleic anhydride plus maleic acid)) 0, 0.001, 0.003, and 0.010 mg/L (target concentrations of maleic anhydride) Vehicle: no vehicle Test substance: Maleic anhydride Exposure: 132 to 136 days of treatment during a 6-months period (6 hrs per day, 5 days per week)	NOAEC (systemic): 0,0033 mg/L air (male/female) (temporarily reduced body weight in both sexes, not considered as adverse effect) LOAEC (systemic): 0.010 mg/L air (male/female) (reduced body weight in both sexes, increased amount of hemosiderin pigment in the red pulp from spleens of female rats) LOAEC (local): 0.0011mg/m³ air (male/female) (nasal and ocular irritations, discharges, hyperplastic and metaplastic changes in the nasal tissue, focal to-multiofocal infiltration of the nasal epithelium)	Klimisch scroe 2: reliable with restrictions Supporting study experimental result Test material (EC name): maleic anhydride	Short, R.D., Johannsen, F.R., Ulrich, C. (1988)

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Four oral repeated dose toxicity reports have been submitted within the REACH registration (full registration, joint submission): one chronic toxicity report with rats and three sub-chronic studies with rats and/or dogs. The studies were carried out equivalent or similar to the OECD TG 408 (Repeated Dose 90-Day Oral Toxicity Study in Rodents), OECD TG 452 (Chronic Toxicity Studies), and OECD TG 409 (Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents) and are considered to be reliable with restrictions (Klimisch score: 2). Furthermore the two-generation reproductive toxicity study (OECD TG 416) is considered as weight of evidence study.

Key study: Humiston et al., 1975

A sub-chronic toxicity study (90 days) of Humiston et al. (1975) was carried out with male/female Sprague Dawley rats (15 animals/sex/dose). Doses of 0, 100, 250 and 600 mg/kg bw/day via the oral route were administered to the laboratory rodents for 90 consecutive days. Because of effects in animals receiving 100 mg/kg bw day an additional study was conducted in rats maintained on diets providing 20 or 40 mg/kg/day. Maleic anhydride has been applied to Sprague-Dawley rats with the diet. The test animals had access to water and diet (containing maleic anhydride) ad libitum. . The studies were carried out according to the the OECD TG 408 (Repeated Dose 90-Day Oral Toxicity Study in Rodents) and can be categorised into Klimisch Score 2.

Parameters, which have been evaluated included clinical signs (appearance and demeanor), body weight, organ weights, gross and histopathology food consumption, , hematological and clinical chemistry evaluations, urinalysis and a urine concentration test.

In summary following overall conclusion can be drawn: at 600 mg/kg/day, increased relative/absolute kidney weights ($p<0.05$) and slight proteinuria was observed in both sexes and increased relative liver weight in males. At 250 mg/kg/day, there was increased relative/absolute kidney weights in males ($p<0.05$). Grossly observed kidney changes were seen in males fed maleic anhydride 100, 250, and 600 mg/kg/day. The changes were characterized by increased size, pale discoloration, and evidence of dilated tubules in the cortex. Microscopically, the kidneys showed varying degrees of nephrosis, being most severe in the high-dose group. These changes consisted of diffuse tubular dilatation, hypertrophy, and degeneration and regeneration of the tubular cells in the cortical portion of the nephron. A decrease in severity of these changes was observed at lower dose levels. Similar changes were observed in the kidneys of females, but were generally limited to the high-dose group and were much less severe (OECD, 2004).

Negative kidney effects were observed in male rats exposed to maleic anhydride from 100 mg/kg bw and in female rats from 250 mg/kg bw onwards, thus, the investigators conducted an additional study in which rats were maintained on diets providing lower amounts of maleic anhydride (20 or 40 mg/kg bw). No dose-related alterations of histopathological or gross-pathological findings were detected in the maleic anhydride treated rats compared to untreated rats. The only statistical significant alterations were increased relative and absolute liver and relative kidney weights in females receiving 40 mg/kg bw maleic anhydride with the diet.

Based on the aforementioned guideline comparable studies and due to renal changes (increased kidney weights, evidence of dilated tubules in the cortex, pale discoloration) which was more severe in the male animals and only observed in the highest dose groups (600 mg/kg bw/day and 250 mg/kg bw/day) in females, a sex specific NOAEL and LOAEL was determined by the registrants.

The NOAEL in this study for females is 100 mg/kg bw/day and for males 40 mg/kg bw/day. The LOAEL for females is 250 mg/kg bw/day and for males 100 mg/kg bw/ day, respectively.

Comparison with the guidance values:

Categorisation into STOT RE Cat. 2 is applicable, when significant toxic effects observed within a 90 day repeated dose study (conducted in experimental animals) are seen to occur within the guidance value ranges oral (rat) $10 < C \leq 100$ mg/kg bw/day. It has to be noted that guidance values and ranges are intended only for guidance purposes, they are according to the guidance on the application of CLP criteria[1] not intended as strict demarcation values.

The negative effects on kidneys in males in the study of Humiston et al. (1975) are observed at 100 mg/kg bw and are increasing dose-dependently.

At 100 mg/kg bw a slight but not significant increase in kidney weights was observed in males and females, a significant increase of kidney weights was observed at dose-levels of 250 mg/kg bw and 600 mg/kg bw in males and at 600 mg/kg bw in females.

At a dose level of 100 mg/kg bw in 5 out of 15 male rats renal tubular dilatation hypertrophy, degeneration of the tubular cells in the cortical portion of the nephron, which have been classified by the study autors as mild changes, have been observed. These aforementioned kidney changes were increased in severity and also by the number of affected animals in a dose dependent manner. At the 250 mg/kg bw dose level 10 out of 15 male rats were affected (5 animals: minimal changes, 4 animals: moderate changes, 1 animal severe changes). At the dose level of 650 mg/kg bw15 out of 15 male rats were affected (5 animals: minimal changes, 5 animals: moderate changes, 5: severe changes).

Supporting study: Humiston et al., 1977

A further sub-chronic toxicity study with male Sprague Dawley rats to which maleic anhydride was applied (0, 250 and 600 mg/kg bw/day) was carried out with 50 animals per dose treated for 90 or 183 days (Humiston et al., 1977).

Parameters, which have been evaluated, included clinical signs (appearance and demeanor), body weight, organ weights, gross and histopathology, food consumption, hematological and clinical chemistry evaluations, urinalysis and a urine concentration test.

No changes in appearance or demeanor were observed in any of the rats, which were observed twice a week. There was no significant difference in body weight or food consumption between treated and untreated rats. Also the haematological parameters were not significantly altered by ingestion of maleic anhydride.

The urine-analyses and the urine concentration tests (specific gravidity) did not reveal any dose related differences between treatment and control group. Small but significant decrease in the alkaline phosphatase activity for both treatment groups at 90 days and for the 250 mg/kg/day group at 183 days was detected. The significance of these decreases in alkaline phosphatase activity in relation to the other toxicological changes observed is uncertain.

There have been significant organ changes (liver, kidney, heart), which are attributed to the compound. At day 90, the relative liver weight (+ 16%) for the rats in the high-dose level and the

[1] 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; Version 4.0, November 2013

absolute/relative kidney weights for both the 250 and 600 mg/kg groups were significantly higher (+ 24% and + 65%, respectively) compared to the controls ($p<0.05$). At 183 days, there was a significant increase in the absolute and relative liver (+ 18% and +10%) and kidney weights (+ 54% and + 119%) for both treatment groups, increased relative testes, brain and heart weight (+ 5%), and decreased fasted body weights for rats at 600 mg/kg/day ($p<0.05$) (the increase in the absolute and relative weights of the liver and kidney and the relative weight of the heart are attributed to treatment).

Following changes of the kidneys of rats sacrificed at day 90 and 183 were observed: the observed changes indicated a marked accentuation of the spontaneously occurring findings seen in the control animals. The changes in the controls and treated animals included: individual tubules that were dilated and contained eosinophilic staining casts, granular degeneration of the epithelial cells lining these tubules, tubular collapse and atrophy with peritubular fibrosis, focal mononuclear inflammatory cell infiltrates, glomeruli that showed thickening of the basement membrane, thickening and epithelialization of Bowman's Capsule, and occasionally showed either focal or diffuse sclerosis of the glomerular tufts. Both tubular and glomerular changes in treated animals were more severe than in controls. In addition to the focal nature of the lesion (in controls), the tubules throughout the cortex of treated rats showed a generalized dilatation and hypertrophy. There were more degenerative tubules and tubules showing mitotic activity in treated versus controls. The degree of degenerative, hypertrophic, and regenerative changes was dose related. In many of the 600 mg/kg dose group animals, there was a marked decrease in the amount of functional tissue in the kidney. Livers of maleic anhydride-treated rats at 183 days showed changes characterized by swollen individual hepatocytes having vacuolated cytoplasm. In some of the smaller rats having decreased amounts abdominal adipose tissue, the liver contained an increased number of focal mononuclear inflammatory cell aggregates surrounded by hepatocytes showing coagulation necrosis. These rats frequently had hepatocytes with vacuolated cytoplasm. The mechanism, by which renal changes occur, i.e. primary tubular or glomerular, is not clear.

Comparison with the guidance values:

The effects seen in the first study carried out by Humiston (Humiston et al., 1975) in relation to kidney changes are verified by the second study, with had a longer study duration (Humiston et al., 1975). The study duration was 183 days. The doses tested (250 mg/kg bw and 600 mg/kg bw) are above the guidance values for STOT RE 2 classification ($5 < C \leq 50$ mg/kg bw/day for 183 day toxicity study). The LOAEL of 250 mg/kg bw/day is based on altered organ weights and on renal changes. No doses in the range of guidance values have been tested. The study is regarded as supporting study. It demonstrates that kidney changes occur in animals exposed to maleic anhydride in a dose-dependent manner.

Supporting study: Short (1982)

In the F0 generation, mortality of adult males and females was 0 to 10% in the low and mid dose groups and 65 to 70% in the high dose group. More deaths were observed in the F1 generation than in the F0 generation. As a result of poor survival, the high dose group was terminated in the F1 generation. Subsequent post-mortem examination (gross and microscopic) revealed bilateral nephrosis/pyelonephritis as the cause of death for the single 20 mg/kg/day male (only one animal died out of 30 animals died) and two 150 mg/kg/day animals (7 males and 13 females out of 30 animals died).

Morphologic evaluation of the post-mortem data (at the terminal sacrifice of F0/F1 generation) revealed that there were clear compound related morphologic changes observed in the kidney and bladder of F0 parents and possible compound related effects in the F1 generation. In the F1 generation, kidney weights were significantly increased in females from the low and mid dose group, however, there were no microscopic changes.

For animals in the F0 generation, toxicologically significant changes observed during post-mortem examination included hydronephrosis/dilated pelvis, kidneys with a mottled appearance or irregular surface and calculi in the urinary bladder. These lesions were randomly distributed among males/females in 20, 55, 100 mg/kg bw/day dosage group. Upon microscopic examination, compound related renal cortical necrosis among males and females in the highest dose group (150 mg/kg bw) was observed. Other compound related changes included hydronephrosis, chronic pyelonephritis, nephrosis, inflammation of the urinary bladder and urinary calculi. Changes were randomly distributed among animals in all dosage groups. The changes to the kidney are attributed to the maleic anhydride exposure.

The study author conclude that, dose levels of 150 mg/kg/day and less were observed to produce morphological changes in the kidney and bladder of F0 parents with similar albeit equivocal findings in F1 parents. On the basis of these results the 20 mg/kg /day level cannot be considered as a definitive no effect dose with respect to administration of maleic anhydride.

Comparison with the guidance values:

The F0 generation was terminated on week 27-32. Hence, the rats were exposed to the compound approximately 210 days. Applying the Haber´s rule the guidance values decrease by a factor of approximately 2.3. The guidance values for STOT RE2 classification for 210 day exposure are therefore 4.3 mg/kg bw < GV < 43 mg/kg bw. The kidney effects have been observed already in the lowest dose group (20 mg/kg bw/day). and had severe outcome at the highest dose (150 mg/kg bw/day).

The observation of the multi-generation study further substantiates the classification of maleic anhydride as STOT RE 2 (kidney) based on the CLP criteria, since adverse effects have been already observed in the lowest dose group. Those effects are within the guidance values.

90-day oral toxicity study-Beagles dog: Braun 1975 et al.

A 90 day oral toxicity study has been carried out with Beagle dogs (4 dogs/sex/dose) according or similar to the OECD TG 408 (Braun et al., ±). The following doses 0, 20, 40 or 60 mg/kg bw/day have been administered to the animals. Body weights and food consumption, as well as clinical parameters and signs of toxicity were recorded. No substance related adverse effects were detected. Therefore, no adverse effects were found to a dose up to 60 mg/kg bw/day. The significant increase in the mean absolute weight of the kidneys of female dogs fed the 60 mg/kg/day dose is considered to be due to the fact that these dogs were larger than the control dogs and thus had larger kidneys. The mean kidney/body weight ratios of females were not significantly different.

Furthermore, parameters which can be associated with kidney toxicity e.g. urinalysis are not significantly altered and gross pathological or histopathological observation did not indicate kidney toxicity.

In Beagle dogs no substance and dose dependent signs of kidney toxicity have been observed. Therefore, it can be assumed, that Beagles are not sensitive towards toxic effects of maleic anhydride to the kidney and it has to be acknowledged that lower dosis up to 60 mg/kg bw has been applied in the dog study in comparison to the aforementioned 90 and 183 day toxicity studies carried out with Sprague Dawley rats (Humiston, 1975, 1977).

2-year chronic toxicity study- F344 rats: Procter & Gamble Company (1983)

In a chronic toxicity study (24 month), which is comparable to the OECD TG 452 (Chronic Toxicity Studies) Fisher F344 rats received 0, 10, 32, 100 mg/kg bw/day maleic anhydride with the feed for a time period of two years (Procter & Gamble Company (1983)). The study has been carried out with a high number of animals (123-126 animals/dose group/sex). Scheduled termination time-points were at 6, 12, and 18 months with final study termination at 24 months. Clinical signs of toxicity, body weights and food consumption were monitored and extensive histo-pathological examinations were conducted. Additionally, the eyes of all animals were examined by ophthalmoscope and hematology, clinical chemistry and urine parameters were assessed in five animals/sex/dose. There was only marginal toxicity which was evidenced by small (<6%), but dose-related, decrease in body weights of male rats fed 32 and 100 mg/kg/day compared to the controls. The female rats fed 32 and 100 mg/kg/day had reduced body weights, but the reductions were smaller and of shorter duration than those observed in males. Food consumption was also slightly reduced during limited periods during the study for animals in the mid- and high-dose groups. Neurologic, ophthalmologic evaluations, haematology, clinical chemistry, cross and histopathological parameters (including the kidney) did not reveal differences between treated and control group. Due to minor but statistically significant reduced body weights in the treatment groups (32 and 100 mg/kg bw) the LOAEL was determined by the registrant(s) to be 32 mg/kg bw/day and the NOAEL was assessed as 10 mg/kg bw/day.

4.7.1.2 Repeated dose toxicity: inhalation

In the frame of REACH registration (full registration, joint submission) two repeated dose inhalation studies have been submitted: a sub-acute inhalation study carried out with rats (Goldenthal et al., 1979) and chronic multi-species toxicity study (Short et al., 1988).

Key study: Goldenthal et al., 1976

The sub-acute inhalation study (1 month/28 days) with male and female Sprague Dawley rats (10 per sex per dose) was conducted according to the OECD TG 412 (Goldenthal et al., 1979). The test animals were exposed to **0, 0.012, 0.032, 0.086 mg/l maleic anhydride** (average analytical exposure concentration determined by gas chromatography) for 6 hours per day (5 days a week). The vapours of maleic anhydride were generated by placing a known quantity of test material in powder form in a stainless steel "boat". This boat was then placed in a horizontally position vaporizer. Upon heating the vaporizer, the maleic anhydride melted and vaporized. The chamber exhaust was filtered through an activated charcoal filter and a Cambridge absolute filter before being discharged outside the laboratory. The overall quantity of maleic anhydride vaporized was determined by weighing the "boat" containing maleic anhydride prior to exposure and upon recrystallization of the compound after exposure. Chamber concentrations were monitored by the use of gas chromatography (by means of a standard curve; at least 5 times over the course of a six hour exposure). There were day-to-day variations of + 33-40% at each concentration. However, there was no over-lapping of mean exposure concentrations between groups.

Following observations and examinations were performed: detailed clinical observations, body weight, food consumption, haematology, clinical chemistry, urinalysis, gross pathology and histopathology.

Details of the results (indicated in REACH registration (full registration, joint submission):

Clinical signs: At the 0.086 mg/l level, the rats exhibited reddish ocular discharge, salivation and nasal discharge along with periodic episodes of nasal bleeding and marked respiratory distress bordering on gasping during exposure in the chamber. Upon removal from the chamber atmosphere, these symptoms disappeared except for nasal discharge.

Body weight and weight gain: All maleic anhydride exposed groups showed decreased body weight directly related to exposure level (low dose group: -3% to -10%.; middle dose group: -3% to -13%; high dose group: -3% to -30%)

Food consumption: Food consumption was significantly lower, relative to the control group, for the female rats exposed to the intermediate (-15% to -27%) and the high concentrations (-13% to -26%) of the compound. The food consumption of the male rats was, in general, not affected.

Opthalmosopic examination: Keratitis and/or corneal vascularization in several rats from the 0.086 mg/l also were probably compound related. Eyes from the intermediate and low dose were not examined microscopicly.

Haematology: Percentage of neutrophils was noticeably higher for the female rats in the high exposure group in comparison to the control group (35% compared to 16%); the percentage of eosinophils was also slightly higher for the high exposure group relative to the control group (these differences indicate a statistical significance but were not considered to be of physiological significance since the values fall within the normal physiological range).

Urinalysis: The mean volume of urine excreted by the female rats in the high exposure group was significantly lower than that of the control group. The pH of the urine was lower for the exposed rats relative to the control rats. However, only the mean pH value for the male rats was statistically significant different (-15%). Occult blood was observed in 3 males and 2 females of the high dose group. No occult blood was observed in the urine of the control group.

Organ weights: There are a few significant changes in the organ weights and relative weights among the female rats of the intermediate concentration group which were probably related also to

the effects of depressed body weight gain. None of the male rats in the intermediate concentration group exhibited any changes in body to organ weight ration. For female rats in the low concentration group, only one parameter, the lung weight - body weight ratio, was statistically higher than the control. This single incidence of change was probably a result of the slightly depressed body weight.

Gross pathology: Higher incidence of hemorrhagic foci in the lunge of rats exposed to the high and intermediate concentrations as compared to the low concentration and the control groups. Dark red lung foci, adhesions, congestion, hemorrhage and localized atelectasis observed at necropsy in the lunge of several rats from the 0.086 and 0.032 mg/l groups were considered compound related.

Histopathology: Compound-related lesions occurred in the upper respiratory tract and lungs of rats from all 3 exposure levels. Findings observed in the upper respiratory tract of all three exposure groups included squamous metaplasia, inflammatory infiltrate in the mucosa of the trachea and nasal turbinates. Epithelial hyperplasia was observed in the nasal turbinates of all three treatment groups while hyperplasia of the tracheal epithelium was observed in only the high and intermediate exposure groups. Compound-related lung lesions included bronchial epithelia hyperplasia and squamous metaplasia in the high and intermediate exposure groups. Localized intraalveolar hemorrhage and presence of foamy macrophages in alveoli were noted in all three exposure groups.

Evidence of nasal and ocular irritation (concentration-dependent) occurred at all treatment levels after one month. Ocular and nasal discharge, periodic nasal bleeding and respiratory distress were seen at the highest concentration levels. Severity of effects is dose dependent.

Gross-pathological examinations demonstrate:

- No lesions in rats exposed to the lowest dose (0.012 mg/l maleic anhydride)
- Lung adhesions, focal atelectasis, dark red foci in 3 out of 10 exposed to the mid dose (0.032 mg/l)
- Atelectasis, congestion, haemorrhage of the lung (0.086 mg/l)

Results of microscopical examinations:

- Squamous metaplasia, inflammatory infiltrate in the mucosa of the trachea and nasal trubinates, epithelial hyperplasia in turbinates (all exposure groups)
- Mucosa inflammatory infiltrate, intravascular haemorrhage and presence of foamy macrophages in alveolar (all exposure groups)
- Haemorrhagic foci in the lung (dose dependent, higher in medium and high exposure group)
- Keratitis and corneal vascularisation (highest dose group)

These examinations show severe damage of the respiratory system due to inhalative exposure of maleic anhydride in all exposure groups in a dose-dependent manner. The most severe effects have been observed in the highest exposure group 0.086 mg/l.

Supporting study: Short et al., 1988

The multispecies inhalation toxicity study was carried out with CD rats (15/sex/group), Engle hamsters (15/sex/group), and Rhesus monkeys (3/sex/group) (Short et al., 1988). The animals were exposed by inhalation (whole body) to 0, 0.0011, 0,0033 and 0,0098mg/L analytical maleic (e.g., maleic anhydride and maleic acid) determined by gas chromatography for 6 hours per day (5 days a week) for a time period of 6 months (132 to 136 days).

Following parameters have been determined: body weight, food consumption, ophthalmoscopic examination, haematology, clinical chemistry, and urinalysis. Complete necropsies were conducted on all animals that died on test and on all survivors. Organ weights and organ/body weight ratios were recorded for adrenals, brain, heart, kidneys, liver, lungs, spleen, pituitary, thyroid, and gonads from all survivors. Histopathologic examinations were performed on tissues and organs from all animals in control and high-exposure groups. The tissues examined were esophagus, stomach, liver, pancreas, small intestine, large intestine, kidneys, urinary bladder, pituitary, thymus, adrenals, thyroid, parathyroids, brain, eye with optic nerve spinal cord, peripheral nerve, gonads, uterus, prostate, seminal vesicle, heart, aorta, skeletal muscle, submandibular (pharyngeal) lymph tissue, thoracic (mediastinal) lymph node, mesenteric lymph node, spleen, trachea, lung, and any other tissue with grossly observable lesions. In addition, nasal turbinate sections from all species at all dose levels were taken immediately posterior to the upper incisors. All sections of the nasal turbinates were approximately 3 to 5 mm thick and contained primarily respiratory epithelium.

Hyperplastic changes in the nasal tissues were present in the mid- and high-exposure groups and metaplastic changes in the nasal tissues were present in all exposure groups of laboratory rodents. There was some mucosal and/or sub-mucosal infiltration of neutrophils into the nasal tissues at all exposure groups and for all test species, including monkey.

The effects of maleic anhydride were more pronounced in the nasal tissue of rats and hamsters than monkeys. Dose-related signs of nasal and ocular irritation (e.g., discharge, sneezing, gasping and coughing) were observed at each test level. These species difference is explained due to the fact that rodents are obligatory nasal breathers and their nasal cavities have a greater surface area to volume ratio than primates.

There are no systemic adverse effects determined in hamsters and monkeys. Systemic adverse effects have been determined in the highest doses group (increased amounts of hemosiderin pigments in the red pulp of spleens and decreased body weight) of rats. The authors of the study conclude that continuous exposure to maleic anhydride at this level during day may produce signs of irritation, regarding systemic toxicity no adverse effects have been observed in this study up to a level of 0.0098 mg/L. The systemic adverse effects at the dose level of 0.010 mg/L mg/m^3 (LOAEC) included reduced body weight in both sexes, increased amount of hemosiderin pigment in the red pulp from spleens of female rats.

However, there have been no histological or clinical evidence of red blood cell destruction that could account for these deposits, hence the toxicological significance of these alterations is not clear.

4.7.1.3 Repeated dose toxicity: dermal

Not assessed for the present dossier.

4.7.1.4 Repeated dose toxicity: other routes

Not assessed for the present dossier.

4.7.1.5 Human information

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4.7.1.6 Other relevant information

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4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

STOT RE 2 (kidney)

Toxic effects to the kidneys were seen to occur within the guidance value range of $10 < C \leq 100$ mg/kg bw/d for classification in STOT Rep. Exp. 2 (CLP Regulation) in male Sprague Dawley rats exposed to maleic anhydride for 90 consecutive days (Humiston, 1975).

Furthermore, grossly observed kidney changes were seen in Sprague Dawley rats fed maleic anhydride 100, 250, and 600 mg/kg/day for 183 consecutive days. The changes were characterized by increased size, pale discoloration, and evidence of dilated tubules in the cortex. Furthermore, kidneys showed varying degrees of nephrosis, being most severe in the high-dose group. These changes consisted of diffuse tubular dilatation, hypertrophy, and degeneration and regeneration of the tubular cells in the cortical portion of the nephron. The severity of these changes is dose dependent. No low doses e.g. within the guidance value range have been tested in the study. (Humiston et al., 1977).

Besides, there is further evidence form a reproductive developmental toxicity studies carried out with Charles River rats, that maleic anhydride provokes kidney damage (Short, 1982).

On the other side, the 90 day study carried out with Beagles, in which maleic anhydride was applied in doses up to 60 mg/kg bw/day did not indicate adverse effects to the kidney, which might be due to the lower doses applied compared to the studies carried out with laboratory rodents. Furthermore, the outcome might be also indicative that dogs are a less sensitive species (Brown, 1975).

The chronic guideline comparable two year toxicity study does not indicate any changes in kidney parameters to doses up to 100 mg/kg bw/day. A possible explanation for this non-consistent observation in laboratory rodents might be that Sprague Dawley rats are more sensitive than F-344 rats, which were used in the chronic (two year) study or that possible adaptation mechanism took place.

Taken into account all available information regarding oral repeated dose toxicity there is evidence that maleic anhydride has an adverse effect on the kidneys in Sprague Dawley rats.

Taken into consideration all available data a classification as STOT RE 2 (oral, kidney) (H373: may cause damage to organs through prolonged or repeated exposure) according to CLP Regulation is warranted, since effects in male Sprague Dawley rats are detected within the guidance values and the results are reproducible. It is acknowledged that the effects at the lower dose concentrations are minor, however since the GV are only for guidance purposes and not strict demarcation values a STOT RE 2 classification is proposed.

STOT RE 1 (respiratory system)

In the frame of the REACH registration (full registration, joint submission) two repeated dose inhalation studies are described. A 28 day inhalative study, in which rats were exposed to different

concentrations of maleic anhydride (Goldenthal, 1979) and a 6-month multispecies study (Short et al, 1981).

In the study of Goldenthal et al. (1976) laboratory rodents at all exposure levels (0.012-0.086 mg/l) had toxic effects due to inhalative exposure. The affected organ is the respiratory system. The study of Short et al. (1981) supports the findings of Goldenthal et al. (1976).

The outcome of inhalative repeated dose toxicity study (Goldenthal et al., 1979, Short et al., 1988) warrant a classification according to criteria laid down in the CLP Regulation as STOT RE 1 (H372: causes damage to respiratory system through prolonged or repeated exposure by inhalation).

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

STOT RE 2 (kidney)

The guidance values (GVs) for STOT RE 2 and STOT RE 1 classification depending on the study duration (derived based on the application of Haber´s rule) are listed in the Table 20. In addition the submitted rat studies for repeated dose toxicity, for which different GVs apply, are listed.

Table 20: Guidance values (GVs) to assist for STOT RE 2 or STOT RE 1 (oral, rat)

Study design	**GVs for STOT RE 2**	**GVs for STOT RE 1**	**Studies presented in the present report for which GVs apply**
90 day repeated- dose study (rat)	10 mg/kg bw/day < GV ≤ 100 mg/kg bw/day	GV ≤ 10 mg/kg bw/day	Humiston et al. 1975
183 day toxicity study (rat) (Factor 2)	5 mg/kg bw/day < GV ≤ 50 mg/kg bw/day	GV ≤ 5 mg/kg bw/day	Humiston et al., 1977
210 day - F0 generation (27-32 week examination) (Factor 2.3)	4.3 mg/kg bw/day < GV ≤ 43 mg/kg bw/day	GV ≤ 4.3 mg/kg bw/day	Short, 1982
Two year toxicity oral toxicity study (rat)	1.25 mg/kg bw/day < GV ≤ 12.5 mg/kg bw/day	GV ≤ 1.25 mg/kg bw/day	Procter & Gamble Company (1983)

According to CLP Regulation classification in STOT RE 2 is applicable, when significant toxic effects observed in experimental animals occur within the guidance value range. The values are intended for guidance purposes and are not strict demarcation values.

Adverse effects have been observed in the 90 day oral toxicity study carried out with Sprague Dawley rats. In the the guideline comparable study a LOAEL of 100 mg/kg bw/day based on renal changes was deduced by the registrants (Humiston et al., 1975). Significant kidney weight alteration as well as microscopical visible kidney changes have been observed in the range of the guidance value for STOT RE 2 (10 mg/kg bw/day < GV < 100 mg/kg bw/day) but not for STOT RE 1 (GV ≤ 10 mg/kg bw/day) in male rats.

A second sub-chronic study carried out with Sprague-Dawley rats substantiates that maleic anhydride has adverse effects on the kidney. At the lowest applied dose in the study 250 mg/kg bw/day (application period 90 and 183 days) negative effects have been observed (Humiston and Quest, 1977). The LOAEL is above the GVs for STOT RE 2 (5 mg/kg bw/day < GV < 50 mg/kg bw/day) and STOT RE 1 (GV ≤ 10 mg/kg bw/day) classification. It has to be noted that only high doses have been tested and only a LOAEL and no NOAEL can be defined.

Furthermore, renal kidney changes have been also observed in the developmental toxicity study (Short, 1982). The data substantiate that repeated oral application of maleic acid provoke kidney damage.

STOT RE 1 (respiratory system)

The guidance value to assist STOT RE 2 or STOT RE 1 are listed in Table 21.

Table 21: Guidance values (GVs) to assist for STOT RE 2 or STOT RE 1 (inhalative, rat vapour)

Study design	**GV for STOT RE 2** mg/litre/6h/day	**GV for STOT RE 1**	**Studies presented in the present report for which GVs apply**
90 day repeated dose toxicity study (rat)	$0.2 < C \leq 1.0$	$C \leq 0.2$	---
28 day repeated dose toxicity study	$0.6 < C \leq 3.0$	$C \leq 0.6$	Goldenthal et al., 1976
6 month repeated dose toxicity study	$0.1 < C \leq 0.5$	$C \leq 0.5$	Short et al., 1988

According to CLP Regulation classification STOT RE 1 is applicable, when significant toxic effects observed in a 90 day repeated dose study conducted in experimental animals occur at or below the guidance value with is for the inhalative route (rat, vapour) ≤ 0.2 mg/l for 6 hours per day.

In the study of Goldenthal et al. (1979) the animals were exposed one month and thus the guidance values for 28 day study is increased by a factor of three corresponding to 0.6 mg/l for 6 hours per day. In the study the animals were exposed to very low concentrations of maleic anhydride (0.012, 0.032, 0.086 mg/l vapour). The applied concentrations are all below the guidance value for STOT RE1 for a 28 repeated dose toxicity study.

The gross pathological examinations include (1) haemorrhagic foci in the lung (dose dependent, higher in medium and high exposure group), (2) dark red lung foci, congestion, haemorrhage and localised atelectasis (medium and high exposure group), (3) squamous metaplasia, inflammatory infiltrate in the mucosa of the trachea and nasal trubinates, epithelial hyperplasia in turbinates (all exposure groups), (4) intravascular haemorrhage and presence of foamy macrophages in alveolar (all exposure groups), (5) keratitis and corneal vascularisation (highest dose group).

The severity of the effects seen in the study of Goldenthal et al. (1979) is dose dependent (e.g. haemorrhagic foci in the lung) but exposure doses are all well below the guidance value.

The outcome demonstrates that significant organ damage has been detected such as dark red lung foci, atelectasis, congestion, which lead to an impairment of the function of the organ. Thus the criteria laid down in the CLP guidance based on which classification is indicated are met (significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination and multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity).

It is stated in the guidance of the application of CLP criteria (ECHA, 2013) that substances classified as corrosive may cause severe toxicological effects following repeated exposure, especially in the lungs following inhalation exposure. It has to be evaluated in such cases whether the severe effect is a repeated exposure toxicity effect or if the effect is due to corrosivity. To distinguish between these effects the dose levels, which causes toxicity needs to be considered. It is stated in the guidance (ECHA, 2013) that if the dose is more than half an order of magnitude lower than that mediating the evident acute toxicity (corrosivity) then it could be considered to be a repeated dose toxicity effect. For maleic anhydride the repeated dose toxicity occurred at a dose level of 0.01 mg/l maleic anhydride (duration of exposure 28 days).

To investigate whether the effects seen are due to repeated exposure toxicity an acute inhalative multispecies toxicity study (4 rats, 1 cat, 1 rabbit, 1 guinea pig and 10 mice) provided via REACH registration (full registration, joint submission) is evaluated and compared to above mentioned data (BASF, 1953).

The registrant(s) declare the acute toxicity study to be reliable with restrictions (Klimisch 2). However, it has to be acknowledged that the acute inhalation toxicity of maleic anhydride is restricted to a study with limited reporting. It is indicated, that the test animals were exposed to a static maleic anhydride atmosphere of 4.35 mg/L for one hour. Vapour pressure was increased by heating up the test substance up to 56°C; the test atmosphere was considered as aerosol. No further details on exposure conditions are provided.

The outcome of the acute inhalative study demonstrates that maleic anhydride possesses potential acute inhalative toxic effects. Five (4 mice and 1 guinea pig) out of 17 animals died attributed to inhalative exposure to maleic anhydride (1 hour, 4.35 mg/l) at day 6 and day 8 after exposure. A LC50 ≥ 4,35 mg/l was assumed by the study authors. A derivation of appropriate LC50 values is not possible. Applying the Haber´s rule (time extrapolation for 4 hours) a concentration of 1.08 mg/l maleic anhydride (present as vapour in the air) would lead to adverse effects (including: inactivity, hyperpnea and sedation) and death of 5 animals out of 17 animals.

Thus, the assumed LC50 (≥ 1.08 mg/l) value is far above the LOAEL (0.001 mg/l) of the repeated dose toxicity study, therefore it can be assumed according to the ECHA guidance (ECHA, 2013), that the adverse effects are repeated dose toxicity effects.

In the repeated dose multi-species study (Short et al., 1988) rats, hamsters and monkeys were exposed (vapour, whole body) to maleic anhydride. The test animals were in a 1 cubic meter chamber and were exposed to different concentrations of maleic anhydride vapour. The concentration was determined with gas chromatography (GC).

Severe effects on the respiratory system were observed already at the lowest dose group of 0,0011 mg/m³. The laboratory rodents had hyperplastic changes in the nasal tissues in the mid- and high-exposure groups and metaplastic changes in the nasal tissues all exposure groups. There was some mucosal and/or sub-mucosal infiltration of neutrophils into the nasal tissues at all exposure groups and in all test animals. A comparison with the guidance value for a 90-day study needs to be divided into 1,5 due to difference in the exposure time, which leads to a guidance value of 0,13 mg/l for 6 hours per day. It has to be considered, that only a LOAEL can be deduced and effects at lower concentrations are not determined within the study. Therefore, for classification purposes the study of Goldenthal et al. (1979) is more appropriate since no time to time extrapolation needs to be carried out. The study of Short et al. (1988) substantiates the adverse outcome.

Due to limited study design description in the BASF study (BASF, 1953) a direct comparison of exposure conditions is difficult. It is acknowledged, that in both studies the animals were exposed to maleic anhydride vapour which was generated by heating up the test compound. In the case of the BASF study the test compound was heated up to 56°C and in the case of the study of Goldenthal et al. 1976 different voltages have been applied to heat the vaporizer.

The deduced “hypothetical” LC 50 values are higher than the chronic LOAEL of the Goldenthal study et. (1976), thus a classification for STOT RE 1 (lung) is proposed.

It is concluded, that the outcome of inhalative repeated dose toxicity study (Goldenthal et al., 1979, Short et al., 1988) warrant a classification according to criteria laid down in the regulation No 1272/2008 as STOT RE category 1 (H372: causes damage to respiratory system through prolonged or repeated exposure by inhalation).

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

STOT RE 2 (kidney)

In one oral toxicity study carried out with male Sprague Dawley rats a NOAEL (male rats) of 40 mg/kg bw has been deduced, which is lower than the guidance value of 100 mg/kg bw (oral exposure). Together with results from a two-generation study, in which adverse effects on the kidney have been observed at a concentrations of 20 mg/kg bw/day, a classification as STOT Rep. Exp. 2, H373 (may cause damage to kidneys through prolonged or repeated exposure) according to Regulation (EC) No 1272/2008 is warranted to respiratory system through prolonged or repeated exposure by oral intake).

STOT RE 1 (respiratory system)

The study outcomes of inhalative respiratory toxicity demonstrate that maleic anhydride has adverse effects to the respiratory system.

Outcomes of repeated dose toxicity studies warrant a classification according to criteria of Regulation (EC) No 1272/2008 as STOT RE 1 (H372: causes damage to respiratory system through prolonged or repeated exposure).

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

4.9 Germ cell mutagenicity (Mutagenicity)

Not evaluated for the present dossier.

4.10 Carcinogenicity

Not evaluated for the present dossier.

4.11 Toxicity for reproduction

Not evaluated for the present dossier.

4.12 Other effects

Not evaluated in the present dossier.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in the present dossier.

6 OTHER INFORMATION

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8 ANNEXES

NON CONFIDENTIAL ANNEX

Solubility and behaviour 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.

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