Committee for Risk Assessment

RAC

Annex 1

Background document

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

Sulfur dioxide

EC Number: 231-195-2
CAS Number: 7446-09-5

CLH-O-0000007055-78-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

26 November 2021

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

Sulfur dioxide

EC Number: 231-195-2

CAS Number: 7446-09-5

Index Number: 016-011-00-9

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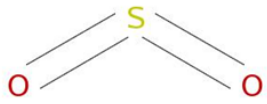
CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Sulfur dioxide
Other names (usual name, trade name, abbreviation)	Sulphur dioxide
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	231-195-2
EC name (if available and appropriate)	Sulfur dioxide
CAS number (if available)	7446-09-5
Other identity code (if available)	-
Molecular formula	SO_2
Structural formula	
SMILES notation (if available)	O=S=O
Molecular weight or molecular weight range	64.0638 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	*Not relevant*
Description of the manufacturing process and identity of the source (for UVCB substances only)	*Not relevant*
Degree of purity (%) (if relevant for the entry in Annex VI)	*Not relevant*

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
Sulfur dioxide	100 %	Press. Gas (Note U), Skin Corr. 1B, H314 Acute Tox. 3*, H331	

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
none				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
none					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	016-011-00-9	sulfur dioxide	231-195-2	7446-09-5	Press. Gas Skin. Corr. 1B Acute Tox. 3*	 H314 H331	GHS06 GHS05 GHS04 Dgr	 H314 H331		*	Note 5 Note U
Dossier submitters proposal					**Retain:** Press. Gas Skin. Corr. 1B **Add:** Skin Sens. 1 Muta. 2 STOT-SE 3 **Modify:** Acute Tox 3	**Retain:** H314 **Add:** H317 H341 H335 **Modify:** H331	**Retain:** GHS04 GHS05 GHS06 Dgr **Add:** GHS08	**Retain:** H314 **Add:** H317 H341 H335 **Modify:** H331		**Add:** Inhalation: ATE: 1041 ppmV (gases)	**Retain:** Note 5 Note U
Resulting Annex VI entry if agreed by RAC and COM					Press. Gas Acute Tox. 3 Skin Corr. 1B Skin Sens. 1 Muta. 2 STOT-SE 3	 H331 H314 H317 H341 H335	 GHS06 GHS05 GHS04 GHS08 Dgr	 H331 H314 H317 H341 H335		Inhalation: ATE: 1041ppmV (gases)	Note 5 Note U

Table 6: Reason for not proposing harmonised classification and status under public consultation

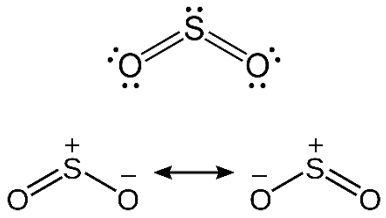
Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not applicable	No
Flammable gases (including chemically unstable gases)	Data conclusive but not sufficient for classification	Yes
Oxidising gases	Data conclusive but not sufficient for classification	Yes
Gases under pressure	Press. Gas, Note U	Yes
Flammable liquids	Hazard class not applicable	No
Flammable solids	Hazard class not applicable	No
Self-reactive substances	Hazard class not applicable	No
Pyrophoric liquids	Hazard class not applicable	No
Pyrophoric solids	Hazard class not applicable	No
Self-heating substances	Hazard class not applicable	No
Substances which in contact with water emit flammable gases	Hazard class not applicable	No
Oxidising liquids	Hazard class not applicable	No
Oxidising solids	Hazard class not applicable	No
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Hazard class not applicable	No
Acute toxicity via oral route	Hazard class not assessed in this dossier	No
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	Acute Tox 3, H331	Yes
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Hazard class not assessed in this dossier	No
Respiratory sensitisation	Data conclusive but not sufficient for classification	Yes
Skin sensitisation	Skin Sens. 1, H317	Yes
Germ cell mutagenicity	Muta. 2, H341	Yes
Carcinogenicity	Data conclusive but not sufficient for classification.	Yes
Reproductive toxicity	Hazard class not assessed in this dossier	No
Specific target organ toxicity-single exposure	STOT-SE 3, H335	Yes
Specific target organ toxicity-repeated exposure	Hazard class not assessed in this dossier	No
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Hazard class not assessed in this dossier	No
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

RAC general comment

Sulfur dioxide (SO_2) is a colourless gas with a distinctive, strong odour. It is obtained from either pyrite or sulfur by burning. SO_2 is an active substance in the context of regulation (EU) 528/2012. It has fungicidal properties and common applications including uses as a preservative in the food industry and as an antibiotic and antioxidant in winemaking. As an industrial chemical, SO_2 is primarily used for the manufacturing of sulfuric acid but also for the production of other sulfur-containing chemicals, in paper industry and in metal refining and water treatment processes.

The structure of SO_2 is shown below, but the bonding can be better described in terms of two resonance structures.



SO_2 has an entry in Annex VI of CLP (016-011-00-9) and is classified as:

- Press. Gas, Notes U and 5 in CLP
- Acute Tox. 3*, H331
- Skin Corr. 1B, H314

The Dossier Submitter (DS), taking into account that SO_2 has an existing harmonized classification, and based on the ECHA - Guidance on the preparation of dossiers for harmonised classification and labelling, v2.0 (2014), section 3.4.3.1, evaluated the endpoints of acute toxicity by inhalation, respiratory sensitisation, skin sensitisation, carcinogenicity and germ cell mutagenicity in the CLH report. The main data sources in the CLH report were:

- Competent Authority Report (2017). Sodium sulfite/metabisulfite releasing SO_2 dossier. Evaluation of active substances.
- REACH registration dossier (accessed in ECHA-REACH-IUCLID: 30 March 2017) on SO_2 (joint submission dated 13 Sep 2010) including the respective CSR.

In addition to the above sources, the *Integrated Science Assessment for Sulfur Oxides—Health Criteria (US EPA-2017)* as well as EFSA (2016) were also considered. In the reference section, the anonymous studies from the CLH report that were publicly available (published in the literature) and were also crucial for the ODD are included with

the publicly available reference, in order for the reader to be able to follow the opinion, response to comments document (RCOM) and background document (BD).

In order to evaluate the toxicological profile of SO_2, an overview of the toxicokinetics including chemistry, metabolites and the read-across assessment is presented in a separate annex.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The substance is an active substance in the meaning of regulation (EU) 528/2012 and shall normally be subject to harmonised classification and labelling.

5 IDENTIFIED USES

Sulfur dioxide is used as a fungicide in the context of BPR. Additonally, it has a broad spectrum of uses within industrial settings including winemaking, water treatment and metal purification.

6 DATA SOURCES

For the toxicological evaluation following data sources were used:

- Competent Authority Report (2017). Sodium sulfitemetabisulfite releasing sulfur dioxide dossier. Evaluation of active substances.
- REACH registration dossier (accessed in ECHA-REACH-IUCLID: 30 March 2017) on sulfur dioxide (joint submission dated 13 Sep 2010) including the respective CSR.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	colourless biting gas	Holleman-Wiberg (1995)	Literature data, information about weight of evidence assessment are given in the IUCLID dossier (If it is not stated otherwise, this comment applies to all endpoints of this table).
Melting/freezing point	-75.5 °C	Lide, D.R. (Ed.) (2007)	Literature data
Boiling point	-10 °C	Holleman-Wiberg (1995) Lide, D.R. (Ed.) (2007)	Literature data
Relative density	2.619 g/L (25 °C), gaseous	Lide, D.R. (Ed.) (2007)	Calculation of ideal gas density in grams per litre at 25°C and 101.325 kPa

Property	Value	Reference	Comment (e.g. measured or estimated)
Vapour pressure	3271 hPa at 20°C	Sax N.; Lewis R.J. 1987.	Vapour pressure is defined as the pressure exerted by a vapour above a liquid. This definition means that vapour pressure data is not relevant for sulfur dioxide because it is a gas under the physical conditions it is being used as a biocide
Surface tension			For SO_2 which is not stable in water the determination of the surface tension is technically not feasible. Sulfur dioxide dissolves in water and forms sulfurous acid.
Water solubility	ca. 22.86 g/100 g water at 101.3 kPa (0 °C, pH 0.63) (calculation) ca. 11.4 g/100 g water at 101.3 kPa (20 °C, pH 0.78 (calculation) Sulfur dioxide dissolves in water and forms sulfurous acid.	Holleman-Wiberg (1995)	
Partition coefficient n-octanol/water			As SO_2 reacts reversible with water to form sulfurous acid (H_2SO_3) the partition coefficient of SO_2 could not be determined, as in water H_2SO_3 is build.
Flash point	Not applicable		Study scientifically unjustified. Due to the fact, that sulfur dioxide is a gas, this endpoint can be waived.
Flammability	non-flammable gas	ISO 10156:2010	Study scientifically not necessary Sulfur dioxide is a non-flammable gas as described in ISO 10156:2010, see Table 1.
Explosive properties	Not applicable		Study scientifically unjustified. Testing is only applicable for solids and liquids. Therefore, gases are out of the scope of the Explosives hazard class.
Self-ignition temperature	Not relevant		Study scientifically not necessary. The study does not need to be conducted because the substance is a gas having no flammable range with air.

Property	Value	Reference	Comment (e.g. measured or estimated)
Oxidising properties	non-oxidising gas	ISO 10156:2010	Study scientifically not necessary. Sulfur dioxide is a non-oxidising gas as described in ISO 10156:2010, see Table 1.
Granulometry			Due to the fact, that sulfur dioxide is a gas, this endpoint can be waived.
Stability in organic solvents and identity of relevant degradation products			Study does not need to be conducted for inorganic substances
Dissociation constant			Sulfur dioxide will not dissociate into two or more chemical species, instead sulfur dioxide reacts reversible with water to form sulfurous acid (H2SO3), with an equilibrium constant K <<< 1.0E-9
Viscosity			Sulfur dioxide is gaseous, which is why viscosity cannot be determined.

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Hazard class not applicable. The study does not need to be conducted because the substance is a gas.

8.2 Flammable gases (including chemically unstable gases)

Flammability shall be determined by tests or, for mixtures where there are sufficient data available, by calculation in accordance with the methods adopted by ISO (see ISO 10156 as amended, Gases and gas mixtures — Determination of fire potential and oxidising ability for the selection of cylinder valve outlets).

8.2.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)

Sulfur dioxide is a non-flammable gas as described in ISO 10156:2010.

8.2.2 Comparison with the CLP criteria

Flammable gas means a gas or gas mixture having a flammable range with air at 20 °C and a standard pressure of 101,3 kPa.

8.2.3 Conclusion on classification and labelling for flammable gases

Sulfur dioxide has no flammable range with air, thus it does not require classification as flammable gas.

8.3 Oxidising gases

8.3.1 Short summary and overall relevance of the provided information on oxidising gases

Sulfur dioxide is a non-oxidising gas as described in ISO 10156:2010.

8.3.2 Comparison with the CLP criteria

Oxidising gas means any gas or gas mixture which may, generally by providing oxygen, cause or contribute to the combustion of other material more than air does.

8.3.3 Conclusion on classification and labelling for oxidising gases

Sulfur dioxide does not cause or contribute to the combustion of other material more than air does, thus it does not require classification as oxidising gas.

8.4 Gases under pressure

8.4.1 Short summary and overall relevance of the provided information on gases under pressure

The critical temperature is the temperature above which a pure gas cannot be liquefied, regardless of the degree of compression.

Critical temperature of sulfur dioxide: 157.5 °C [CHEMSAFE (2016)]

8.4.2 Comparison with the CLP criteria

Gases under pressure are gases which are contained in a receptacle at a pressure of 200 kPa (gauge) or more at 20 °C, or which are liquefied or liquefied and refrigerated. They comprise compressed gases, liquefied gases, dissolved gases and refrigerated liquefied gases.

Liquefied gas: A gas which, when packaged under pressure, is partially liquid at temperatures above – 50 °C. A distinction is made between: (i) high pressure liquefied gas: a gas with a critical temperature between – 50 °C and + 65 ° C; and (ii) low pressure liquefied gas: a gas with a critical temperature above + 65 °C.

8.4.3 Conclusion on classification and labelling for gases under pressure

Sulfur dioxide requires classification as “Gases under pressure” when put on the market in accordance with Note U. Due to the critical temperature of 157.5 °C, sulfur dioxide shall be classified as Press. Gas (Liq.), H280: Contains gas under pressure; may explode if heated.

8.5 Flammable liquids

Hazard class not applicable. The study does not need to be conducted because the substance is a gas.

8.6 Flammable solids

Hazard class not applicable. The study does not need to be conducted because the substance is a gas.

8.7 Self-reactive substances

Hazard class not applicable. The study does not need to be conducted because the substance is a gas.

8.8 Pyrophoric liquids

Hazard class not applicable. The study does not need to be conducted because the substance is a gas.

8.9 Pyrophoric solids

Hazard class not applicable. The study does not need to be conducted because the substance is a gas.

8.10 Self-heating substances

Hazard class not applicable. The study does not need to be conducted because the substance is a gas.

8.11 Substances which in contact with water emit flammable gases

Hazard class not applicable. The study does not need to be conducted because the substance is a gas.

8.12 Oxidising liquids

Hazard class not applicable. The study does not need to be conducted because the substance is a gas.

8.13 Oxidising solids

Hazard class not applicable. The study does not need to be conducted because the substance is a gas.

8.14 Organic peroxides

Hazard class not applicable. The study does not need to be conducted because the substance is a gas.

8.15 Corrosive to metals

Hazard class not applicable. The study does not need to be conducted because there is no established suitable test method for gases.

8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

Anhydrous sulfur dioxide is generally considered non-corrosive to steel and other common metals, however it reacts with atmospheric moisture and water to form corrosive acids (sulfurous acid, which will rapidly convert to sulfuric acid) and this cause rapid corrosion of some metals.

8.15.2 Comparison with the CLP criteria

Neither the corrosivity of gases nor the formation of corrosive gases is currently covered by CLP.

8.15.3 Conclusion on classification and labelling for corrosive to metals

No classification and labelling is proposed.

RAC evaluation of physical hazards

SO_2 is a gas and consequently the physical hazard classes concerning liquids and solids are not applicable and were not evaluated by the DS.

Summary of the Dossier Submitter's proposal

Flammable gases

A flammable gas is defined as a gas or gas mixture having a flammable range with air at 20 °C and a standard pressure of 101.3 kPa. SO_2 has no flammable range with air, thus it does not require classification as flammable gas (ISO 10156:2017).

Oxidising gases

An oxidising gas is defined as gas or gas mixture which may, generally by providing oxygen, cause or contribute to the combustion of other material to a greater extent than air. SO_2 does not cause or contribute to the combustion of other material more than air does, thus it does not require classification as oxidising gas (ISO 10156:2017).

Gases under pressure

The definitions as given in the CLP Regulation were followed.

SO_2 is a gas with a critical temperature of 157.5 °C and is categorized as a low pressure liquefied gas. Thus, SO_2 requires classification as "Gases under pressure" when put on the market in accordance with Note U. Due to the critical temperature of 157.5 °C, SO_2 shall be classified as **Press. Gas.**

Corrosive to metals

The hazard class is not applicable since there are no suitable test methods established for gases. However, although anhydrous SO_2 is generally considered non-corrosive to steel and other common metals, it reacts with atmospheric moisture and water to form corrosive acids (sulfurous acid, which will rapidly convert to sulfuric acid) which cause rapid corrosion of some metals. Since neither the corrosivity of gases nor the formation of corrosive gases is currently covered by CLP classes no classification was proposed by the DS.

Comments received during consultation

No comments were received for the physical hazard endpoints.

Assessment and comparison with the classification criteria

The physical hazards flammable gases, oxidising gases, gases under pressure and corrosive to metals were re-evaluated by RAC and its assessment is in full agreement with the DS section. RAC supports the DS' proposals.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Sulfur dioxide

Only non-guideline-conform study on toxicokinetics were available. As the dossier relies nearly exclusively on published literature information, the vast amount of studies deal with some aspects of toxicokinetics, but were often conducted for other/special purposes. Therefore, data from various kinds of studies were compiled to provide some information on classical toxicokinetic endpoints (absorption, distribution, metabolism, elimination).

Please also note that all references to concentration on ppm unit refers to gas volume (ppmV).

Table 8: Summary table of toxicokinetic studies

Method **Guideline, GLP status,** **Reliability**	**Species,** **Strain,** **Sex,** **No/Group**	**Test substance** **Dose levels** **Duration of exposure**	**Results**	**Remarks** (*e.g. major deviations*)	**Reference**
Sulfur dioxide					
Non-guideline; non-GLP; Reliability 2 Key study	Healthy human volunteers (age: 27 – 39 y), M, 7 in total (1 group)	SO_2, mean: 16.1±4.1 ppm (conc. of SO_2 in the mask, mean of 13 samples), 25 – 30 min exposure, **inhalation via mask**	Conc. within the nose: 13.8±3.4 ppm (n = 19 determinations); conc. in pharynx: not detectable 0.3 ppm estimated in comparison to controls. Essentially complete initial deposition in nasal mucosa, desorption and expiration via air ~15 %, estimated systemic absorption 85 %.	Temperature: 21°C, determination of SO_2 concentrations via changes in electro conductivity	Speizer and Frank 1966 Arch Environ Health 12: 725-728
Non-guideline; non-GLP Reliability 2 Key study	Rat, Sprague Dawley, M, 40-48/group	SO_2, 0, 10, 30 ppm: 6 h/d; 5 d/wk, 21 wks; measured conc.: 10.1 ± 0.3 29.9 ± 1.2 (means of daily average); Recovery period: 4 wks following treatment	Conc. of R-S-SO_3^- + SO_3^{2-} in trachea: 30 ppm: 173 ± 59 nmol/g wet weight. 30 ppm (recovery period): 65 ± 16 nmol/g wet weight. In large bronchi: 30 ppm: 156 ± 78 nmol/g wet weight. No accumulation of sulfite in plasma in rats with intact sulfit oxidase at wk 6, 14, and 21 of exposure.	Concentrations determined in lung only slightly higher than in controls – finding is in accordance with results of studies demonstrating nasopharyngeal absorption (Speizer & Frank 1966, Anonymous38, 1969, see above).	Anonymous40
Non-guideline; non-GLP Reliability 2 Key study	Rabbit, New Zealand White, F, 4/group	SO_2: Group 1: 10.3 ± 0.5 ppm SO_2 for 10 days Group 2: 9.7 ± 0.4 ppm for 3.7 days **Inhalation** exposure	Plasma concentration at equilibrium (nmol/mL): 21 – 59 (n = 8) Half-life (days; min - max): 0.8 – 8.7 mean: 3.2 ± 2.3, equilibrium at approx. 3-5 days	Exponential clearance of exogenous S-sulfonate proceeded to a conc. of approx. 10 nmol/ mL above endogenous conc. followed by a plateau for	Anonymous42

Method Guideline, GLP status, Reliability	**Species, Strain, Sex, No/Group**	**Test substance Dose levels Duration of exposure**	**Results**	**Remarks** (*e.g. major deviations)*	**Reference**
				several days – afterwards decrease to endogenous conc.	
Non-guideline; non-GLP Reliability 2 (e.g. untypical study design) Key study	Dog, no information on strain and sex, 10 per group	$^{35}SO_2$; Inhaled $^{35}SO_2$ (min – max): 7.2 – 132.3 µcurie ^{35}S **Inhalation** via tracheal cannulation	Distribution in organs % of administered ^{35}S (20 – 30 min after end of exposure; min – max): Trachea: 1.6 – 20.3 % Lung: 1.8 – 6.9 % Liver: 2.8 – 27.4 % Spleen: 0.1 – 5.4 % Kidney: 0.2 – 12.4 % Brain: 0.2 – 7.2 % Lymph nodes of pulmonary hilar: 0.003 – 1.7 %	Uncommon administration	Anonymous57
Non-guideline; non-GLP Reliability 2 (reporting de-ficiencies) Key study	Dog, Mongrel, no information on sex, 5 or 4 per group, respectively	$^{35}SO_2$; **Inhalation exposure** Group 1; 5 dogs: exposure: 22 ± 2 ppm Group 2; 4 dogs: exposure: 50 ppm, exposure duration: 30 or 60 min	Absorption: Percentage of dialyzable ^{35}S in plasma: 64.4 ± 2.3 % (SE), content of ^{35}S after precipitation: 74.7 % ± 8.8 % (SE). Blood levels increased continuously during exposure (60 min exposure) Excretion: Within a 3 hour post-exposure period, radio-activity of whole blood decreased little despite con-tinuous renal excretion of ^{35}S. An average of 84.4 % of the urinary-^{35}S was in the form of inorganic sulfate; 92.4 % was present as total sulfate. Main excretion via urine: **total ^{35}S** mean: 92.4 %, as **inorga-nic sulfate (^{35}S)** mean: 84.4 %; 7.6 % as esters of sulfuric acid; rest not determined (e.g. neutral sulfur) Protein binding predominantly to α-globulins and albumin Binding to RBC: 35.1 % ± 3.6 % (SE; in vivo) of which 2/3 intracellular, 63.6 % ± 10.3 % (SE; in vitro)		Anonymous37
Non-guideline; non-GLP, Reliability 2	Rat, Wistar, M, 4 - 16/group	Sulfur dioxide; 0; 5; 50; 100 ppm 5 h /day; 7 – 28 days	Depletion of GSH due to formation of S-sulfo-glutathione as detoxification product. GSH depleted in lung, liver, kidney and heart tissue at 5 or 100 ppm sulfur dioxide	* Rats exposed to 50 ppm SO_2 maintained tissue GSH status	Anonymous44

Method Guideline, GLP status, Reliability	**Species, Strain, Sex, No/Group**	**Test substance Dose levels Duration of exposure**	**Results**	**Remarks** (*e.g. major deviations)*	**Reference**
Key study			(sulfitolysis of GSSG to $GSSO_3^{2-}$*. Enzyme activity: Lung activity of GCS (γ-glutamylcysteine synthetase), GPx (glutathione peroxidase), GRed (glutathione reductase) and γ-glutamyltranspeptidase reduced; Liver: GRed and GPx sign. reduced.		
Non-guideline, non-GLP Reliability 2 Key study	Rabbit, NZW, M; 8 rabbits in total 1 Rhesus Monkey/ ♀;	SO_3^{2-}: Injected doses: 0.15, 0.30 and 0.60 mmol SO_3^{2-} /kg bw; Single injections	Rapid equilibration of the central and tissue compartments, Rapid distribution and elimination, rate constants: 0.1 – 1.0 /min, clearance and elimination inversely related to dose - sulfate inhibits SO *in vitro*. Sulfite distribution fits two-compartment model. Clearance by direct oxidation to sulfate – metabolic clearance: 22 mL/min/kg bw.	-	Gunnison and Palmes 1976. Toxicol. Appl. Pharmacol. 38:111-126.
Non-guideline; non-GLP, Reliability 2 inhalation whole body	Mouse (Kunming albino)/ ♂; # of animals not clearly given (3 animals / group suggested): 4 equal groups of mice 3 groups exposed to SO_2, 1 control group.	Sulfur dioxide in mg/m^3; 0) control 1) 14 ± 1.25 2) 28 ± 1.98 3) 56 ± 3.11 corresp. to 0; 5.3±0.5; 10.5±0.7; 21.1 ±1.2 ppm Exposure duration: 4 hours per day, 7 days.	Dose-dependent increases of sulfite levels in tissues µg/mg protein): **Brain:** 0): 0.174±0.008 1): 0.275±0.05 2): 0.299±0.073 3): 0.352±0.06 **Heart:** 0): 0.147±0.004 1): 0.236±0.029 2): 0.362±0.061 3): 0.397±0.062 **Lung:** 0): 0.187±0.015 1): 0.335±0.059 2): 0.354±0.018 3): 0.512±0.055	-	Anonymous45
Non-guideline; non-GLP; Reliability 2	Adult dogs, 7 in total (1 group. cross-over design), 5 evaluated, sex and strain not reported	SO_2, 1 - 50 ppm; 5 min exposure: **inhalation via mask** (nose or mouth) airways surgically isolated,	**Nose breathing**: Initial nasal retention of sulfur dioxide almost complete (99 %) in dogs irrespective of concentration (1-50 ppm) and of air flow **Mouth breathing**: Uptake by mouth: 95 % on average at 3.5 L/min; < 50 % at 35 L/min	Initial and systemic uptake of SO_2 depends on airflow and mode of breathing (mouth *vs* nose, fast or slow breathing).	Anonymous38

Method Guideline, GLP status, Reliability	**Species, Strain, Sex, No/Group**	**Test substance Dose levels Duration of exposure**	**Results**	**Remarks** (*e.g. major deviations*)	**Reference**
		Airflow: 3.5 and 35 L/min	airflow, higher desorption from respiratory tissue Mouth breathing and high flow rates (e.g. when exercising) results in increased exposure of lower airways		
Non-guideline; non-GLP; Reliability 4	Healthy male volunteers, N = 15, age not reported	SO_2, slowly increasing concentrations up to 1, 5, and 25 ppm on days 1, 2, 3 respectively, 6 h exposure **Inhalation**	< 1 % of conc. reached the oropharynx; 99 % absorption by nose. Concentration dependent decrease in mucus flow rate	SO_2 concentration in expired air not determined. Study predominantly to study respiratory function.	Andersen et al. 1974 Arch. Environ. Health 28: 31-39
Non-guideline; non-GLP Reliability 4	Rabbit, Strain not specified, A):10 in total cross-over design; B) 5 in total, cross-over design	SO_2, **inhalation** in exposure chamber – 2 methods: A (air sucked from cannulated trachea - : 100, 250 ppm B (cannulated trachea, free breathing) 100 – 200 ppm / 300 ppm	Efficient absorption of SO_2 in the upper respiratory tract: 100-200 ppm: 95 – 98 % absorption by nasal cavities 300 ppm: 51 – 86 % absorption by nasal cavities, only 2 – 5 % reached the trachea.	-	Dalhemn and Strandberg 1961; Int J Air Water Pollut 4: 154-167
Non-guideline; non-GLP Reliability 2	Rats/Wistar/ no data on sex; 10/group, 7 groups exposed.	SO_2, 40 to 750 ppm. analytical concentration: 41 ± 2 ppm, 64 ± 4 ppm, 83 ± 2 ppm, 145 ± 1 ppm, 231 ± 3 ppm, 426 ± 4 ppm and 751 ± 17 ppm.; **inhalation via mask**	Capacity to retain SO_2, inversely related to exposure concentration (range 41 to 751 ppm). Absorption at the 1 ppm level would be anticipated to be about 93 % (exponential extrapolation of retention for lower sulfur dioxide concentrations).	-	Anonymous19
Non-guideline; non-GLP, Reliability 2	Human healthy volunteers, M, 13/group (group 1: 13 non smokers, mean age: 22). 7/group	SO_2, Group 1: 12 subjects: 0, 0.3; 1.0, 3.0, ppm (cross-over design):	Plasma levels increased by 1.1 ± 0.16 nmol S-sulfonate/ ml plasma (mean + SE) for each 1-ppm SO_2 increment in chamber.	-	Gunnison and Palmes 1971

Method Guideline, GLP status, Reliability	**Species, Strain, Sex, No/Group**	**Test substance Dose levels Duration of exposure**	**Results**	**Remarks** (*e.g. major deviations*)	**Reference**
	(group 2: 7 heavy smokers, mean age: 34)	Group 1: 12 subjects 120h continuous exposure: **inhalation in chamber**: 1 subject: 3.0, 6.0 ppm 48 h exposure Group 2: 96 h continuous exposure: 0, 0.3; 1.0, 3.0 ppm;			
Non-guideline; non-GLP Reliability 2	Rabbits (New Zealand White)/ ♂; 3-11 animals/group 12 rabbits exposed to 3 ppm	SO_2, 0, 3, 10 ppm: measured conc. max. ± 5 %)	Conc. of R-S-SO_3^- + SO_3^{2-} in trachea (nmole/g dry wt) at: **10 ppm** and exposure duration (hr): 0 hr: 14±11 1 hr: 39±14 3 hr: 107±36 10 hr: 100±58 24 hr: 116±54 48 hr: 152±37 72 hr: 163±37 **3 ppm:** 3 hr: 45±17 24 hr: 61±41(n.s. from 3 hours exposure)	Several rabbits showed signs of infections and had clearly higher elevated levels of R-S-SO_3^- + SO_3^{2-} at 48 and 72 hr: 294±147 – they were evaluated separately and excluded from overall evaluation.	Anonymous46
Non-guideline; non-GLP, reliability 4	2 dogs, no information on sex and strain	$^{35}SO_2$; 15 ppm, 42.5 min exposure 33 ppm, 40 min exposure	≥90 % $^{35}SO_2$ per unit of gas-air mixture inhaled was retained in the respiratory tract 2 days following inhalative exposure. Still detectable 7 days after exposure. $^{35}SO_2$ readily excreted in urine, not detected in faeces	Exposure via tracheal tube. Adverse effects: decrease in lung compliance and increases in pulmonary resistance	Anonymous56 1960b
	1 dog	$^{35}SO_2$; 11.2 ppm, 20 min exposure	Distribution in organs 40 min following end of exposure: Trachea: 7.2 % Lung: 6.9 % Liver: 27.4 % Spleen: 5.4 % Kidney: 2.8 % Brain: 0.6 %	Dogs tracheostomied	
	1 dog	$^{35}SO_2$; 29.8 ppm, 35 min exposure	Distribution in organs 40 min following end of exposure		

Method Guideline, GLP status, Reliability	**Species, Strain, Sex, No/Group**	**Test substance Dose levels Duration of exposure**	**Results**	**Remarks** (*e.g. major deviations)*	**Reference**
			Trachea: 6.0 % Lung: 2,4 % Liver: 10.2 % Spleen: 1.0 % Kidney: 1.9 % Brain: -		
Sulfites and sulfates					
Non-guideline; non-GLP Reliability 2 Key study	Rabbit (New Zealand White), F	8.9 or 26 **$Na_2S_2O_5$** µmol/mL drinking water, corresponding to approx. 0.9 or 2 mmol sulfite/kg bw/d, corresp. to approx.. 72 or 160 mg/kg bw/d sulfite **Oral** exposure	Plasma concentration at equilibrium (nmol/mL; min - max) **at 8.9 µmol/mL**: 24 – 31 (n = 2); mean concentration at equilibrium: 28 ± 5 Half-life (days; min – max): 0.45 – 1.69 **at 26 µmol/mL**: 46 – 120 (n = 8); mean concentration at equilibrium: 82 ± 25 Half-life (days; min – max): 1.12 – 1.58 Mean half-life: 1.3 ± 0.3 days (n = 10)	Exponential clearance of exogenous S-sulfonate proceeded to a conc. of approx. 10 nmol/ mL above endogenous conc. followed by a plateau for several days – afterwards decrease to endogenous conc.	Anonymous42
	Rabbit (New Zealand White), F, 4	0.9 mmol $Na_2S_2O_5$ /kg bw **Intravenous** exposure	T_{max}: 20-40 min C_{max}: 110 – 180 nmol/mL		
Non-guideline, non-GLP Reliability 2 Key study	Human, 8 healthy male subjects, bw: 59.1 – 99.0 kg	**Sodium sulfate** (^{35}S), 60 – 80 µCi ^{35}S/mL, administered volume: 1 mL, **i.v. and p.o**.	**Volume of distribution:** **Intravenous:** 16.8±1.1 L **Oral:** 15.3±1.2 L **Excreted within 24 h:** **Intravenous:** 86.3 ± 1.8 **oral:** 79.9±2.2 **Time to reach equilibrium with C_{max}:** **Intravenous:** 60 – 90 min **Oral:** 60 – 105 min	Slow absorption from the GI tract over 10-30 min, followed by a rapid absorption phase until C_{max} is reached.	Bauer 1976 J. Appl. Physiol 40:648-650
Non-guideline, non-GLP	Rat, Wistar, F, Group 1: n=8 Group 2: n=12 4 treatment	Group 1: $Na_2S_2O_5$, 2000 ppm (as SO_2) in	Excretion in urine within 4 h following	-	Anonymous55

Method Guideline, GLP status, Reliability	**Species, Strain, Sex, No/Group**	**Test substance Dose levels Duration of exposure**	**Results**	**Remarks** (*e.g. major deviations)*	**Reference**
Reliability 2	groups a) NaCl b) $Na_2S_2O_3$ x 5 H_2O c) Na_2SO_4 x 10 H_2O d) $Na_2S_2O_5$ Group 3: n=12, as group 2 but i.p. administration	**drinking water**, Group 2: **p.o. gavage**, single dose; volume administered: 5 % of bw Group 3: **i.p.**; volume admin.: 3 % of bw; single dose urine collection 4 h following administration	**- oral** application (group 2): **sulfur (%):** a) NaCl: - b) $Na_2S_2O_3$: 23.1±3.11 c) Na_2SO_4: 7.1±1.15 d) $Na_2S_2O_5$: 55.1±6.24 **- i.p.** application (group 3) **sulfur (%):** a) NaCl: - b) $Na_2S_2O_3$: 84.9±11.7 c) Na_2SO_4: 87.7±14.16 d) $Na_2S_2O_5$: 88.6±5.29 urinary excretion predominantly as inorganic sulfate		
Non-guideline, non-GLP Reliability 2	Human ileostomied, healthy subjects 3M/3F; mean age: 60 (48 – 74 y), mean weight 63 kg (57 – 76 kg) 3 healthy subjects, age and weight not reported	**Sulfate,** dietary exposure; 1.6 – 16 mmol/d	Maximum net intestinal absorption of dietary sulfate: **Ileostomied subjects** - plateau at 5 mmol/day with dietary intakes of 7 mmol/day; **Healthy subjects**: > 16 mmol/day; Urinary excretion of sulfate correlation lineary with dietary sulfate: 97 % excreted via urine , 19.4 mmol/ day excretion from endogenous sulfate production (zero dietary sulfate) Faeces: Faecal losses of sulfate <0.5 mmol/day in the normal subjects at all doses.		Florin et al. 1991; Gut; 32:766-73
Non-guideline, non-GLP Reliability 2	Human, 5 healthy men, age: 25 – 36 y, bw: 66 – 79 kg	18.1 g **Sodium sulfate** (equiv. 8 g anhydrous sodium sulfate, single **oral dose** or 4 divided **oral** doses in hourly intervals	**Baseline sulfate excretion** (prior to external sulfate intake; min – max: **13.0±2.1 – 24.3±6.5 mmol/24 h** **Mean excretion after single dose (cumulative, % of dose):** 24 h: 36.4±15.4 48 h: 49.5±15.6 72 h: 53.4±15.8 **Mean excretion after divided doses (cumulative, % of dose):** 24 h: 43.5±12.0 48 h: 53.1±7.5 72 h: 61.8±7.8	Values are amounts of excreted sulfate minus baseline values. No radiolabelling of sulfur.	Cochetto and Levy 1981, J Pharm Sci. 70/3: 331-333

Method Guideline, GLP status, Reliability	**Species, Strain, Sex, No/Group**	**Test substance Dose levels Duration of exposure**	**Results**	**Remarks** (*e.g. major deviations)*	**Reference**
Non-guideline, non-GLP Reliability 2	Human, 18 healthy volunteers 10M/8F; age: 16- 62 y; Dog, Mongrel; 16	**H_2SO_4** (^{35}S), 20 µCi ^{35}S/mL administered volume: 1 mL, **i.v**.	**$T_{1/2,elimination}$** (average) human: 5.9 h, dog: 7.5 h **Clearance human**: 24-49 mL/min **Clearance dog**: not reported **V_D human** (18 min): 5.2 – 14.6 L **V_D dog** (25-30 min): 1.12 – 4.27 L	-	Walser et al. 1953 J. Clin. Invest. 32: 299-311
Non-guideline, non-GLP Reliability 2	Human, 33 healthy volunteers 25M/8F; age: 17- 72 y; **A)** 11 soldiers: mean age: 18.6±1.6 y **B)** 9 young male students: mean age: 26.3±3.5y **C)** 5 elderly men: mean age: 62.6±1.7 **D)** 8 elderly women: mean age: 65.3±7.8 y	**Na_2SO_4** (^{35}S), 100 µCi ^{35}S **i.v.** single dose	**V_D**: A: 13.4±1.5 L B: 12.0±2.1 L C: 10.6±1.3 L D: 10.7±1.5 L	-	Ryan et al. 1956 J Clin Invest. 35(10):1119-30
Non-guideline, non-GLP Reliability 2	Mammals (pig, sheep, bovine, horse, rat, rabbit (without further details).	No exposure, general investigation on sulfite oxidase distribution	High activities of sulfite oxidase in liver, kidney and heart of mammals (tissues with a high catabolic activity for amino acids) - very low activities in brain, spleen, lung and testis.	-	Cabré, F. et al., 1990, Biochem Medicine Metabol Biol. 43:159-62

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Sulfur dioxide:

Absorption

Inhalation is the predominant route of exposure for SO_2 as a gaseous substance. Sulfur dioxide is rapidly absorbed in the moist epithelium of the upper respiratory tract. Virtually all of the inspired sulfur dioxide was absorbed by the nasal mucosa following nasal respiratory exposure in seven healthy men. A part of approximately 15 % was subsequently desorbed and eliminated with exhaled air (Speizer and Frank 1966).

^{35}S enters the circulation from the mucosa of the upper airways. Radioactivity of whole blood decreased little during postexposure periods of up to 3 hours (Anonymous37). Anonymous57 detected ^{35}S in the airway tissues

of a dog one week after the animal had been exposed to $^{35}SO_2$ through a tracheal cannula. However, dogs not exposed via the trachea retained most of the inhaled ^{35}S in the nasal mucosa following nasal exposure and blood levels were below the limit of detection (Anonymous57).

In tracheal tissue in the rat a concentration-dependent steady-state of sulfite and S-sulphonates was reached within 6 weeks of exposure to 10 or 30 ppm sulfur dioxide in rats. Tracheal concentrations declined as exposure stopped but were still elevated after a 4-week post exposure period. S-sulphonate compounds ($RS\text{-}SO_3^-$) were not detectable in blood in this study (Anonymous40), whereas plasma S-sulphonate levels increased progressively during exposure with SO_2 until equilibrium was reached (Anonymous42; Gunnison and Palmes 1971).

Plasma contained more ^{35}S than red blood cells. During the postexposure period, plasma levels decreased slightly whereas level of ^{35}S in red blood cells increased (see also Anonymous39). Approximately one third of the plasma ^{35}S was bound to proteins (albumin; Anonymous37).

Following nasal exposure to sulfur dioxide in dogs, the blood concentrations reached its maximum at the end of the short exposure period of 38 min (Anonymous39).

Anonymous38 and Brain (1970, cited by US EPA 2007) investigated the oral and nasal absorption of SO_2 in the surgically isolated upper respiratory tract of anesthetized dogs. Radiolabelled SO_2 at concentrations of 1; 10; and 50 ppm were passed separately through mouth and nose at a flow of 3.5 and 35 L/min, respectively. Nasal absorption was complete independent of air flow, whereas oral absorption of SO_2 was 95 % on average at 3.5 L/min but only 34 % at 34 L/min. However, differences might be due to methodological variations as it was apparently difficult to fix the mask closely over the snout of the dogs.

Distribution

Absorbed sulfur dioxide metabolites are readily distributed throughout the body (distribution and elimination rate constants: 0.1 – 1.0/min; Gunnison and Palmes 1976). Clearance and elimination inversely related to dose following nasal administration (Anonymous39; Anonymous37) or exposure via tracheal cannulation (Anonymous56). In the latter investigation, radioactivity levels were highest in trachea, lungs, and liver followed by spleen, kidney brain and the pulmonary hilar lymphnodes. Within the blood, ^{35}S is distributed in the plasma and in the cellular compartments (Anonymous38; Anonymous39). Gunnison and Palmes (1974) showed a positive correlation with air concentrations of sulfur dioxide and plasma levels of S-sulphonate in human subjects following continuous exposure to 0.3, 1.0, 3.0, 4.2, or 6.0 ppm SO_2 in a chamber for periods of up to 120 hours.

Metabolism

Sulfur dioxide readily dissolves in water forming sulfurous acid which dissociates to form bisulfite and sulfite ions in a ratio depending on the pH of the solution (Menzel et al. 1986).

$$SO_2 + H_2O \leftrightarrows `H_2SO_3´$$

$$H_2SO_3 \leftrightarrows H^+ + HSO_3^- \leftrightarrows 2H^+ + SO_3^{2-}$$

$$2HSO_3^- \leftrightarrows H_2O + S_2O_5^{2-}$$

Following absorption, inhaled sulfur dioxide dissolves on the walls of the moist airways (Gunnison & Jacobsen 1987). Sulphite (sulfite, bisulphate) reacts with cellular molecules especially by sulphitolysis of disulfite bonds in molecules such as cysteine, albumin, and glutathione (Gunnison & Jacobsen 1987; Menzel et al. 1986).

Sulphitolysis reaction:

$$RS - SR + SO_3^{2-} \leftrightarrows \quad RS\text{-}SO^-_3 + RS$$

At pH 7.4 the forward reaction is essentially irreversible. Detection of elevated levels of S-sulphonate (RS-SO^-_3) compounds in an organ or tissue is an indication for recent exposure to sulfite (Gunnison & Jacobsen 1987).

Sulphite oxidase (SO) catalyses the oxidation of sulfite to sulphate with ferricytochrome c being the physiological electron acceptor. Sulphite oxidase is located in the intermembrane space of mitochondria. Sulphite oxidation is performed in the Mo (molybdenum) centre, and the reducing equivalents are passed on the b5 haem, where, in turn, the terminal electron carrier cytochrome $c_{(ox)}$ is reduced. This is the final step in the oxidative degradation of the sulfur containing amino acids cysteine and methionine. The enzyme also plays an important role in detoxifying exogenously supplied sulfite and sulfur dioxide (Feng et al. 2007).

High activity of this enzyme has been found in the liver, kidney, and heart, whereas activity is low in brain, spleen, lungs, and testis (Anonymous40). Human lung has an approximately 135-fold lower capacity to oxidise sulfite than human liver (Beck-Speier et al. 1985). Sulphite oxidase activity was high in liver and hepatocytes and low activity was detectable in lung samples and in phagocytic cells. ATP level decreased following 30 min incubation (pH 6, 37°C) with sulfite dependent on the SO activity of the tissue of approximately 10 % in hepatocytes and rat liver slices compared to an decrease > 90 % in rat lung slices, alveolar and peritoneal macrophages (Anonymous41). Sulfite was cleared by direct oxidation with a metabolic clearance rate of 22 mL/min/kg bw (Gunnison and Palmes, 1974)

Compared to other animal species, rats have approximately three and five time greater SO activity than rabbits and rhesus monkeys (e.g. Anonymous46). Sulphite oxidase activity in liver was determined to be highest in rats (rat > horse > cattle > sheep > rabbit > pig) whereas pigs showed the highest SO activity in the kidney (Cabré et al. 1990). Hepatic SO activity in rats is about 10-20 fold higher than that in humans.

Rats with impaired SO activity showed higher *in vivo* plasma concentrations of sulfite than normal rats (Anonymous40). SO activity has been shown to be lower in young than in adult rats as molybdenum - which is the cofactor of sulfite oxidase - is present in low levels in newborns (Johnson and Rajagopalan 1976). Deficiency of SO leads to accumulation of SO_3^{2-}, a strong nucleophile, capable of reaction with a wide variety of cell components (Feng et al. 2007).

Glutathione (GSH) is suggested to play a role in SO_2 detoxification through the sulphitolysis of glutathione disulphide (GSSG) to S-sulphoglutathione ($GSSO_3^{2-}$). Repeated inhalation exposure to 5 ppm of SO_2 did lead to depletion of GSH pools in lung, liver, heart, and kidney (Anonymous44). In addition, a variety of authors demonstrated depletion of GSH levels and increased lipid peroxidation and oxidative stress in various organs (lung, heart, liver, kidneys, spleen, retina, lens tissue, testis, intestinal tissues, various regions of the brain, testicles; e.g. Anonymous47; Anonymous43; Anonymous48; Anonymous53 ; Anonymous51, Anonymous11; Anonymous49) following repeated exposure to SO_2 in various species (guinea pig, rabbit, mouse, rat). These results are in agreement with the wide distribution of metabolites of sulfur dioxide within the body.

Elimination

The majority of inhaled sulfur dioxide was excreted in the urine as inorganic sulphate (84.4 %) with a total urinary excretion of 92.4 % (Anonymous37). Anonymous39 determined maximal levels in urine approximately 90 min following onset of a 30-min exposure. In the study performed by Anonymous37, blood concentrations were steadily increased during exposure of 60 min whereas peak excretion in urine was not reported but apparently depends on exposure duration. Mean half-life of SO_2 was: 3.2 ± 2.3 days with an equilibrium at approx. 3-5 days following inhalation exposure to approximately 10 ppm SO_2 in rabbits (Anonymous42).

Sulphite/bisulfite:

Absorption

Sodium metabisulfite: Ji et al (1995) determined endogenous plasma concentrations of 4.87 ± 2.49 µmol/L as total plasma sulfites of 76 donors (reference range for total serum sulfite in normal subjects is 0 – 9.85 µmol/L). Two subjects received an oral single dose (with vegetable juice) of 20 mg/kg bw of sodium metabisulfite (no information on the content of sulfite of vegetable juice). A rapid rise in total serum sulfite was observed which reached a maximum of 112 and 38 µmol/L in subject 1 and 2, respectively within approximately 30 min.

Sodium sulphate: Maximal plasma concentration with equilibrium was reached within 60 – 105 min following oral application of sodium sulphate in healthy male volunteers whereas equilibrium with maximal plasma concentrations are achieved within 60 – 90 min following intravenous application. A biphasic absorption is assumed with a slow phase from the GI within 10 – 30 min followed by rapid absorption in which plasma peak levels are reached (Bauer 1976).

Distribution

Sulfite distribution (investigated in rabbits) can be described by a two-compartmental model, characterized by rapid equilibration of the central and tissue compartments with elimination occurring predominantly by the metabolic route from the central compartment. The authors suggest that the fast component represents the diffusion of a low molecular weight S-sulphonate from the blood vessels while the slow component probably corresponds to clearance of protein S-sulphonate (Gunnison and Palmes 1976).

Metabolism

Inhaled, ingested or injected sulfite is metabolized by sulfite oxidase to sulphate.

Only small amount of unchanged sulfite is cleared via kidney and excreted with urine following single i.v. injection of sodium metabisulfites in rabbits (Gunnison and Palmes 1976). In human polymorphonuclear leukocytes (neutrophils) two alternative pathways have been observed following incubation of human neutrophils with sulfite in vitro: one enzymatic route dependent on sulfite oxidase and one non-enzymatic route which involves intermediate formation of sulfur trioxide anion radicals (Constantin et al. 1996).

Endogenous sulphate production

Endogenous production of sulphate in the lung may take place in the human alveolar epithelial cells with cysteine dioxygenase (CDO) and sulfite oxidase (SO) being the responsible enzymes for possible pulmonary in situ production. Sulphate can then be converted to the substrate for phase II sulphotransferases or be used for the sulphation of structural components of the alveolus. Sources for endogenous sulphate production are sulfur containing amino acids, predominantly cysteine (Millard et al. 2003). The authors showed that both enzymes were expressed in alveolar cells but whether the activity of the enzymes would be sufficient for endogenous sulphate production remains speculative.

Elimination

Mean urinary excretion from endogenous produced sulphate (oxidation from S-amino acids) amounted to approximately 15 mmol/day in healthy volunteers (Florin et al. 1991). Maximum net intestinal absorption in ileostomised subjects reached a plateau at 5 mmol/day with dietary intakes of 7 mmol/day and above. Provided colonic absorption of sulphate is similar in healthy volunteers, a net absorption of 10 mmol/day was calculated during a period of high sulphate (16.6 mmol/day) intake. The authors assumed that diet and intestinal absorption are the principal factors affecting the amounts of sulphate reaching the colon. Endogenous secretion of sulphate by colonic mucosa may also contribute to the amounts of sulphate determined in the colon of healthy and ileostomised subjects (Florin et al. 1991).

Similar to sulfur dioxide, orally ingested sodium metabisulfite, sodium sulphate and hydrated sodium bisulphate in rats are predominantly excreted via the kidney as anorganic sulphate. Approximately 55 % of $Na_2S_2O_5$ is excreted within 4 hours following oral administration compared to 89 % following percutanous administration (Bhagat and Lockett 1960).

Protein binding

Sulfite binds to fibronectin and to serum albumin in vitro and in vivo (Gregory and Gunnison 1984).

Conclusions:

The sulfites oral absorption can be considered 100 % (approx. 80 % within 24 h) based on sulfur dioxide high solubility in water and on the most reliable study on oral and intravenous administration of sulfites (sodium sulphate), which demonstrated urinary excretion of 80 % of dose within 24 hours (Bauer 1976). Complete absorption can be assumed.

Studies on dermal absorption of sodium metabisulfite were not available. Default values according to EFSA guidance on dermal absorption (EFSA 2012) are applied. Based on physico-chemical properties of sodium metabisulfite, the substance is not likely to penetrate skin to a large extent as the substance is highly water soluble (negative $logP_{ow}$). Therefore, dermal absorption of sulfites, metabisulfites, bisulfites and sulphates can be assumed as below 25 / 75 % (at concentrations > 5 % and ≤ 5 %, resp.) based on the EFSA Guidance on Dermal Absorption (2012). With respect to the gaseous appearance of sulfur dioxide, exposure via skin is not a relevant exposure route. Sulfur dioxide has a harmonised classification as Skin Corr. 1B, H314.

Various studies indicate complete inhalation absorption of SO_2 (Anonymous38; Anonymous39; Andersen et al. 1974, Anonymous37). Sulfur dioxide is highly soluble in water. It is, therefore, readly absorbed by the mucous of the upper respiratory tract. Parts of the absorbed SO_2 is subsequently exhaled (~ 15 %) via expired air. The values considered were 85 – 92 %.

Inhaled and ingested sulfur dioxide/sulfites are systemically available. Distribution in blood in plasma and cellular compartments. Accumulation of sulfite in plasma is not to be expected.

Sulphite oxidase is the most important enzyme in sulfite metabolism, oxidizing SO_3^{2-} to SO_4^{2-}. Liver, kidney, and heart are tissues with high activity of sulfite oxidase, whereas e.g. lung, brain, spleen, and testes show low activity (Gunnison & Jacobson 1987). Deficiency of sulfite oxidase results in accumulation of SO_3^{2-} which is a strong nucleophile that can react with a variety of cell components (Feng et al. 2007). Inhaled sulfur dioxide dissolves on the walls of the moist airways producing a mixture of sulfite, bisulfite, and hydrogen ions. Lung is the predominant target organ for local effects of SO_2 exposure (port of entry) presumably due to its low activity of sulfite oxidase. Organs with low activity of sulfite oxidase are suggested to be target organs.

All studies which addressed elimination following sulfur dioxide or sulphate exposure identified urinary excretion of inorganic sulfur as predominant route of elimination. Amount of expired SO_2.

10 EVALUATION OF HEALTH HAZARDS

Taking into account that sulfur dioxide has an existing harmonized classification, only the endpoints which need to be amended are addressed[1]. Therefore, only the data for acute toxicity by inhalation, skin irritation, respiratory sensitisation, skin sensitisation, carcinogenicity and germ cell mutagenicity assessments are presented. The REACH Registration report on Sulfur dioxide was also taken into account (c.f., section 6) and is generally in agreement with the information presented in this CLH report.

Sulfur dioxide is a well-known pollutant in ambient air as result from anthropogenic and natural emissions. In addition, sodium metabisulfite has a long tradition as preservation agent and antioxidant in food and cosmetics

[1]ECHA- Guidance on the preparation of dossiers for harmonised classification and labelling, vs 2.0 (2014), section 3.4.3.1, page 18: "The dossiers proposing revisions to Annex VI entries need only focus on the specific hazard classes that are proposed to be revised. If one or several of the CMR and respiratory sensitisation hazard classes were not assessed in the past when the current harmonised classification was adopted and included in Annex VI, it may be considered (in line with Article 36(1), CLP) that these are included in the updated dossier, in addition to the hazard class(es) for which the revision is proposed. The process for updating Annex VI entries is the same for active substances used in BP and PPP as for other substances, and hence CLH dossiers proposing a revision of an existingentry for active substances in BP and PPP do not need to include data on all hazard classes but only data relevant for the revision proposal".

(e.g. Us-EPA 2008, JECFA 1964/65/66, Nair et al. 2003). Furthermore, considerable quantities of sulfite are generated in the body by normal catabolic processing of sulfur-containing amino acids (e.g. cysteine) and other sulfur-containing substances (Gunnison and Jacobsen 1987).

Consequently, extensive research has been performed on the toxicology of sulfur dioxide and sulfites and a vast amount of human and animal toxicity data has been accumulated. Unfortunately, little of the available data has been acquired and reported in a way complying with current OECD and EU guidelines for the testing of chemicals. Therefore, appropriate care needs to be taken in its interpretation. Nevertheless, it provides the information required for an assessment of the human health effects of sulfur dioxide and sodium metabisulfite.

As sodium metabisulfite is another substance, its classification has to be addressed in, a separate CLH dossier.

As human health effect assessment bases almost completely on published information, reliability can rarely be scored better than “reliable with restrictions” which is equivalent to Klimisch score 2. As a consequence, key studies are generally defined on the basis of studies with reliability scores of 2 if the results of these are supported by other studies.

Not all references available were considered relevant for hazard assessment. Due to the vast amount of studies submitted and additionally retrieved from scientific literature search, the DS refrained from listing of all studies that were not used for hazard assessment (e.g. due to poor reliability).

Read-across concept for sulfur dioxide, sulfites, hydrogensulfites, in aqueous solution:

Quadrivalent-sulfur substances (S^{IV}, sulfurous acid: H_2SO_3 and salts of sulfite: SO_3^{2-}) and bisulfite (HSO_3^-)) are produced when SO_2 dissolves in water and exist in a pH-sensitive equilibrium as shown in the following equations (Anonymous46, Shapiro 1972, Hayon et al. 1972; Beets and Voss, 1970). In addition, the active substance is sulfur dioxide generated by hydrolysis in situ of sodium metabisulphite. The following reactions occur, when sulphur dioxide is generated:

$$S_2O_5^{2-} + H_2O \leftrightarrows 2\ HSO_3^-$$

$$HSO_3^- + H_2O \leftrightarrows H_3O^+ + SO_3^{2-}$$

$$SO_3^{2-} + 2\ H_3O^+ \leftrightarrows SO_2 + 3\ H_2O$$

At pH 0.9, 24.7°C (Beets and Voss, 1970):

$$SO_2 + H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} H^+ + HSO_3^- \qquad 2\ HSO_3^- \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} S_2O_5^{2-} + H_2O$$

$K_{-1} = 2.48 \pm 0.27 \times 10^9$ mole^{-1}1s^{-1} $K_2 = 7.00 \pm 0.21 \times 10^2$ mole^{-1}1s^{-1}

$K_1 = 1.06 \pm 0.13 \times 10^8$ s^{-1} $K_{-2} = 10^4$ s^{-1}

This dossier concerns SO_2 as gas and in aqueous solution. A comprehensive read-across concept was developed for sulfur dioxide, sulfites, and hydrogensulfites. It is expected that the cation (i.e., sodium, potassium, ammonium) contributes to a lesser extent to the toxicity and solubility (all compounds are very soluble in water). Therefore, chemical and biological properties of the "sulfite" anion are predominantly considered as relevant determinant and information from sulphates with other cations than sodium are included in the evaluation.

The species that dominates among these rapidly interconvertible hydration products depends primarily upon pH but also on ionic strength and temperature (Gunnison and Jacobsen, 1987). Therefore, sulfur dioxide is transported through aqueous systems at neutral pH almost totally in its hydrated form. Because of this rapid

hydration, the interactions of sulfur dioxide with biological molecules in an aqueous medium are probably those of sulfite and bisulfite.

Acidification will liberate sulfur dioxide vapours; in alkalis, sulfites, bisulfites, and metabisulfites are produced (Green, 1976). At concentrations > 1M, bisulfite anions will dimerize with the elimination of water to form metabisulfite (S2O5)2-; at low concentrations metabisulfite will hydrolyse to form bisulfite (HSO3)- (Shapiro 1983; Gunnison and Jacobsen 1987, Nair et al., 2003).

10.1 Acute toxicity - oral route

Endpoint not addressed.

10.2 Acute toxicity - dermal route

Endpoint not addressed.

10.3 Acute toxicity - inhalation route

Currently, there are no acute inhalation studies available according to OECD Guideline 403, but sufficient information on acute inhalation toxicity can be derived from some older studies (Anonymous17, Anonymous23; Anonymous24). The most reliable studies with the lowest LC_{50} values were re-assessed and the conclusion was the same as the previous classification on Acute Tox. Cat. 3.

Table 9: Summary table of animal studies on acute inhalation toxicity

Summary table of animal studies on acute inhalation toxicity with sulfur dioxide					
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/group**	**Test substance, form** *(gas, vapour, dust, mist)* **Actual and nominal concentration, Type of administration** *(nose only / whole body/ head only)*	**Results** LC_{50}	**Remarks** *(e.g. major deviations)*	**Reference**
Pre-guideline OECD 403, Non-GLP, Reliability 2. Key study	Rat, CD outbred, M, 8/group	**Sulfur dioxide** (CAS 7446-09-5), air containing SO_2, 4 h exposure: whole-body dose levels: 224; 593; 965; 1168; 1319 ppm	Effects of various concentrations of inhaled SO_2 on the mortality of rats: Concentration of SO_2 (ppm) / 2-week mortality 224 / 0/8 593 / 0/8 965 / 3/8 1168 / 5/8 1319 / 8/8 965 ppm < LC_{50} < 1168 ppm (approx. 2.57 mg/L < LC_{50} < 3.11 mg/L) at 965 ppm and higher: Respiratory difficulties followed by exhaustion and death	Acute Tox 3 A LC50 value of 1041 ppmV was calculated post-hoc by log-probit regression using BMDS software version 2.6.0.1.	Anonymous17
Method: Specific investigation on time-course of airway hyperreactivity and inflammatory changes in bronchoalveolar lavage (BAL) after exposure to high concentration; Non-guideline;	Dogs (Beagle)/ (M+F); 8 animals in total (4/group – control and treated).	**Sulfur dioxide** (CAS 7446-09-5); air containing SO_2 2-h exposure: endotracheally intubated, artificially respired Conc.: 400 ppm	LC_0 (2 h): > 400 ppm; an immediate increase of bronchial responsiveness to histamine that lasted for about 2 hours post-exposure. Cell numbers in BAL were increased up to 1 hour for epithelial cells and from 1-4 hours for neutrophils. There	-	Anonymous18

Summary table of animal studies on acute inhalation toxicity with sulfur dioxide					
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/group**	**Test substance, form** *(gas, vapour, dust, mist)* **Actual and nominal concentration, Type of administration** *(nose only / whole body/ head only)*	**Results** LC_{50}	**Remarks** *(e.g. major deviations)*	**Reference**
Non-GLP; Reliability of 2.			was no significant change of lymphocytes, macrophages, eosinophils, goblet cells, or mast cells in lavages.		
Method: investigation of ventilatory parameters and histological changes of the respiratory tract; Non-guideline; Non-GLP; Reliability of 2.	Rats (Wistar) / no data on sex; Seven groups of ten rats. Further control group.	**Sulfur dioxide** (CAS 7446-09-5); air containing SO_2 2-h exposure: head only, inhalation by face mask Conc.: 41 ± 2 ppm, 64 ± 4 ppm, 83 ± 2 ppm, 145 ± 1 ppm, 231 ± 3 ppm, 426 ± 4 ppm and 751 ± 17 ppm	LC_0 (2 h): > 700 ppm LOAEC (2 h): 40 ppm (decrease of respiratory rate); Effects: sneezing, coughing and lachrymation, intermittent burst of quick and deep inspirations and expirations; 750 ppm: animals became grievously labored 0 and 40 ppm: no adverse histological changes of lungs 64-231 ppm: 10-30 % of the lungs showed pulmonary edema; 426-751 ppm: 70-80 % of the lungs showed pulmonary edema A positive correlation between the frequency of occurrence of pulmonary damage and the concentration of SO_2 was shown.	-	Anonymous19
Method: investigation on time-course of inflammatory changes; Non-guideline; Non-GLP; Reliability of 2.	Dogs (Beagle)/ (F+M); 7 animals.	**Sulfur dioxide** (CAS 7446-09-5) air containing SO_2 2-h exposure: endotracheally intubated, artificially respired	LOAEC: 200 ppm; no mortality Airway hyperreactivity to histamine induced in dogs after a 2 hour inhalation of 200 ppm sulfur dioxide was associated with significant	-	Anonymous20

Summary table of animal studies on acute inhalation toxicity with sulfur dioxide					
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/group**	**Test substance, form** *(gas, vapour, dust, mist)* **Actual and nominal concentration, Type of administration** *(nose only / whole body/ head only)*	**Results** LC_{50}	**Remarks** *(e.g. major deviations)*	**Reference**
		Conc.: 200 ppm	inflammatory changes lasting up to the end of the observation period of 22 h.		
Method: investigation of respiratory rate; Non-guideline; Non-GLP; Reliability of 2.	Mice (dd strain)/ no data on sex 4 mice / test concentration, 7 test groups (including controls).	**Sulfur dioxide** (CAS 7446-09-5) air containing SO_2 10-min exposure: whole body Conc.: 0, 23, 38, 75, 128, 250, 500 ppm	LOAEC: 23 ppm; Sensory irritation, decrease of respiratory rate.	-	Anonymous21
Method: investigation of microscopic lesions of respiratory tract; Non-guideline; Non-GLP; Reliability of 2.	Mice (Ha/ICR)/ ♂; 3 DF-mice and 2 CO-mice/timepoint of sacrifice; controls: 9 DF-mice, 7 CO-mice	**Sulfur dioxide** (CAS 7446-09-5) Air containing SO_2 Exposure period: 4, 24, 48, 72 hours continuously (gas): whole body Conc.: 10 ppm	LOAEC: 10 ppm (24 h exp., not after 4h exp.); Severe injury of respiratory and olfactory epithelium of the nasal cavity (oedema, necrosis and desquamation).	-	Anonymous22
Method: investigation of survival time and histological changes of the lower respiratory tract; Non-guideline; Non-GLP; Reliability of 2.	Rats (Sprague Dawley)/ ♂; 12 animals/dose.	**Sulfur dioxide** (CAS 7446-09-5) Exposure period: until death: whole body Conc.: 1.975, 3.498, 5.052 ppm	LC_{100}: 1975 ppm: 198 min; 3.498 ppm: 72 min; 5.052 ppm: 41 min; Deaths: time-dependent, 100% mortality at all concentrations.; Early deaths: acute asphyxia; Late deaths: pulmonary failure (oedema, consolidation of lung tissue).	-	Anonymous23

Summary table of animal studies on acute inhalation toxicity with sulfur dioxide					
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/group**	**Test substance, form** *(gas, vapour, dust, mist)* **Actual and nominal concentration, Type of administration** *(nose only / whole body/ head only)*	**Results LC_{50}**	**Remarks** *(e.g. major deviations)*	**Reference**
			Mean survival time: Susceptibility to lethal toxic action of SO_2 highest in mice, intermediate in guinea pigs, least in rats		
Method: investigation of survival time and histological changes of the lower respiratory tract; Non-guideline; Non-GLP; Reliability of 2.	Mice (Connaught Medical research laboratory mice) /♂; 12 animals/dose.	**Sulfur dioxide** (CAS 7446-09-5) Exposure period: until death: whole body Conc.: 610, 913, 1178 ppm	LC_{100}: 610 ppm: 286 min; 913 ppm: 75 min, 1178 ppm: 39 min; Mortality: time-dependent, 100% at all concentrations ; Early deaths: acute asphyxia; Late deaths: pulmonary failure (oedema, consolidation of lung tissue). Mean survival time: Susceptibility to lethal toxic action of SO_2 highest in mice, intermediate in guinea pigs, least in rats	-	Anonymous23
Method: investigation of survival time and histological changes of the lower respiratory tract; Non-guideline; Non-GLP; Reliability of 2.	Guinea pigs/♂; 12 animals/dose.	**Sulfur dioxide** (CAS 7446-09-5) Exposure period: until death: whole body Conc.: 2.207, 2.508, 2.750 ppm	LC_{100}: 2.207 ppm: 68 min; 2.508 ppm: 39 min; 2.750 ppm: 36 min. Mortality: time-dependent, 100% at all concentrations; Early deaths: acute asphyxia; Late deaths: pulmonary failure (oedema, consolidation of lung tissue). Mean survival time: Susceptibility to lethal toxic action of SO_2 highest in mice,	-	Anonymous23

Summary table of animal studies on acute inhalation toxicity with sulfur dioxide					
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/group**	**Test substance, form** *(gas, vapour, dust, mist)* **Actual and nominal concentration, Type of administration** *(nose only / whole body/ head only)*	**Results LC_{50}**	**Remarks** *(e.g. major deviations)*	**Reference**
			intermediate in guinea pigs, least in rats		
Method: investigation of mortality and histological changes of the respiratory tract; Non-guideline; Non-GLP; Reliability of 2.	Hamsters (Syrian) /♂; No. of animals: 8 at 40 ppm, 12 at 40 ppm + carbon, 8 controls (without carbon).	**Sulfur dioxide** (CAS 7446-09-5) Exposure time 4h: whole body Conc.: 40 ppm SO_2, 40 ppm SO_2 + 0.74 g carbon dust/m³, control: 0.74 g carbon dust/m³	Exposure to SO_2 alone: No leucocyte recruitment Exposure to SO_2 and carbon dust: Numerous polymorphonuclear leukocytes within bronchial walls and in lumen (in part reversible)	Experiments with hamsters are not reliable (pneumonia, pathogenic bacteria in trachea/lungs). Repeated exposure of hamsters to SO_2 was also reported. A LC_{50} value cannot be derived due to reporting deficiencies. Epithelial changes in trachea and large bronchi were observed after exposure to 100, 200, 400 ppm for up to 6 weeks.	Anonymous24
Method: investigation of ciliary beat in trachea; Non-guideline; Non- GLP; Reliability of 2.	Rabbits (no data on strain and sex); 10 animals/ dose.	**Sulfur dioxide** (CAS 7446-09-5) 45-min exposure: Head-only or whole body Conc. (nominal and analytical): about 100, 200, 300 ppm or about 100, 250 ppm	NOAEC (NOEC): 100 ppm (analytical: 99 ppm); LOAEC (LOEC): 200 ppm (analytical 210 ppm); (based on ciliar activity stop) high retention of sulfur dioxide in nose, mouth and pharynx; only about 1-2 % of the initially inhaled sulfur dioxide (at up to 250 ppm, analytical: 241 ppm) reached tracheal region	-	Anonymous25
Method: investigation of haematological changes;	Rats (Swiss) /♂; 50 animals (experimental group) /51 animals (control).	**Sulfur dioxide** (CAS 7446-09-5) 24-h exposure:	LOAEC: 0.87 ppm: haematocrit ↑; Sulfhaemoglobin ratio ↑;	-	Anonymous26

Summary table of animal studies on acute inhalation toxicity with sulfur dioxide					
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/group**	**Test substance, form** *(gas, vapour, dust, mist)* **Actual and nominal concentration, Type of administration** *(nose only / whole body/ head only)*	**Results** LC_{50}	**Remarks** *(e.g. major deviations)*	**Reference**
Non-guideline; Non-GLP; Reliability of 2.		whole body Conc.: 1 ppm (nominal)	Viscosity (whole blood/packed cell): ↓; Erythrocyte counts, Hb methemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration (no significant difference).		
Method: fixed concentration procedure; Non-guideline; Non-GLP; Reliability of 2.	Rats (Sprague Dawley)/ ♂; 15 animals (pretreated with tracer particles, divided into 3 groups [one control, one SO_2, one HCHO] after exposure).	**Sulfur dioxide** (CAS 7446-09-5) 4-h exposure (SO_2 gas after inhalation of radioactive tracer particles): nose only Conc.: 20.1 ± 0.6 ppm	LOAEC: 20.1 ppm; delayed upper respiratory tract particle clearance whereas clearance from the deep lung was not affected.	-	Anonymous27
Method: investigation of ultrastructural histological changes in different regions of the respiratory tract; Non-guideline; Non-GLP; Reliability of 2.	Rats (Wistar)/ ♂; 5 gnotobiotic rats, 5 further gnotobiotic control rats.	**Sulfur dioxide** (CAS 7446-09-5) 8-h exposure: whole body Conc.: 800 ppm (2.16 g/m³)	LOAEC: 800 ppm; upper trachea represented the most affected region of epithelial damage; gradient of decreasing cellular damage was observed in the tracheobronchial tree in peripheral direction accompanied by decreasing mitotic and metabolic activity of surviving cells.	-	Anonymous28

Summary table of animal studies on acute inhalation toxicity with sulfur dioxide					
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/group**	**Test substance, form** *(gas, vapour, dust, mist)* **Actual and nominal concentration, Type of administration** *(nose only / whole body/ head only)*	**Results** LC_{50}	**Remarks** *(e.g. major deviations)*	**Reference**
Method: investigation of time-dependence and reversibility of histological changes in nose tissues; Non-guideline; No data on GLP; Reliability: 2 (reliable with restrictions).	Mice (ICR) / ♀; 56 healthy mice: 44 mice were exposed to SO2, 12 were used as controls	**Sulfur dioxide** (CAS 7446-09-5) 30-, 60- and 120-min exposure: whole body Conc.: 20 ppm	LOAEC: 20 ppm; severe injury of respiratory and olfactory epithelium of the nasal cavity (depending on exposure/observation time); The changes were primarily degenerative rather than inflammatory.	-	Anonymous29
Method: investigation of blood pressure; Non-guideline followed; Non-guideline; No data on GLP; Reliability: 2 (reliable with restrictions).	Rats (Wistar) /♂; 10/conc. group (3 exposure groups and 3 control groups)	**Sulfur dioxide** (CAS 7446-09-5) 6-h exposure: Whole body Conc.: 28.6 ± 1.0 mg/m³ (about 10 ppm) 57.3 ± 2.0 mg/m³ (about 20 ppm) 114.4 ± 2.0 mg/m³ (about 40 ppm)	NOAEC: 10 ppm; LOAEC: 20 ppm; Dose-dependent significant decreases of blood pressure in comparison to control values.	-	Anonymous31
Sodium metabisulfite: Studies with sodium metabisulfite aerosol in mice (Anonymous33), sodium sulfite aerosol in mice (Anonymous34), rats (Anonymous35), and guinea pigs (Anonymous36) were not conducted to derive an LC_{50}.					

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

The LC_{50} value of 965 ppm < LC_{50} < 1168 ppm originates from the most reliable study (eq. to Klimisch score of 2) available for sulfur dioxide (Anonymous17). Although the study was conducted prior to OECD 403 it is considered sufficient for classification and labelling. No further acceptable study reporting LC_{50} values for sulfur dioxide is available. At 965 ppm and higher, the animals presented respiratory difficulties followed by exhaustion and death. A LC_{50} value of 1041 ppmV was estimated by the DS post-hoc using log-probit regression. The value requires classification according to Reg (EC) No. 1272/2008: Acute Tox. 3, H331: Toxic if inhaled.

10.3.2 Comparison with the CLP criteria

The following table presents the critical results for acute inhalative toxicity used for classification and labelling and further lists the criteria required from CLP regulation.

Toxicological result*	**CLP criteria**
Sulfur dioxide: rat, M: Inhalation LC_{50}: 965 ppm < LC_{50} < 1168 ppm (approx. 2.57 mg/L < LC_{50} < 3.11 mg/L) Based on log-probit regression an ATE of 1041ppmV is proposed.	Cat. 4 (H332): 2500 < LC_{50} ≤ 20000 (ppmV) Cat. 3 (H331): 500 < LC_{50} ≤ 2500 (ppmV) Cat. 2 (H330): 100 < LC_{50} ≤ 500 (ppmV) Cat. 1 (H330): LC_{50}1 ≤ 100 (ppmV)

*Only studies used for classification are listed.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on the results listed above, the proposed classification and labelling for the inhalation LC_{50} endpoint is:

Sulfur dioxide:

Acute Tox. 3, H331: Toxic if inhaled. (ATE: 1041 ppmV)

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute inhalation toxicity

The DS proposed classification as Acute Tox. 3, H331: Toxic if inhaled, based on the most reliable study (reported by the DS as reliability 2) available in the sources used for the evaluation of SO_2 (Anonymous17). Although the study was conducted before the OECD TG

403 was published, it was considered of sufficient quality for classification. The LC_{50} was calculated to be 1041 ppmV based on a log-probit regression.

Comments received during consultation

There was one comment from an industry or trade association agreeing with the proposed classification.

Assessment and comparison with the classification criteria

Although there were numerous studies on short term exposure to inhaled SO_2, none of them were conducted according to guidelines. In the table below, the studies where mortality was observed and that were considered relevant for the evaluation of acute inhalation toxicity by RAC, are shown.

***Table**: Summary table of animal studies on acute inhalation toxicity with SO_2*

Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/group**	**Test substance, form (gas, vapour, dust, mist) Actual and nominal concentration, Type of administration (nose only / whole body/ head only)**	**Results LC_{50}**	**Remarks (e.g. major deviations)**	**Ref.**
Pre-guideline OECD TG 403, Non-GLP, Reliability 2. Key study	Rat, CD outbred, M, 8/group	**SO_2** (CAS 7446-09-5), air containing SO_2, 4 h exposure: whole-body, concentrations (ppm): 224, 593, 965, 1168 and 1319 ppm	Effects of various concentrations of inhaled SO_2 on the mortality of rats: Conc. of SO_2 (ppm) / 2-week mortality 224 / 0/8 593 / 0/8 965 / 3/8 1168 / 5/8 1319 / 8/8 965 ppm < LC_{50} < 1168 ppm (approx. 2.57 mg/L < LC_{50} < 3.11 mg/L) at 965 ppm and higher: Respiratory difficulties followed by exhaustion and death	Acute Tox 3 A LC_{50} value of 1041 ppmV was calculated post-hoc by log-probit regression using BMDS software version 2.6.0.1.	Anonymous17
Method: Survival time and histological	Rats (Sprague Dawley)/M,	**SO_2** (CAS 7446-09-5)	LC_{100}: 1975 ppm: 198 min 3.498 ppm: 72 min 5.052 ppm: 41 min;	Mean survival time: Susceptibility to lethal toxic	Anonymous23

changes of the lower respiratory tract; Non-guideline; Non-GLP; Reliability of 2.	12 animals/dose	Exposure period: until death whole body Conc.: 1.975, 3.498, 5.052 ppm	Deaths: time-dependent, 100% mortality at all concentrations; Early deaths: acute asphyxia; Late deaths: pulmonary failure (oedema, consolidation of lung tissue).	action of SO_2 highest in mice, intermediate in guinea pigs, least in rats	
	<u>Mice</u> (Connaught Medical research laboratory mice)/M; 12 animals/dose	**SO_2** (CAS 7446-09-5) Exposure period: until death whole body Conc.: 610, 913, 1178 ppm	LC_{100}: 610 ppm: 286 min 913 ppm: 75 min 1178 ppm: 39 min; Mortality: time-dependent, 100% at all concentrations; Early deaths: acute asphyxia; Late deaths: pulmonary failure (oedema, consolidation of lung tissue).		
	<u>Guinea pigs</u> ♂; 12 animals/dose	**SO_2** (CAS 7446-09-5) Exposure period: until death whole body Conc.: 2.207, 2.508, 2.750 ppm	LC_{100}: 2.207 ppm: 68 min 2.508 ppm: 39 min 2.750 ppm: 36 min Mortality: time-dependent, 100% at all concentrations; Early deaths: acute asphyxia; Late deaths: pulmonary failure (oedema, consolidation of lung tissue).		

There was only one study from which an LC_{50} could be calculated. The study (Anonymous17) although conducted before the OECD TG 403, was considered reliable for classification purposes.

The proposed LC_{50} value of 1041 ppmV was estimated by the DS post-hoc using log-probit regression and is supported by RAC, rounded to 1000 ppmV based on mathematical reasons (significant digits). Based on this value, classification according to Regulation (EC) No. 1272/2008 as **Acute Tox. 3, H331: Toxic if inhaled** is warranted.

It should be noted, that from the Anonymous23 study there is evidence that the susceptibility to the lethal toxic action of SO_2 is highest in mice, intermediate in guinea pigs and least in rats and that the LC_{50} could be lower than the one estimated in rats. However, data is lacking for further evaluation. Nevertheless, rounding the calculated ATE to 1000

ppmV takes also partial care of this concern. Consequently, RAC proposes an **ATE = 1000 ppm (gases)**.

10.4 Skin corrosion/irritation

Hazard class not assessed in this dossier.

10.5 Serious eye damage/eye irritation

Sulfur dioxide has a harmonised classification: Skin Corr. 1B; H314: Causes severe skin burns and eye damage.

10.6 Respiratory sensitisation

For justification on a Read-across from metabisulfite, please refer to section 10.

Table 10: Summary table of animal studies on respiratory sensitisation

Sulfur dioxide/Sodium metabisulfite

Method, Guideline, GLP status, Reliability	Species, Strain, Sex, No/group	Test substance Dose levels, Duration of exposure	Results	Reference
Method: *in vivo* (specific investigation of effect of low-level sulfur dioxide exposure on allergic sensitisation to inhaled allergen); Non-guideline; No data on GLP; Reliability: 2 (type of study)	Guinea pig (Dunkin-Hartley); (A) ovalbumin (OA) and sulfur dioxide exposure, N=12 ♂, (B) sulfur dioxide exposure, N=12 ♂, (C) ovalbumin exposure, N=11 ♂, Controls: (D) saline exposure, N=7 ♂	**Sulfur dioxide**; 0.1 ppm sulfur dioxide on 5 consecutive days, 5 h/d (Groups A and B) and 0.1 % ovalbumin aerosol in exposure chamber for 45 min on day 3, 4 and 5 (Groups A and C).	No allergic response was observed in case of SO_2 exposure only. The OA-and SO_2-exposed group showed airway obstruction. Results confirm findings with respect to SO_2 and OA reported by Anonymous2 (1988).	Park et al., 2001; Ann Allergy Asthma Immunol.. 86:62-7
Method: *in vivo* (specific investigation of effect of sulfur dioxide on allergic sensitisation to inhaled allergen); Non-guideline; Non-GLP; Reliability: 2 (type of study)	Guinea pig (Perlbright-White); Three groups of animals were exposed to different concentrations: (A): N=6 ♀ (B): N=5 ♀ (C): N=6 ♀ (D): Control group N=14 ♀	**Sulfur dioxide** followed by ovalbumine exposure on day 3; Conc. of SO_2: (A): 0.1 ± 0.05 ppm (B): 4.3 ± 1.2 ppm (C): 16.6 ± 3.5 ppm; Exposure duration: 8 hours on 5 consecutive days.	Low concentrations of SO_2 can facilitate local allergic sensitisation against ovalbumine in guinea pigs. 67 – 100 positive bronchial reactions to inhaled OA, depending of SO_2 concentrations, compared to 7 % in controls without prior SO_2 exposure.	Anonymous2
Method: *in vivo* (specific investigation of effect of sulfur dioxide on allergic sensitisation to inhaled allergen and effect of anti-inflammatory agents); Non-guideline; GLP; Reliability: 2 (type of study).	Guinea pig (Perlbright-White); - Number of animals: N = 6 ♀/group; 4 groups - Controls: N = 6 ♀	**Sulfur dioxide**; Exposure: 5 ppm sulfur dioxide on 5 consecutive days, 8 hours/day, in exposure chamber; Concomitant exposure to ovalbumin and anti-inflammatory agents: - indomethacin; - methylprednisolone; - nebulized nedocromil sodium - control: clean air and OA	Sulfur dioxide-induced enhancement of allergic sensitisation to ovalbumine was inhibited by treatment with anti-inflammatory agents simultaneously to sulfur dioxide exposure (mechanism not investigated).	Anonymous3

Method, Guideline, GLP status, Reliability	Species, Strain, Sex, No/group	Test substance Dose levels, Duration of exposure	Results	Reference
Method: *in vivo* (specific investigation of effect of 5 ppm sulfur dioxide exposure on allergic sensitisation to injected allergen), Non-guideline; No data on GLP; Reliability: 2 (type of study).	Guinea pig (Hartley); 12 ♂/group, 3 groups in total	**Sulfur dioxide** (or NO_2); Mean concentration: 4.92 ± 0.51 ppm; (NO_2: 4.76 ± 0.48 ppm Duration of exposure: 4 h/d, 5 d/w; 30 exposures; Simultaneous exposure to *Candida albicans* (sensitisation agent) from the 1st day of treatment.	Exposure to SO_2 increased sensitisation rate to *C. albicans* resulting in significantly increased numbers of animals with prolonged expiration and/or inspiration and in a decrease of respiratory rate. Delayed-type dyspneic symptoms even lead to mortality in 3/12 sulfur dioxide exposed animals.	Anonymous4

Table 11: Summary table of human data on respiratory sensitisation

(Please refer to section 12 for further details (tables and figures) on key studies.)

Type of data/report, Reliability	Test substance	Relevant information about the study	Observations	Reference
Published study Reliability: 2 (reporting deficiencies). Key study	**Sodium metabisulfite**, oral administration in capsules: 0, 5, 10, 25, 50, 100, 200 mg, concentration increase every 30 min	Single-blind study design: 44 patients with history of sulfite sensitive asthma, 27 asthmatics without sulfite sensitivity, 8 controls without asthma	39 % of patients with a history of sulfite-sensitive asthma showed significant broncho-constriction after ingestion of metabisulfite (PD_{20} $FEV_{1:}$ 34±56 mg; min: 5, max: 200 mg; n=17); specificity: 100 %, sensitivity: ca. 40 % Onset of SMB reaction minimal 60, maximal 210 min, average 150 min.	Hein et al. 1996 Pneumologie 50/6: 394-8
Published study Reliability: 2 (reporting deficiencies). Key study	**Metabisulfite** (MB) (sodium or potassium) oral administration: 50 mg in 30 mL 0.5 % citric acid (pH2) and **SO_2 inhalation:** 0.5; 1.5; 5 ppm (or 3 ppm if large decrease in	Single-blind, placebo controlled 3 groups of 10 subjects, each 1: asthmatics sensitive to oral MB, 2: asthmatics, not sensitive to oral MB, 3: non-asthmatics controls	% fall after MB: Group 1: 35±14; group 2: 6±6; group 3: 5±3 Pc20 SO_2 (ppm): group 1: 1.19±0.78 (0.5 – 2.9); group 2: 2.3±1.42; group 3: >5; Pc20 SO_2 does not correlate with	Delohery et al. 1984 Am Rev Respir Dis; 130:1027-32

Type of data/report, Reliability	Test substance	Relevant information about the study	Observations	Reference
	PEFR occurred) for 4 min on separate days	Endpoint 20 % decrease in PEFR (Pc20, SO_2, PD_{20} MB)	MB PEFR fall. Asthmatics whose asthma is provokes by ingestion of acid MB solutions, are not supersensitive to inhaled SO_2 gas SO_2 sensitivity does not correlate with histamine reactivity.	
Published study Reliability: 2 (reporting deficiencies). Key study	**Potassium metabisulfite**: aerosol challenge: 0.05; 0.5; 5.0 mg/mL; 2 mL inhaled Oral challenge: 10, 25, 50 mg	Objective: Responses in 8 asthmatic patients (2M/6F) to aerosolised metabisulfite Endpoint: 50 % change in specific airway resistance	Bronchospastic response at 1.2 ppm Aerosol challenge 2/8 negative; 3/8 positive at 0.5 mg/mL, and 5.0 mg/mL, respectively 3/8 positive reactors to 0.5 mg/mL aerosol reacted at 10, 25, 50 mg oral SMB, respectively. All patients negative in prick tests.	Schwarz and Chester 1984; J Allergy Clin Immunol. 74:511-3
Published study (Survey (asthmatic and non-asthmatic patients; inhalation exposure route), reliability not assignable	**Sodium metabisulfite** (aerosol): in increasing doubling concentrations (0.3 to 160 mg/ml) in normal saline	13 asthmatic (9M, 4F) and five atopic non-asthmatic subjects, , endpoint: PD_{20} FEV_1	>20 mg/ml metabisulfite: Mild irritation and cough noticed by all volunteers. 3 subjects (2 asthmatics, 1 non-asthmatic) did not achieve PD_{20} no further response. Inhalation of > 160 mg/ml of metabisulfite not possible due to cough and irritation. Molar Sign. linear correlation between , PD_{20} FEV_1 metabisulfite and methacholine ($r = 0.714$; $p < 0.05$) but	Nichol et al. 1989; Thorax. 44: 1009-1014

Type of data/report, Reliability	Test substance	Relevant information about the study	Observations	Reference
			potency of metabisulfite approx.. 6x lower than of methacholine. PD20 FEV_1 response reproducible over days and weeks.	
Published case reports Reliability: 2 (type of study: case report).	**Sulphites**, unspecified Case 2: sodium bisulfite	4 cases of occupational exposure to sulfites Case 1: F, 36 y, involved in production of beverages and oenology Case 2: M, 41 y, printer, atopic history, sulfite related asthma Case 3: sex and age not specified, press photographer Case 4: sex not specified, 25 y, atopic, console operator, history,	Case 1: Rhinorroeha, anosmia, ageusia negative skin tests, blood basophilia, nasal eosinophilia, IgE negative Case 2: Obstructive rhinorroeha, anosmia, ageusia, basophilia, asthma, eczema Case 3: urticaria, IgE negative, basophilia, histamine release Case 4: asthma (severe crisis), no eosinophilia, positive for basophilie, obstrucion not beta2 reversible, sulfite specific IgE increased	Vallon et al. 1995. Allergie et Immunologe 27/3:83-7
Published case reports Reliability: 2 (type of study: case report).	**Sodium bisulfite, sodium metabisulfite** Oral challenge: 0.1, 0.5, 1, 5 mg SMB in 5 mL water	Patient (1F, age: 18 y) with perennial asthma and intra-alveolar infiltration of eosinophils and histioocytes – diagnosis: chronic eosinophilic pneumonia Symptom treatment with isoeharine aerosol and metoclopramide (containing sodium bisulfite resp. SMB). Double-blind oral challenge with SMB	After treatment intubation for acute respiratory failure required. Oral challenge: 10 min following 5 mg SMB, FEV_1 decreased 52 %	Twarog et al. 1982. JAMA 248: 16:2030-1
Published study: oral challenge with sulfites	**Potassium bisulfite**, capsule: 1, 5, 10, 25, 50, 100, 200 mg) at 30 min intervals	56 asthmatic children (35M/21F) age: 6 – 14 y (10.2 ± 3.4)	Positive reactions after challenge with solution: 2 of 56	Boner et al. 1990; J. Allergy Clin. Immunol. 85:479-83

Type of data/report, Reliability	Test substance	Relevant information about the study	Observations	Reference
Reliability: 2 (reporting deficiencies).	solution: 30 mL of 0.5 % citric acid with 1, 10, 25, 50, 100 mg at 15 min intervals	Pulmonary function tests at 2, 5, 15 min following each dose and at 30, 60, 90 min following dosing Endpoint: Pc20 FEV_1	children (3.6 % 1x at 50 mg, 1x at 100 mg) Capsules: 4 positive reactions at 200 mg	
Published study Reliability: 2 (reporting deficiencies).	**Potassium metabisulfite** in capsules	Prospective single-blind screening study: 120/83 non-/steroid dependent asthmatics Endpoint: Pc20 FEV_1	5 non-steroid dependent and 9 steroid dependent with positive reactions Best estimate of prevalence of sulfite sensitivity in asthmatics is 3.9 %	Bush et al. 1986. Am J Med. 81: 816-820
Published study Reliability: 2 (reporting deficiencies).	**Sodium metabisulfite**, potassium metabisulfite, sodium bisulfite (and tartrazine)	40 patients with clinical diagnosis of chronic urticaria: 29 F; 7M; 4-62 y	63.8 % (23/36) with positive oral challenge tests 36.1 % (13/36) to sodium metabisulfite, 33.3 % (12/36) to sodium bisulfite and 30.5 % (11/36) to potassium metabisulfite. (47.2 % (17/36) positives to tartrazine)	Jimenez-Aranda et al. 1996 Rev Alerg Mex 43/6:152-6
Published study Reliability: 2 (reporting deficiencies).	**Sodium metabisulfite** (SMB) diluted in lemon juice: 1, 10, 25, 50, 75, 100, 150 mg MBS in 15 mL of lemon juice	Oral challenge tests with sodium metabisulfite diluted in lemon juice at pH 4.2 and at pH 3.3 if no reaction at pH 4.2). Spanish and Dutch pickled onions used for oral challenge in 7/9 patients. Total # of patients: 18 (10M/8F; age 12-23 y)	MBS, pH 4.2: positive response in 6 patients (33.3 %); at pH 3.3: positive 3/12 patients. 3/7 positive responses to Spanish pickled onions (SO2 conc: 765 and 1182 ppm) no reaction against Dutch pickled onions; SO_2 conc.: 200 ppm). Inhalation of SO_2 while consuming food with high SMB conc. with acid pH is considered as critical conditions	Gastaminze et al. 1995. Clin Exp Allergy. 25(8):698-703.
Case reports				

Type of data/report, Reliability	Test substance	Relevant information about the study	Observations	Reference
Published case report Reliability: 2 (type of study: case report).	**Potassium metabisulfite**	Case report and double blind placebo-controlled food challenge, 49 y male patient	Case of severe hypotension after food ingestion Anaphylaxis following potassium metabisulfite challenge (300 mg; approx. 4 mg/kg bw)	Cifuentes et al. 2013; Int. Arch. Allergy Immunol. 162/1:94-6
Published case report Reliability: 2 (type of study: case report).	**Sulphites** in food and wine, challenge with potassium metabisulfite: 1, 5, 10, 25, 50, 100 mg in capsules, skin prick test with 10 and 1 mg/mL	F, age: 22 y with reported 2-y-history of episodes of urticaria-angiooedema Challenge test: sequential administration at 30 minutes intervals, FEV_1 and blood pressure determined every 10 min	Urticaria and angioedema of face, neck, upper thorax, disphonia without asthma skin prick test: negative oral challenge: positive at 25 mg dose: urticaria on face and upper thorax after 12 min, nasal itching, rhinorrhoea, dysphonia, relief of symptoms after s.c. adrenaline injection Prevalence in asthmatics: 2 - 6 %	Belchi-Hernandez et al. 1993; Ann Allergy; 71/3:230-2

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10.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

Sulfur dioxide (SO_2) and sulfites including sodium metabisulfite have been recognised to induce bronchial hyperresponsiveness (BHR), in sensitive and healthy persons (e.g. van Schoor and Pauwels 2000 and tables above). Subjects with mild asthma develop airflow limitation at a lower threshold concentration of SO_2 and with greater magnitude than do non-asthmatic subjects (Sheppard et al., 1980). In an animal study, repeated exposure of guinea pigs to sulfur dioxide (0.1 ppm) alone did not result in a sensitisation response, although animals pretreated with ovalbumin developed asthmatic reactions (Park et al., 2001). Similar findings were observed by Anonymous2, Anonymous3 and Anonymous4.

Cases of sulfite induced asthma (mild and life-threatening) are described in literature for decades in the general population and in occupationally exposed workers (e.g. van Schoor et al. 2000, John and Linn 2010). Severe life-threatening asthmatic, urticarial and anaphylaxis-like attacks have been documented after exposure to sulphiting agents while eating a restaurant meal, different foods, drinking wine or after receiving parenteral medication containing sulfites as preservatives (Gillman, 1982; Schwartz and Chester, 1984, Delohery et al., 1984; Nichol et al., 1989; Wüthrich and Huwyler, 1989; Wright et al., 1990; Wüthrich et al., 1993; Vallon et al., 1995; Jiménez-Aranda et al., 1996; Gastaminza et al., 1995; Kounis et al., 2014; Cifuentes et al., 2013; Cussans et al., 2015). Patients, who also have had asthma attacks and gastrointestinal distress after eating restaurant meal, were mostly positive to sodium metabisulfite challenge by inhalation, although some persons were negative by aerosol and oral challenge despite their history (Schwartz and Chester, 1984). Some asthmatic persons can develop airways obstruction to ingested sodium metabisulfite while the other asthmatics do not (Delohery et al., 1984). Nichol et al. (1989) reported that asthmatic and non-asthmatic but atopic persons reacted similarly to challenge by sodium metabisulfite aerosol in a dose-dependent manner. It seems that inhaled sulfite aerosols can induce asthma in sensitive persons, although this effect is not restricted to patients with a clinical history of sulfite sensitivity or to subjects who demonstrated sensitivity to oral ingestion of metabisulfite (van Schoor et al., 2000; Schwartz and Chester, 1984).

Cases of metabisulfite induced asthma in occupationally exposed persons have been reported in radiographer (Merget and Korn, 2005), wine tester, pressman, photographer (Vallon et al., 1995), technician handling chemicals in a water treatment plant (Valero et al., 1993) and in persons who worked in fishing and fish processing industry (Steiner et al., 2008; Pougnet et al., 2010; Uriarte et al., 2015). The patients reacted positive to inhalation challenge by sodium metabisulfite (Merget and Korn, 2005; Steiner et al., 2008; Uriarte et al., 2015), whereby control non-occupationally exposed asthmatic persons could also possess a high susceptibility to sodium metabisulfite and sulfur dioxide (Merget and Korn, 2005).

In conclusion, exposure to aerosolized sodium metabisulfite can induce asthma-like symptoms mostly in sulfite-sensitive population. Sensitisation of healthy subjects is also described, especially following frequent exposure e.g. in occupational settings.

Furthermore, sulfur dioxide exposure elicitates asthma-like symptoms in sulfite-sensitive populations and/or asthmatics.

According to Guidance on the Application of the CLP Criteria (Version 4.1 – June 2015), "*Substances shall be classified as respiratory sensitisers if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity. This is further described in the CLP Annex I, 3.4.2.1.2"*

"Annex I: 3.4.2.1.2 Human evidence

Annex I: 3.4.2.1.2.1. Evidence that a substance can lead to specific hypersensitivity will normally be based on human experience. In this context, hypersensitivity is normally seen as asthma, but other hypersensitivity reactions such as rhinitis/conjunctivitis and alveolitis are also considered. The condition will have the clinical character of an allergic reaction. However, immunological mechanisms do not have to be demonstrated.…"

The underlying mode of action is still under debate. Different mechanisms may be involved in SO_2-induced asthma which at least partly differs in humans and animals. As long as an allergic mechanism cannot be excluded, the afore-mentioned criteria from CLP guidance (2015) apply. Inflammatory processes are clearly

involved in hypersensitivity reactions (e.g. see citation below). Classification for respiratory irritation alone is not sufficient to protect vulnerable persons.

Possible mode of actions of sulfur dioxide were described by US EPA and are cited here as follows (US EPA Report: Integrated Science Assessment for sulfur oxides – Health Criteria, September 2008 and references cited therein):

"In humans, the mechanisms responsible for SO_2-induced bronchoconstriction are not fully understood. In non-asthmatics, near complete attenuation of bronchoconstriction has been demonstrated using the anticholinergic agents atropine and ipratropium bromide (Snashall and Baldwin, 1982; Tan et al., 1982; Yildirim et al., 2005). However, in asthmatics, these same anticholinergic agents (Field et al., 1996; Myers et al., 1986), as well as short- and long-acting β2-adrenergic agonists (Gong et al., 1996;Linn et al., 1988), theophylline (Koenig et al., 1992), cromolyn sodium (Myers et al., 1986), nedocromil sodium (Bigby and Boushey, 1993) and leukotriene receptor antagonists (Gong et al., 2001; Lazarus et al.,1997) only partially blocked SO_2-induced bronchoconstriction (see Annex Table D-1, (U.S. EPA, 1994c). That none of these therapies have been shown to completely attenuate the effects of SO_2 implies the involvement of both parasympathetic pathways and inflammatory mediators in asthmatics. Strong evidence of this was borne out in a study by Myers et al. (1986), in which asthmatic adults were exposed to SO_2 following pretreatment with cromolyn sodium (a mast cell stabilizer), atropine (a muscarinic receptor antagonist), and the two medications together. While both treatments individually provided some protection against the bronchoconstrictive effects of SO_2, there was a much stronger and statistically significant effect following concurrent administration of the two medications. It has been proposed that inflammation contributes to the enhanced sensitivity to SO_2 seen in asthmatics by altering autonomic responses (Tunnicliffe et al., 2001), enhancing mediator release (Tan et al., 1982) and/or sensitizing C-fibers and RARs (Lee and Widdicombe, 2001). Whether local axon reflexes also play a role in SO_2-induced bronchoconstriction in asthmatics is not known (Groneberg et al., 2004; Lee and Widdicombe, 2001; Widdicombe, 2003). However, differences in respiratory tract innervation between rodents and humans suggest that C-fiber mediated neurogenic inflammation may be unimportant in humans (Groneberg et al., 2004; Widdicombe and Lee, 2001; 2003)."

In addition to the observations indicating the presence of direct allergic reactions by exposure to SO_2 and metabisulfite, an important feature of the clinical syndrome asthma, the airway hyperresponsiveness (AHR) has to be considered as well. The variable part of AHR is associated with acute inflammation, the persistent component of AHR is connected with chronic inflammation and airway remodeling (Cockcroft and Davis, 2006). However, the mechanism of action is in both cases far from clear and can include factors such as mast cells and histamine release like in allergic reactions. Atopic IgE-mediated allergic responses are the most common inducers of AHR. The indirect stimuli such as chemicals inducing indirect AHR were considered to be more clinically relevant.

Acute toxicity inhalation studies are available and demonstrate clinical signs of AHR induced by SO_2 in dogs (Anonymous18; Anonymous20). Although these studies were attributed to the endpoint acute toxicity inhalation, the observation of AHR signs as a syndrome of asthma in this endpoint is not foreseen to be included by the CLH template nor is it in the endpoints respiratory irritation or respiratory sensitization. However, DS decided to assign AHR effects such as bronchoconstriction to Specific Target Organ Toxicity (STOT SE 3: Respiratory Tract Irritation) and propose classification for this endpoint. Moreover, AHR is a severe adverse outcome that should in any case be considered not only for risk assessment but also for classification and labelling (Cockcroft, D.W. and Davis, B.E., 2006).

10.6.2 Comparison with the CLP criteria

Toxicological result	**CLP criteria**
Sulfur dioxide: -human data, metabisulfite oral administration and sulfur dioxide inhalation: **:** 0.5; 1.5; 5 ppm for 4 min on separate days (Delohery et al. 1984): Inhalation elicitation: ≤ 0.5 ppm (1.3 mg/m^3)	<u>Category 1 :</u> Substances shall be classified as respiratory sensitisers (Category 1) where data are not suficient for sub-categorisation in accordance with the following criteria :

Toxicological result	CLP criteria
-human data, 4-8 % of asthmatics are intolerant to sulfites according to Hein et al. 1996. Prevalence of asthmatics in Europe: between 1.6 % in Romania and 7 % in France (OECD statistics 2012), corresponding to a prevalence of sulfite sensitive subjects between 0.064 and 0.56 %. The frequency of occurrence is only roughly estimated.- elicitation of asthma-like symptoms/bronchoconstriction following SO_2 inhalation - sulfur dioxide is used as an example of respiratory tract irritant substance in the Guidance on the Application of the CLP Criteria (2017, section 3.8.5.1.3., page 456), based on the broad, well documented human experience on irritating effect to respiratory system. - as described above, airway responses cannot be solely assigned to the corrosive properties of the substance already covered by Skin Corr 1 classification. Proposed classification as Respiratory Tract Irritant Cat. 3 (see section 10.11)	– if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity ; and/or – if there are positive results from an appropriate animal test <u>Sub-category 1A :</u> – Substances showing a high frequency of occurrence in humans; or a probability of occurrence of a high sensitisation rate in humans based on animal or other tests. Severity of reaction may also be considered. <u>Sub-category 1B :</u> – Substances showing a low to moderate frequency of occurrence in humans; or a probability of occurrence of a low to moderate sensitisation rate in humans based on animal or other tests. Severity of reaction may also be considered.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

Sulfur dioxide is considered not to be a sensitiser itself, but unequivocally exacerbates existing asthma in sulfite-sensitive populations and/or asthmatics by the inhalation route and after single exposure to concentrations ≤0.5ppm. Thereby, sulfur dioxide can cause asthma symptoms and breathing difficulties as described in hazard sentence H334: "*May cause allergy or asthma symptoms or breathing difficulties if inhaled*.". As sulfur dioxide is not an allergen itself and requires an existing allergy is a prerequisite for the observed asthma symptoms, classification as respiratory sensitisation does not apply according to current CLP criteria and guidance. However, the DS would like to point out that the observed effects are not completely in line with the criteria in CLP regulation. The definition of sensitisation under CLP is quoted in the CLP guidance (2017, p 333) as follows:

> "**Annex I:** ***3.4.1.3.*** *For the purpose of* section 3.4*, sensitisation includes two phases: the first phase is induction of specialised immunological memory in an individual by exposure to an allergen. The second phase is elicitation, i.e. production of a cell-mediated or antibody-mediated allergic response by exposure of a sensitised individual to an allergen.*
> **Annex I*: 3.4.1.4.*** *For respiratory sensitisation, the pattern of induction followed by elicitation phases is shared in common with skin sensitisation. […]."*

In contrast, regarding the clinical character of the observed symptoms the following will apply (citation from CLP guidance 2017, p 333): "**Annex I*: 3.4.2.1.2 Human evidence***
Annex I: ***3.4.2.1.2.1.*** *Evidence that a substance can lead to specific hypersensitivity will normally be based on human experience. In this context, hypersensitivity is normally seen as asthma, but other hypersensitivity reactions such as rhinitis/conjunctivitis and alveolitis are also considered. The condition will have the clinical character of an allergic reaction. However, immunological mechanisms do not have to be demonstrated."*

It is further noted that respiratory sensitisation may be induced not only by inhalation but also by skin contact (Dotson et al. 2015 as quoted from CLP guidance 2017).

In summary, sulfur dioxide does not meet the criteria given in the CLP regulation and respective guidance (see citation above) for respiratory sensitisation. Nevertheless, it should be evaluated how the hazard potential of substances inducing asthma-like symptons through inducing airway-hyperresponsiveness, such as sulfur dioxide, can be adequately reflected by classification under the CLP regulation.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The DS described in the CLH report, that SO_2 and sulfites have been recognised to induce bronchial hyperresponsiveness (BHR), in both sensitive and healthy persons. Cases of sulfite induced asthma (mild and life-threatening) have been described in the literature for decades in the general population and in occupationally exposed workers (van Schoor and Pauwels, 2000, published study not used for classification purposes). Subjects with mild asthma develop airflow limitation at a lower threshold concentration of SO_2 and with greater magnitude than do non-asthmatic subjects (Sheppard *et al.*, 1980, published study not used for classification purposes).

From the studies included in Table 11, pages 33-37 of the CLH report, the DS observed that severe life-threatening asthmatic, urticarial and anaphylaxis-like attacks have been documented after exposure to sulfiting agents while eating a restaurant meal, different foods, drinking wine or after receiving parenteral medication containing sulfites as preservatives (Schwartz and Chester, 1984; Delohery *et al.*, 1984; Nichol *et al.*, 1989; Vallon *et al.*, 1995; Jiménez-Aranda *et al.*, 1996; Gastaminza *et al.*, 1995; Cifuentes *et al.*, 2013; etc). Patients, who also have had asthma attacks and gastrointestinal distress after eating a restaurant meal, mostly were positive to sodium metabisulfite challenge by inhalation, although some persons were negative by aerosol and oral challenge despite their history (Schwartz and Chester, 1984). Some asthmatic people can develop airway obstruction to ingested sodium metabisulfite while other asthmatics do not (Delohery *et al.*, 1984). Nichol *et al.* (1989) reported that asthmatic and non-asthmatic but atopic people reacted similarly to challenge by sodium metabisulfite aerosol in a dose-dependent manner. It seems that inhaled sulfite aerosols can induce asthma in sensitive people, although this effect is not restricted to patients with a clinical history of sulfite sensitivity or to subjects who demonstrated sensitivity to oral ingestion of metabisulfite (van Schoor *et al.*, 2000, published study not used for classification purposes; Schwartz and Chester, 1984). Cases of metabisulfite induced asthma in occupationally exposed people have been reported in a radiographer (Merget and Korn, 2005, published study not used for classification purposes), wine tester, pressman, photographer (Vallon *et al.*, 1995), technician handling chemicals in a water treatment plant (Valero *et al.*, 1993) and in people who worked in fishing and fish processing industry (Steiner *et al.*, 2008; Pougnet *et al.*, 2010; Uriarte *et al.*, 2015; all published studies not used for classification purposes). The patients reacted positively to inhalation challenge by sodium metabisulfite (Merget and Korn, 2005; Steiner *et al.*, 2008; Uriarte *et al.*, 2015; all published studies not used for classification purposes), whereby control non-occupationally exposed asthmatic people could also possess a high susceptibility to sodium metabisulfite and SO_2 (Merget and Korn, 2005, published study not used for classification purposes).

The DS also used non-guideline animal studies on SO_2 (described as reliability 2) as supporting evidence (Table 10, pages 32-33 of the CLH report). More specifically, in an animal study, repeated exposure of guinea pigs to SO_2 (0.1 ppm) alone did not result in a sensitisation response, although animals pre-treated with ovalbumin developed asthmatic

reactions (Park *et al.*, 2001). Similar findings were observed by Anonymous2, Anonymous3 and Anonymous4.

In conclusion, exposure to aerosolized sodium metabisulfite can induce asthma-like symptoms mostly in sulfite-sensitive populations. Sensitisation of healthy subjects is also described, especially following frequent exposure e.g. in occupational settings. Furthermore, SO_2 exposure elicits asthma-like symptoms in sulfite-sensitive populations and/or asthmatics.

When considering the mechanism of action for SO_2, the DS described different mechanisms that may be involved in SO_2-induced asthma, which at least partly differs in humans and animals. An allergic mechanism cannot be excluded, but inflammatory processes are clearly involved in hypersensitivity reactions. In addition to the observations indicating the presence of direct allergic reactions by exposure to SO_2 and metabisulfite, an important feature of the clinical syndrome asthma, the airway hyperresponsiveness (AHR) has to be considered as well. The variable part of AHR is associated with acute inflammation while the persistent component of AHR is connected with chronic inflammation and airway remodelling (Cockcroft and Davis, 2006). However, the mechanism of action is in both cases far from clear and could include factors, such as mast cells increase and histamine release which is seen in allergic reactions (US EPA, 2017). Atopic IgE-mediated allergic responses are the most common inducers of AHR. The indirect stimuli such as chemicals inducing indirect AHR were considered to be more clinically relevant. The DS pointed out that AHR induced by SO_2 in dogs has been reported in acute toxicity inhalation studies (Anonymous18; Anonymous20).

Based on the above, the DS concluded that classification for respiratory sensitisation alone is not sufficient to protect vulnerable individuals from SO_2 exposure. Moreover, AHR is a severe adverse outcome that should in any case be considered not only for risk assessment but also for classification and labelling (Cockcroft and Davis, 2006). Moreover the DS stated that the AHR signs as a syndrome of asthma are not foreseen to be included in the endpoint of respiratory sensitization in the CLP regulation. In summary, SO_2 does not meet the criteria given in the CLP Regulation for respiratory sensitisation. Nevertheless, the DS noted that it should be evaluated how the hazard potential of substances inducing asthma-like symptoms through inducing airway-hyperresponsiveness, such as SO_2, can be adequately reflected by classification under the CLP Regulation.

Comments received during consultation

No comments directly addressing classification for Respiratory Sensitisation were provided either by Industry or MSCAs. Nevertheless, there were indirect comments supporting no classification for this hazard class. More specifically,

- Under Skin Sensitisation, the LR and SAC referred to a recent Substance Evaluation as required by REACH Article 48 for Disodium disulfite (EC No 231-673-0, CAS No 7681-57-4) by the Evaluating Member State Hungary, where it is stated that "*Based on the evaluated literature data it is unlikely that disodium disulfite is a skin sensitiser or induces respiratory sensitization but may enhance symptoms of asthma in sensitive individuals. The information related to the skin and respiratory sensitising properties of the disodium disulfite presented by the Registrant is*

sufficient for evaluation. Based on the available data the evaluating Member State concludes that there is no concern for respiratory sensitisation."

Assessment and comparison with the classification criteria

For the evaluation of the Respiratory Sensitisation properties of SO_2, there are two sets of data presented in the CLH report:

1. Animal data on SO_2 exposed Guinea pigs (Table 10 of the CLH report). All 4 non-guideline, non-GLP studies were performed to investigate the effect of SO_2 on allergic sensitisation to inhaled allergen and the effect of anti-inflammatory agents. The findings support the fact that no allergic response was observed in case of SO_2 exposure only. When co-exposure to a known allergen (i.e. ovalbumin, *C. albicans*) took place, the animal group with combined exposure showed airway obstruction and prolonged expiration and/or inspiration and a decrease in the respiratory rate. In some cases, delayed-type dyspnoeic symptoms even led to mortality in 3/12 SO_2 exposed animals. SO_2-induced enhancement of allergic sensitisation to ovalbumin was inhibited by treatment with anti-inflammatory agents simultaneously with SO_2 exposure (mechanism not investigated).
2. Human data, all on sulfites (Table 11 of the CLH report), comprising 8 studies on asthmatics or patients with a history of sulfite-sensitive asthma and asthmatic children, 4 cases of occupational exposure to sulfites and 3 case reports on a male and 2 female individuals, orally exposed to potassium metabisulfite and sulfites, respectively, via food/ wine exposure. In the former studies (7 with oral administration, 3 with inhalation of aerosol) the number of patients enrolled varied from 7 to 120. Delohery *et al.* (1984), also reported on 10 asthmatics inhaling three different concentrations of SO_2 in a study investigating metabisulfite sensitivity in patients with asthma. The authors stated that SO_2 exposure did not correlate with the peak expiratory flow rate decrease caused by metabisulfite co-exposure. In addition, asthmatics whose asthma is provoked by ingestion of acid metabisulfite solutions, were not supersensitive to inhaled SO_2 gas. Finally, SO_2 sensitivity did not correlate with histamine reactivity, as measured by PC_{20} (20% drop in FEV1).

In evaluating the respiratory sensitisation properties of SO_2, RAC has also considered results from human studies on healthy subjects (presented in Table 19 of the CLH report), which were used by the DS to evaluate the STOT SE hazard endpoint. These findings are summarised in the Table mentioned in the STOT-SE section of this opinion and are considered to represent signs of inflammation/irritation both of the upper and the lower respiratory tract and not hypersensitivity of the airways.

In the US EPA Report: "Integrated Science Assessment for sulfur oxides – Health Criteria" (September, 2008), the following possible mode of actions of SO_2 induced bronchoconstriction were described:

- Different mechanisms may be involved in SO_2 induced respiratory effects seen in asthmatics and non-asthmatics, as indicated by the fact that in non-asthmatics, near complete attenuation of bronchoconstriction has been demonstrated using the anticholinergic agents atropine and ipratropium bromide, while in asthmatics, these

same anticholinergic agents, as well as short- and long-acting β2-adrenergic agonists, theophylline, cromolyn sodium, nedocromil sodium and leukotriene receptor antagonists only partially blocked SO_2-induced bronchoconstriction.

- Both parasympathetic pathways and inflammatory mediators are involved in SO_2 exposed asthmatics. In asthmatic adults exposed to SO_2 following pre-treatment with cromolyn sodium (a mast cell stabilizer), atropine (a muscarinic receptor antagonist), and the two medications together, while some protection against the bronchoconstrictive effects of SO_2 was provided by both treatments individually, there was a much stronger and statistically significant effect following concurrent administration of the two medications.
- It has been proposed that inflammation contributes to the enhanced sensitivity to SO_2 seen in asthmatics by altering autonomic responses, enhancing mediator release and/or sensitizing C-fibres and RARs (Rapidly Adapting Receptors or simply Irritant Receptors). Whether local axon reflexes also play a role in SO_2-induced bronchoconstriction in asthmatics is not known.

In conclusion, based on all the above, RAC recognises that SO_2 unequivocally exacerbates existing asthma in sulfite-sensitive populations and/or asthmatics by the inhalation route. Whether SO_2 can be considered as a respiratory sensitiser itself, is not fully demonstrated. Based on the available data, all three key events (allergic sensitisation, airway inflammation and airway remodelling) involved in the observed increased airway responsiveness (main clinical effect) triggering asthma (health effect at the organism level) coexist and are difficult to differentiate. In all human studies available in the CLH report and used for respiratory sensitisation classification purposes, the study population is limited and co-exposure to other confounding factors such as particulate matter or environmental pollutants is not accounted for. Therefore, due to inconclusive data, RAC agrees with the DS and proposes **no classification for SO_2 for respiratory sensitisation**.

10.7 Skin sensitisation

For justification of Read-across from metabisulfite please refer to section 10. Moreover, case reports are published describing contact dermatitis from sodium metabilsulfite suspected to be caused by sulfur dioxide evaporated from sodium metabisulfite solutions (e.g. Jacobs and Rycroft 1995, Vallon et al. 1995) and not by direct skin contact to the solution.

Table 12: Summary table of animal studies on skin sensitisation

Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/group**	**Test substance, Vehicle, Dose levels, Route of exposure** *(topical/intradermal, if relevant)***, Duration of exposure**	**Results** *(EC3-value or amount of sensitised animals at induction dose)*	**Remarks** *(e.g. major deviations)*	**Reference**
OECD 429 GLP; Reliability: 1	Mouse (NMRI / Crl:NMRI); 6 animals/group (♀).	**Sodium metabisulfite** (purity: 99.1 %); vehicle: Application: 25 µL on the dorsum of animal's left and right ears (10 % w/w, 25 % w/w, and 50 % w/w); Duration of exposure: 3 consecutive days)	Not sensitising: all stimulation index (SI) values are under the trigger values for C&L: 10 % w/w: SI: 0.854 (cell count); SI: 0.800 (lymph node weight); SI: 0.934 (ear weight) 25 % w/w: SI: 0.970 (cell count); SI: 1.200 (lymph node weight); SI: 1.086 (ear weight) 50 % w/w: SI: 0.878 (cell count); SI: 1.171 (lymph node weight); SI: 1.020 (ear weight)	Alternative endpoints were chosen: lymph node weight, lymph node cell count, ear weight, ear thickness: modified OECD 429, method according to Ehlings et al. 2005 Study not considered a key study due to minor relevance regarding human exposure. Sufficient studies with human exposure available.	Anonymous1

Table 13: Summary table of human data on skin sensitisation (Please refer to section 12 for further details (tables and figures) on key studies.)

Type of data/report, Reliability	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
Published case series and literature review Key study	**Sodium metabisulfite** 2 %, later 1 % in petrolatum	Retrospective study with 2763 patients patch-tested between 1990 and 2010	124 (4.5 %) positive results (77F/47M), most frequently on the face and the hands, median age: 50; 13 cases (10.5 %) occupational exposure.	Garcia-Gavin et al. 2012; Contact Dermatitis 67:260-9
Published report Key study	**Sodium metabisulfite and sodium sulfite**	Prospective small study in a patient population patch tested with sodium sulfite 1 % pet. and SMB 1 % pet.	183 patients tested: 5.5 % (n=10) positive to sodium metabisulfite, 3.8 % (n=7) positive to sodium sulfite.	Oliphant et al. 2012 Contact Dermatitis 66/3:128-30
Published retrospective analysis of positive patch test cases Key study	**Sodium metabisulfite** (SMB); 1 % in petrolatum	1751 patients in a Contact Dermatitis Investigation Unit in Manchester, UK	71 (4.1 %) positive reactions, interpreted as allergic. 33/71 with identifiable source (group A), 38 with unknown sources (group B). 47	Madan, V., Walker, S.L., Beck, M.H. 2007, Contact Dermatitis; 57:173-6.

Type of data/report, Reliability	Test substance	Relevant information about the study	Observations	Reference
			cases with known sources after reanalysis (3 %). Sensitization to sodim metabisulfite from parenteral solutions and occupational exposure from food handling may account for some of the otherwise unexplained positive patch test reactions.	
Published study	**Sodium metabisulfite** 1 % in petrolatum (1 % pet.), prick and intradermal testing with 10 mg/mL If positive: subsequently tested with sodium sulfite, sodium bisulfite 1 %/5 % pet, potassium metabisulfite 1 % pet.	2894 (953M/1941F) consecutive patients Incidence of delayed hypersensitivity (type IV allergy) in patients with eczematous dermatitis	50/2894 (1.7 % positive reactions), also positive after potassium metabisulfite and sodium bisulfite but only 2 (4 %) positive after sodium sulfite, no positive reaction after prick test or intradermal test or oral challenge with 30 and 50 mg sodium metabisulfite - 7 patients with occupational contact.	Vena et al. 1994; Contact Dermatitis. 31:172-5
Published retrospective study at Department of Occupational and Environmental Dermatology in Stockholm, Sweden	**Sodium metabisulfite** 2 % in petrolatum	1518 consecutive patients (839; 55.3 % F; 679, 44.8 % M) patch test	51/1518 patients (3.4 %) reacted positive to SMB	Kaaman et al. 2010; Contact Dermatitis. 63: 110-112
Retrospective case review	**Sodium metabisulfite** 2 % in petrolatum	1751 patients are patch tested to the standard series, including SMB [1 % in petrolatum (pet.)].	71/1751 patients (4.1 %) reacted positive to SMB	Madan et al. 2007. Contact Dermatitis. 63/2:110-112
Published study	**Sodium metabisulfite, potassium metabisulfite, sodium bisulfite (and tartrazine)**	40 patients with clinical diagnosis of chronic urticaria: 29 F; 7M; 4-62 y	63.8 % (23/36) in positive oral challenge tests36.1 % (13/36) to sodium metabisulfite, 33.3 % (12/36) to sodium bisulfite and 30.5 % (11/36) to potassium metabisulfite. (47.2 % (17/36) positives to tartrazine)	Jimenez-Aranda et al. 1996 Rev Allerg Mex 43/6:152-6
Case reports (probably IgE-mediated allergic reactions)				
Published case report	**Potassium metabisulfite**	Case report and double blind placebo-controlled food challenge, 49 y male patient	Case of severe hypotension after food ingestion Anaphylaxis following potassium metabisulfite challenge (300 mg; approx.. 4 mg/kg bw)	Cifuentes et al. 2013; Int. Arch. Allergy Immunol. 162/1:94-6

Type of data/report, Reliability	Test substance	Relevant information about the study	Observations	Reference
Published case report	**Sulfites** in food and wine, challenge with potassium metabisulfite: 1, 5, 10, 25, 50, 100 mg in capsules, skin prick test with 10 and 1 mg/mL	F, age: 22 y with reported 2-year-history of episodes of urticaria-angiooedema Challenge test: sequential admini-stration at 30 minutes intervals, FEV_1 and blood pressure determined every 10 min	Urticaria and angioedema of face, neck, upper thorax, disphonia without asthma skin prick test: negative oral challenge: positive at 25 mg dose: urticaria on face and upper thorax after 12 min, nasal itching, rhinorrhoea, dysphonia, relief of symptoms after s.c. adrenaline injection Prevalence in asthmatics: 2 - 6 %	Belchi-Hernandez et al. 1993; Ann Allergy; 71/3:230-2
Published case reports	**Potassium metabisulfite** 10 and 50 mg oral challenge	4 asthmatic patients acutely sensitive to potassium metabisulfite (present in restaurant food)	Severe wheezing, chest tightness, flushing, weakness + 1 case of generalised urticarial, angioedema of the tongue and constriction of the chest.	Gillman 1982; Epitomes – Allergy 137/2: 120-1
Published case report	**Sodium metabisulfite** 1 % in patch test	54 y, M with 6-week history of a non-pruritic rash affecting axillae and groins following food consumption in restaurants	Positive reaction to 1 % SMB in patch test According to authors: first reported case of type IV allergy following consumption of high-sulfite diet.	Cussans et al. 2015 Contact Dermatitis; Jun 2015 (epub)
Case report + oral and skin provocative tests	**Sodium metabisulfite** 10 mg/mL in PBS in patch test; Prick and intradermal testing with 10-fold serial dilutions. Oral challenge: 1, 5, 10, 25, 50, 100, 200 mg SMB	34 y, F with of metabisulfite-induced anaphylaxis : convincing evidence of an IgE-mediated mechanism of action	Symptoms: urticaria, angioedema, nasal congestion, and apparent nasal polyp swelling following provocative challenge with sodium metabisulfite. Skin test to metabisulfite was positive as was a basophil histamine release test when the patient's cells were incubated with metabisulfite. Oral challenge test with 50 mg SMB resulted in angioedema, urticarial, nasal congestion. After 3^{rd} oral challenge reactions occurred already with 1 mg SMB and 10 mg SMB in 4^{th} and 5^{th} oral challenge after 6 months.	Sokol and Hydick, 1990 Annals of Allergy 65:233-238

Type of data/report, Reliability	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
Case report	**Sodium metabisulfite; oral challenge with single dose of 50 mg SMB**	47 y, M with recurrent severe episodes of acute urticarial, angioedema and dyspnoea. + Placebo-controlled oral challenge test with 50 mg SMB resulted in an acute urticaria attack.	Placebo-controlled oral challenge test with 50 mg SMB resulted in an acute urticaria attack.	Wüthrich et al. 1993. Dermatology 187: 290-292

Reliability of the studies is "not assignable" according to Klimisch, because no OECD guideline was followed. However, all listed studies are considered reliable from the scientific point of view. PEFR: Peak expiratory flow rates, PD_{20} FEV_1: provocative dose that produce 20 % decrease in FEV_1, SMB: Sodium metabisulfite.

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

Sodium metabisulfite is widely used as an antioxidant in oral, topical, and parenteral pharmaceutical formulations; it is also widely used in food products (Rowe et al., 2009) and cosmetics (Nair and Elmoore, 2003). Although it is extensively used in a variety of preparations, sodium metabisulfite and other sulfites have been associated with a number of severe to fatal adverse reactions. There are reports of hypersensitivity, anaphylaxis, and even death from Kounis syndrome from sulfite administration (Kounis et al., 2014). Anaphylactoid shock has been reported during epidural anesthesia for cesarean section, in which the responsible agent was metabisulfite, as additive agent of adrenaline-containing local anesthetic (Soulat et al., 1991, cited in Kounis et al., 2014). The reactions are usually hypersensitivity-type reactions and include bronchospasm, angioedema, anaphylactoid reactions, urticaria, and asthmatic attacks (Jacobs and Rycroft, 1995; Wüthrich et al, 1993). Allergy to sulfite antioxidants is estimated to occur in 5–10 % of asthmatics, although adverse reactions may also occur in non-asthmatics with no history of allergy (Rowe et al., 2009). According to Nair and Elmoore (2003) between 2 % and 5 % of asthmatics are sulfite-sensitive.

Sokol and Hydick (1990) reported a case of a patient with a history of allergic rhinitis who demonstrated anaphylactic clinical reaction to sodium metabisulfite after eating a restaurant meal. The patient demonstrated urticaria, angioedema, nasal congestion, and apparent nasal polyp swelling following provocative challenge with sodium metabisulfite. Skin test to metabisulfite was positive in all cases (Sokol and Hydick, 1990). Sokol and Hydick presented also a literature review of allergic IgE-mediated reactions in sensitive individuals. IgE mediated nature of basophil activation was also detected in patients with sulfite intolerance (Saint-Laudy et al., 1994). Wüthrich and Huwyler (1994) suggested also IgE-dependent mechanism of allergic reactions in their patients, while Belchi-Hernandez et al. (1993) found that IgE mediated mechanism was not involved in eliciting of urticaria -angioedema, nasal itching, rhinorrhea, and dysphonia in a patient who consumed sulfite containing foods and drinks. They believe that the stimulation of cholinergic receptors, either directly by sulfites or by accumulation due to partial sulfite oxidase deficiency, could cause the clinical manifestations of sulfite-induced allergic reactions (Belchi-Hernandez et al., 1993). The IgE-mediated allergy has not been demonstrated either in a recent study in a patient who reacted with anaphylaxis (severe hypotension) after consuming sulfite containing foods (Cifuentes et al., 2013). The patient reacted with anaphylaxis to potassium metabisulfite in an oral provocative test. In the last study, the patient had a diagnosis of monoclonal mast cell activation syndrome (MCAS) and therefore, the authors suggest that monoclonal MCAS may be involved in the mechanism of sulfite-intolerance (Cifuentes et al., 2013).

Other cases of severe, life-threatening asthmatic and urticarial reactions are described in asthmatic patients after ingestion of wine, salads and other foods containing sulfites (Gillman, 1982; Wüthrich and Huwyler, 1989; Wüthrich et al., 1993; Jiménez-Aranda et al., 1996). All the patients with chronic urticaria and asthma reacted positively to sodium metabisulfite in oral provocation test. Furthermore, a case of a type IV systemic allergic reaction to dietary sulfites is reported in a patient who had a high dietary intake of sulfite-rich foods (Cussans et al., 2015).

Roberts et al. (2012) proposed a probable mechanism for the in cutaneo modification of proteins by sodium metabisulfite which involves the sulfite di-anion acting as a nucleophile towards electrophilic centres in proteins. This is a rare mechanism, as most known skin-sensitizing chemicals behave as electrophiles.

Sodium metabisulfite is present as antioxidant in oral, topical and parenteral medicines (Riemersma et al., 2004). In this regard, several cases of contact allergic reaction to local anesthetics containing sodium metabisulfite are reported in sensitive persons: two cases of Burning Mouth Syndrome in two patients who underwent several dental interventions (Levanti et al., 1996) and in a patient receiving an anaesthetic injection for a biopsy (Riemersma et al., 2004). Other cases of contact sensitivity have been attributed to the use of hydrocortisone, hydroquinone (i.e. bleaching cream), ketoconazole creams as well as Trimovate ® and Timodine ® creams, in which sodium metabisulfite serves as a preservative (Madan et al. 2007; Huang and Chu, 2007). Sodium metabisulfite produced positive reactions in a patient under patch test after use of cosmetic creams (Malik et al., 2007).

Cases of occupational contact dermatitis are described in photographers, in a pharmaceutical technician, baker, caterer, salad maker, wine producer, agronomist, carpenter, chemical factory worker, radiographer and hairdresser (Vena et al., 1994; Jacobs and Rycroft, 1995; Lee and Nixon, 2001; Merget and Korn, 2005; Madan

et al., 2007; Aalto-Korte et al., 2009; Madan and Beck, 2009; Sasseville and El-Helou, 2009). All the patients reacted positively to sodium metabisulfite in patch test. Madan and Beck (2009) believe that allergic skin reactions point more to a diagnosis of contact allergy rather than irritancy.

The incidence of delayed hypersensitivity reactions to sodium metabisulfite was investigated in several studies in a large number of patients. Vena et al. (1994) present results of patch testing of 2894 eczematous patients. Positive patch test to sodium metabisulfite was considered to be high: 50 out of 2894 subjects (1.7 %) reacted positively (Vena et al., 1994). The dermatitis was considered to be occupational in 7 cases, while only 5 out of the 43 non-occupational cases were considered to be relevant. According to the authors, the relevance of positive reactions is difficult to establish due to the ubiquity of the substance in drugs and foods. In another study of Madan et al. (2007) 71 out of 1751 patients (4.1 %) reacted positively to sodium metabisulfite, whereby 33 (46.5 %) were originally reported as relevant and 38 (53.5 %) were of unexplained relevance. A careful re-analysis of data by the authors revealed a higher incidence of potentially relevant cases due to sodium metabisulfite as it firstly was interpreted: cases of 47 patients were retrospectively regarded as relevant (instead of 33 after first analyses). In 2007, patch test positivity of 6.6 % (8 persons) to sodium metabisulfite was described in a consecutive series of 117 patients in Ireland (Malik et al., 2007; Davies and Johnson, 2011). 4 cases were considered relevant (3.4 %). In a retrospective study in 1518 patients with hand eczema, sodium metabisulfite produced positive reaction in 3.4 % of subjects in patch test (Kaaman et al., 2010). The majority of incidences could probably be ascribed to occupational exposure, although the relevance of positive cases was difficult to establish because not all patient records enabled a complete evaluation. In a cross-sectional study in 63 workers with occupational contact dermatitis at two Indonesian tanneries, sodium metabisulfite was found to be occupationally relevant sensitizer (2.6 % persons showed positive skin reactions) (Febriana et al., 2012). Garcia-Gavin and coworkers (2012) analysed results of patch testing of patients from 1990 to 2010 in a retrospective study and found that 124 (4.5 %) of 2763 were positive to sodium metabisulfite. Of these, 76 persons (61.3 %) reacted only to sodium metabisulfite, while the others presented one or more concomitant positive test reactions. The reactions were considered relevant in 80 cases of which 11 were occupational. A relationship of allergenicity of sodium metabsiulfite and sodium sulfite was investigated in a study with 180 patients (Oliphant et al., 2012). The authors found that the majority of patients with positive reactions to sodium metabisulfite were also positive to sodium sulfite. It should be mentioned that sodium metabisulfite is part of the standard series of substances used in patch testing (e.g. Madan et al. 2007).

10.7.2 Comparison with the CLP criteria

The following table presents the critical results for skin sensitisation used for classification and labelling and further lists the criteria required from CLP regulation.

Toxicological result	**CLP criteria**
Sulfur dioxide: Elicitation and Sensitisation cannot be differentiated on the basis of available data. Value for elicitation/sensitisation: dermal: ≤ 1 % (lower concentrations not tested in patch test), no elicitation at 10 mg/mL in prick/intradermal testing. Values represent LOAELs from a considerably study population in peer reviewed scientific journals. High frequency of occurrence in humans (2-5% unselected patients) and 5–10 % of asthmatics According to CLP, high frequency of occurrence: ≥ 1 % in unselected dermatitis patients,) classifies as Skin Sens 1A.	<u>Category 1 :</u> – if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons, or – if there are positive results from an appropriate animal test <u>Sub-category 1A :</u> – Substances showing a high frequency of occurrence in humans; or a probability of occurrence of a high sensitisation rate in humans based on animal or other tests. Severity of reaction may also be considered high frequency criteria :. - 0.2 % general population study - 1.0 % dermatitis patients (unselected, consecutive) - 2.0 % selected dermatitis patients (aimed testing, usually special test series).

Toxicological result	CLP criteria
	Sub-category 1A (non-human data) : – GPMT : ≥ 30 % responding at ≤ 0.1 % intradermal induction dose, or ≥ 60 % responding at > 0.1 % to ≤ 1 % intradermal induction dose <u>Sub-category 1B :</u> – Substances showing a low to moderate frequency of occurrence in humans; or a probability of occurrence of a low to moderate sensitisation rate in humans based on animal or other tests. Severity of reaction may also be considered. Sub-category 1B (non-human data) : – GPMT : ≥ 30 % to < 60 % responding at > 0.1 % to ≤ 1% intradermal induction dose, or ≥ 30 % responding at > 1 % intradermal induction dose

10.7.3 Conclusion on classification and labelling for skin sensitisation

The DS proposes classification of Sulfur dioxide as Skin sens. 1.

According to the guidance on the Application of CLP criteria (section 3.4.2.2.1.2., page 336, 2017), "when considering human evidence, it is necessary to take into account the size of the population exposed and the extent of exposure and frequency, and thus the consideration is on a case by case basis".

However, the extent of exposure and the frequency of occurrence of allergic reactions in the general population cannot be established due to lack of information. Regarding the ubiquity of the substance in drugs, foods and cosmetics, a high extent of exposure to sodium metabisulfite can be assumed.

Even though the CLP criteria for unselected dermatitis patients are fulfilled and might require subcategorization to 1A, no subclassification is proposed on the basis of the afore mentioned assumption.

Furthermore, the available data are based on metabisulfite. As sulfur dioxide is a gas, skin sensitisation would be expected for an aqueous solution of sulfur dioxide due to the formation of (bi-)sulphite under such conditions. Positive reactions with sodium metabisulfite were predominantly observed after testing a 1 % solution in petrolatum. Classification for skin sensitisation category 1 is thus proposed for sulfur dioxide aqueous solutions.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

This CLH report was based on the assessment of SO_2 as a biocidal active substance and thus includes all studies submitted by the applicant(s) or included by the applicant(s) into the dossier on request by the authority. Although there are more skin sensitisation studies

available from the open literature for SO_2, the DS argued that these would not have an impact on the classification proposal and consequently have not been included.

The DS proposed to read-across data from sulfites (mainly sodium metabisulfite) to SO_2 and therefore these studies were included in their evaluation.

Positive reactions with sodium metabisulfite were predominantly observed after testing a 1% solution in petrolatum. As SO_2 is a gas, skin sensitisation would be expected for an aqueous solution of SO_2 due to the formation of (bi-)sulfite under such conditions.

Analyses of human patch tests with sodium metabisulfite in different populations of patients formed the basis for the classification proposed by the DS according to the criteria of the CLP Regulation.

Case reports are discussed separately by the DS as probable IgE-mediated allergic reactions, in order to differentiate in the assessment from pseudo-allergic food intolerances or food allergy.

Human studies on the relevant dermal route were distinguished from studies on the oral route. Dermal sensitisation (type IV reaction, patch test) was distinguished from mechanistically different IgE-mediated type I reactions (prick test) and the proposal for classification was based on relevant human data as required by the CLP Guidance.

The DS regarded the only animal study available, a modified LLNA test, as less relevant, and therefore data from human patch tests were given priority in the DS's assessment.

Although the frequency of dermal allergic reaction from some reports on patch tests (4.5% in Garcia-Gavin *et al.,* 2012, 5.5% in Oliphant *et al.,* 2012, and 4.1% in Madan *et al.,* 2007) is not low, the DS is of the opinion that the extent of exposure and the frequency of occurrence of allergic reactions in the general population cannot be established with accuracy due to lack of information. Based on the ubiquity of the substance in drugs, foods and cosmetics, a high extent of exposure to sodium metabisulfite can be assumed.

Therefore, since "when considering human evidence, it is necessary to take into account the size of the population exposed and the extent of exposure and frequency, and thus the consideration is on a case by case basis" (Guidance on the Application of CLP criteria, section 3.4.2.2.), and even though the CLP criteria for unselected dermatitis patients are fulfilled and might justify sub-categorization to 1A, no sub- categorization is proposed by the DS on the basis of the aforementioned assumption. Consequently, classification for skin sensitisation category 1 is proposed for aqueous solutions of SO_2.

Comments received during consultation

Four (4) comments were submitted during consultation, all coming from Industry (IND).

The Sulfuric Acid REACH Consortium (SAC), which also represents the REACH Lead Registrant for SO_2 (LR), claimed that the DS had cited in the CLH report an arbitrary selection of references on human case reports, thus rendering this assessment essentially incomplete. In addition, SAC reported that the DS had omitted to verify whether the criteria for actual sensitisation are met in the studies the CLH proposal refers to. SAC also made

reference to the scientific opinions of several reputable scientific organisations (including EFSA) which altogether do not conclude that there is a concern for sensitisation. In conclusion, both SAC and the LR are of the opinion that the classification criteria for skin sensitisation are not met.

AFEPASA (Azufrera y Fertilizantes Pallarés, S.A.U.) and another IND representative (name confidential), raised the following points in support of no classification:

(i) the lack of differentiation between "contact allergy and hypersensitivity"

(ii) the existence of numerous reliable reports confirming the lack of skin sensitisation (SCF (1997), SCCNFP (2003), CIR (2003), EFSA, 2004, MAK (2014), EFSA (2016), OECD SIDS (2001))

(iii) IgE mediated reactions have been discussed but were never confirmed

(iv) the very low prevalence of susceptible individuals with sulfite oxidase deficiency

(v) the absence of epidemiological study on the general population

(vi) disodium disulfite was evaluated in 2015 by the MS Hungary who concluded that it is "unlikely that disodium disulfite is a skin sensitiser,"

(vii) EFSA 2016 conclusion that "IgE tests were usually negative indicating that the reactions were not immune-mediated, and sensitivity reactions were mostly intolerance reactions".

Micro-Pak Europe BV (IND representative) questioned the applicability of the case reports listed in the CLH report on acute, immediate-type systemic reactions after sulfite exposure via injection of sulfite-containing anaesthesia or via ingestion of sulfite-containing food or wine. Furthermore, cases of occupational contact dermatitis in photographers, in a pharmaceutical technician, baker, caterer, salad maker, wine producer, agronomist, carpenter, chemical factory worker, radiographer and hairdresser are poorly described in the CLH report. In order to show the potential of sulfites to induce systemic pseudo-allergic effects, including symptoms visible on the skin, a robust evaluation of the dataset for clear indications for the induction of skin sensitization as prerequisite for delayed-type allergic contact dermatitis is required and is critical for the evaluation of SO_2. Industry is of the opinion that pseudo-allergic food intolerances, i.e. mimicking symptoms of allergy but with no underlying specific immune-mediated responses as e.g. as described by the WHO (WHO IPCS, Guidance for Immunotoxicity Risk Assessment for Chemicals, 2012) is the dominant mode of action for the clinical manifestations observed. The potential to induce systemic non-immune intolerances after other than dermal exposure does not meet the CLP criteria for classification of a substance as Skin Sens. In line with this, sodium metabisulfite has been evaluated as not sensitizing by the MS Hungary (CoRAP report, 2014), supported by earlier evaluation of inorganic sulfites e.g. by the SCCNFP (2003) and the German MAK Commission (1997, 2014). Industry noted that none of the human studies provide any indication for the induction of dermal responses after contact with SO_2. As SO_2 is a gas under standard conditions with a considerable high vapor pressure, skin penetration and thus dermal bioavailability as prerequisites for the induction of skin sensitization can reasonably be expected to be negligible. Finally, no animal study exists that indicates any

skin sensitizing potential of inorganic sulfites. A modified local lymph node assay (LLNA) in mice, conducted according to OECD TG 429 and under GLP conditions, on sodium metabisulfite is mentioned in the CLH report, yielding a clear negative result for this substance. This is supported by a negative result obtained for sodium metabisulfite in a standardized test for skin sensitization in guinea pigs, reported in the OECD SIDS report on sodium metabisulfite (OECD, 2001). Thus they concluded that appropriate predictive animal tests consistently indicate the absence of a skin sensitization potential of inorganic sulfites.

The DS clarified that the CLH report was based on the assessment of SO_2 as a biocidal active substance and thus it included all studies submitted by the applicant(s) or included by the applicant(s) in the dossier on request of the evaluating authority. Furthermore, in the CLH report, human studies on the dermal route, which is relevant for classification, were distinguished from studies on the oral route. Dermal sensitisation (type IV reaction, patch test) was separated from mechanistically completely different IgE-mediated type I reactions (prick test) and the medical assessment performed by dermatologists in clinics is not to be questioned. Case reports are listed in the CLH report in a separate section.

The DS is of the opinion that the various skin sensitisation studies available from the open literature for SO_2 would not change the classification proposal. More importantly, recent data in 12156 patients (Uter *et al.*, 2018) report a sensitisation rate of 3% and other studies in the CLH report high sensitisation frequency 3-6% (Garcia-Gavin *et al.*, 2012; Oliphant *et al.*, 2012; Madan *et al.*, 2007).

For sodium metabisulfite, the DS claimed that it is a standard allergen included in testing baseline series number 38 for preservatives of the German Contact-Allergy-Group (DKG). The studies cited by the IND, for which confirmation on IgE-mediated reactions is uncertain (Sokol and Hydick (1990), Wüthrich and Huwyler (1994), Hernandez *et al.* (1993) did not evaluate ACD – Allergic Contact Dermatitis) were, according to the DS, all much older and performed under previous guidelines and therefore not used in this CLH report.

Regarding the issue raised by IND on pseudo-allergic food intolerances and food allergy, the DS explained that these manifestations are mediated by different routes and represent different mechanisms of action:

- Food allergy: IgE by plasma cells, mast cells release vasodilating factors, anaphylaxis)
- Skin allergy: less to no IgE and mast cells increase; killer cells, macrophages cause eczema

For the majority of skin allergy-causing substances (not inducing rare cases of cross-reactions), IgE tests are negative. Therefore, a lack of increase in IgE does not necessarily indicate the absence of a skin sensitisation potential.

The DS explained that in the EFSA report, the only study on skin allergy mentioned is the one by García-Gavín *et al.* (2012), and quoting EFSA's assessment on page 68, the said study "reported that 124 (4.5%) of 2,763 patients patch tested positively to sodium metabisulfite. A total of 13 cases (10.5%) were occupational with 10 of them presenting hand eczema. Sodium metabisulfite was the single allergen found in 76 cases (61.3%). The reactions were considered to be relevant in 80 cases (64.5%), of which 11 were

occupational." Therefore, the DS found the assessment of the García-Gavín study in the CLH dossier completely in agreement with EFSA's.

The DS also explained that the only animal LLNA study was regarded as less relevant, and therefore data from human patch tests was given priority in the DS assessment. Reference is made also to the CLP Guidance Chapter 3.4.2.2.6. Decision logic for classification of substances.

Assessment and comparison with the classification criteria

Read-across from sulphites

All data on skin sensitisation included in the CLH report (both animal and human) refer to studies with sulphites. RAC considers, as explained in a previous section of the present opinion, that read-across from sulfites is justified for systemic routes of exposure.

Regarding read-across for dermal exposure which is relevant for the evaluation of skin sensitisation, there is no direct evidence that a gas, such as SO_2, which is a very common environmental pollutant and air impurity in industrial settings, can lead to sufficient concentrations of sulfites on the skin to cause sensitisation.

Two factors could theoretically affect the formation of sulphites from SO_2 on the skin: the SO_2 concentration in the air and the water availability in the skin.

Skin has three layers: the epidermis, the outermost layer of skin, provides a waterproof barrier and creates the skin tone. The dermis, beneath the epidermis, contains tough connective tissue, hair follicles, and sweat glands. The deeper subcutaneous tissue (hypodermis) is made of fat and connective tissue. In general, the water content of the epidermis and the dermis is approximately 20% of the water in the inner milieu of the body, with 60–70% of this amount being accumulated in the dermis (Kacalak-Rzepka *et al.*, 2008). Water from the deeper epidermal layers moves upward to hydrate cells in the outermost skin layer, the stratum corneum, and is eventually lost to evaporation. Then, an evaporation barrier is needed to maintain body water homeostasis. Variable skin pH values are reported in literature, all acidic but with a broad range from pH 4.0 to 7.0, with pH values below 5.0 being optimum (Lambers *et al.*, 2006). Such conditions could favour the transformation of gaseous SO_2 to sulfites in the skin, in case SO_2 could permeate the skin.

Permeation of SO_2 through skin and the consequences of dermal exposure need further consideration. According to a recently published study, no evidence of skin absorption or penetration was found following exposure to SO_2 at 100 ppm for up to 30 min exposure. The surface of skin exposed to 3000 ppm of SO_2 for up to 30 mins showed negligible skin absorption or penetration. Fresh air ventilation following exposure of bare skin did not reduce the skin load. The influence of temperature and relative humidity on skin absorption and penetration was also negligible. The barrier integrity remained intact with no reduction in electrical impedance following exposure to 3000 ppm of SO_2 for 30 min (Gaskin *et al.*, 2019).

Therefore, although the conditions of water availability and pH in the skin could favour transformation of SO2 to sulfites there is no evidence that SO2 is available at the concentrations required to form sufficient quantity of sulfites to cause an effect.

Regarding other skin effects of SO_2, it should be noted that SO_2 is classified as skin corrosive, category 1B. Whether SO_2 itself was shown to be corrosive or, read-across from sulfites or H_2SO_4 was considered in the originally classification, is unclear. No evidence could be retrieved on the rationale for this previous classification of SO_2. It is noted that for the formation of H_2SO_4 (sulfuric acid, as opposed to H_2SO_3, sulfurous acid), an additional oxidation step is required, while sulfites have not been shown to be corrosive.

A corrosive "mode of action" is very different from a sensitising substance. A corrosive substance would destroy the material it contacts with, rather than penetrating through the material. For the effects of corrosivity to be noticed, the chemical would not need to go as deep in the dermis to cause an effect, as it would need to go in case of skin sensitisation, where it needs to completely traverse the skin to activate the immune system. Based on the above it could be explained why a substance can be skin corrosive but not skin sensitiser.

Animal testing

There is no animal data in the literature regarding effects of SO2 on the skin. The reason is likely due to the physical state of SO2 (gas).

Epidemiological data

Epidemiological data on SO_2 skin effects retrieved by RAC are not based on patch testing or other diagnostic protocols in dermatological clinics but are rather descriptive reports. These epidemiological data are circumstantial and not according to the specifications set in the Guidance for Application of CLP criteria, version 5.0.

More specifically, large occupational cohorts (n > 100000 workers) included in the CLH report for the evaluation of the carcinogenicity and mutagenicity endpoints (Tables 16 and 18 of the CLH report) do not report any skin effects or contact dermatitis for workers. Although such effects may not have been the subject of observation and reporting, any such effects would be clearly visible, despite the possible use or not of personal protective equipment by workers. Hence, the absence of reporting on skin effects or contact dermatitis on such a large cohort provide an indication that SO_2 would not be a skin sensitiser.

Similarly, in a recent review article, where several studies have looked into the relationship between traffic-related air pollutants (TRAP) exposure (including SO_2) and the development of atopic dermatitis and aeroallergen sensitization, no specific reference to SO_2 effects is made, while various limitations are presented in making a firm conclusion about the causative link between air pollution and atopic disease (Hassoun *et al.*, 2019). In addition, when the association between Asian Dust (AD)-borne air pollutants (including SO_2), and daily reported subjective symptoms on the skin in 42 healthy subjects was investigated in Japan, no significant correlation was observed between SO_2 and skin symptoms (e.g. rash, itching, etc.), although the daily skin scores were statistically higher in days with AD prevalence (Majbauddin *et al.*, 2016).

On the other hand, in a random sample of Chinese pupils (n=2335) enrolled in a two-year follow-up of a cohort with repeated questionnaires, outdoor concentration of SO_2 was positively associated with new onset of dermal symptoms (facial and hand rash or itching; eczema) (Zhang *et al.*, 2014).

Furthermore, association between environmental factors in Turkey (air monitoring parameters measured for the Turkish national air quality network: particulate matter PM_{10}, SO2, air temperature, air pressure and relative humidity) and outpatient clinic visits for eczema is published in the literature. More specifically, data on dermatology clinic outpatient visits for eczema in Düzce province, Turkey, between January 2013 and July 2019, show that SO_2 atmospheric values, after adjusting for temperature and PM_{10} (particulate matter) values, had significantly positive effects on the number of daily outpatient visits over a total 5 days of lag after adjusting for temperature (5.34%) (Karagun *et al.*, 2020).

In addition, two case reports on sodium metabilsulfite exposure, included in the CLH report, describe contact dermatitis located in parts of the body, where direct skin contact to the metabisulfite solutions themselves could not have occurred. Therefore, the authors of the studies reported that contact dermatitis is suspected to be caused by SO_2, which was evaporated from these sodium metabisulfite solutions, and reached the skin (Jacobs and Rycroft 1995; Vallon *et al.* 1995).

However, RAC does not consider that the cases described in (Zhang *et al.*, 2014), (Karagun *et al.*, 2020) and (Jacobs and Rycroft 1995; Vallon *et al.* 1995) provide sufficient and clear evidence to dispute the absence of reported on skin effects or contact dermatitis from the large cohort of workers (n > 100000 workers).

In conclusion, RAC recognises the fact that no measurements are available on the extent, if any at all, of SO_2 transformation to sulphites on the skin and that no relevant mechanistic evidence is provided in the literature to support read-across from sulfites. Epidemiological data on SO_2 exposure are abundant and do not report skin sensitisation effects due to SO_2 dermal exposure.

Therefore, RAC concludes that read-across from sulphites is not substantiated and based on the available data on SO_2, **no classification of SO_2 for skin sensitisation** is warranted.

10.8 Germ cell mutagenicity

For justification of Read-across from metabisulfite, please refer to section 10.

Table 14: Summary table of mutagenicity/genotoxicity tests *in vitro* (Further details on key studies, such as data tables and figures, are provided in section 12 for clarity.)

Method, Guideline, GLP status, Reliability	**Test substance, Doses**	**Relevant information about the study (e.g. cell type, strains)**	**Results**	**Remarks** (e.g. major deviations)	**Reference**
Sulphite, bisulfite, metabisulfite					
In vitro gene mutation studies in bacteria					
Bacterial reverse mutation assay, similar to OECD 471 (1983) Non-GLP Rel. 2 (reporting deficiencies) Key study	Plate incorporation method: 0.3-3.3-33.3-100-333.3-1000-3333.3-10000 µg/plate **sodium metabisulfite** (± S-9 mix) Positive controls: Concurrent positive controls were run with each test in compliance with OECD 471 (see remarks)	*S. typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, TA 100 *E. coli:* WP2 (uvrA) Solvent: 0.067 M potassium phosphate buffer, pH 7.0	**Negative** **Cytotoxicity :** TA 1535: –S9: toxic range 100 - 10,000 µg/plate; +S9: toxic range 3,333 - 10,000 µg/plate TA 100: –S9: toxic range > 333.3 µg/plate +S9: only at the highest doses tested. WP2: +S9: Toxicity at the highest dose tested. No observed toxicity +/-S9	Difference in outcome to study performed by Pagano and Zeiger (1987) might be due to pH-effects (here: neutral pH) because apparently mutation was observed in medium with pH 5-6. No purity (but batch) is given in the report. Only two plates per concentration (SD not available; cytotoxicity difficult to determine). Positive controls for strains TA 1537 and TA1538 (+S9) were reported in the publication - Prival et al. 1991, but not in the study report	Simmon, V.F. and Eckford S.L. (1978), NTIS Report PB89-193684 Published as Prival et al. 1991; Mutation Research 260: 321-329

Method, Guideline, GLP status, Reliability	**Test substance, Doses**	**Relevant information about the study (e.g. cell type, strains)**	**Results**	**Remarks** (e.g. major deviations)	**Reference**
Gene mutation in vitro and host-mediated assay; non-guideline Non-GLP; Reliability: 2 (no test guideline, missing relevant tester strains). Key study	**Sodium metabisulfite**; 5 % (w/v) for *in vitro* part host mediated assay: single and repeated dose (5 days): 30, 700, 1200 mg/kg bw	*Salmonella typhimurium* G 46, TA 1530 Host animals: random-bred Swiss-Webster male mice (28 – 30 g bw), 10 mice/treatment group	**Negative** *in vitro* and in host-mediated assay	Positive control: 0.1 % EMS: ok Well conducted early non-guideline study	NTIS 1972 NTIS Report PB221825 Published as Maxwell and Newell; Mol. Environ. Aspects Mutagenesis, Proc. Publ., Rochester Int. Conf. Environ. Toxic. 6th, 223-252, 1974
Bacterial reverse mutation assay, complies with OECD 471 (1983) Non-GLP Rel. 2 (Reporting Deficiencies) Key study	Preincubation test: 500 µL of **sodium metabisulfite** dilutions up to 0.64M in sodium phosphate buffer (pH 5, 6, 7, or 8)	*S. typhimurium:* G46 (Target *his*G46): TA92, TA1535, TA100, SB2802, SB2061, TR3243 (Target *his*D6610): , TA88, TA110, TA90, TA97, D3052 (Target *his*D3052), TA1538, TA98, C3076 (Target *his*C3076), TA1537, TA1977 (strains recommended by OECD 471 are labelled)	**Positive** – slight but dose-related increase in # of revertants – increase < 2-fold, with 60 min incubation, >2-fold after 90 or 120 min incubation) Reproducible weak mutagenic response in *S. typhimurium* strains carrying the *his* D6610 or *his*G46 mutations. Peak mutagenic response in G46 stains at 0.1 M and in TR3243 at 0.3 M. Number of induced revertants per dose, the ***his*D6610** site was most responsive, with **TA 97** being the most active. Mutagenic response highest with 0.1 M sodium phosphate buffer at pH 5.0-6.0. Base-pair substitution and frameshift mutations Base-pair substitution (deamination of cytosine): At higher concentrations (1 M): cytosin bisulfite adducts leading to base substitution At lower concentrations (approx. 0.01 M) deamination of cytosine via oxidative damage assumed.	Examination of conditions under which sodium bisulfite is mutagenic (pH 5-6, phosphate buffer, see results). Reporting deficiencies (e.g. purity of test substance lacking)	Pagano and Zeiger (1987). Mutation Research 179: 159-166

Method, Guideline, GLP status, Reliability	**Test substance, Doses**	**Relevant information about the study (e.g. cell type, strains)**	**Results**	**Remarks** (e.g. major deviations)	**Reference**
Bacterial reverse mutation assay, OECD 471 (1983) Non-GLP Rel. 2 (relevant tester strain missing)	Standard plate test: 0-20-100-500-2500-5000 µg/plate **96-98 % pure sodium sulfite (anhydride)** (± S-9 mix) Preincubation test: 0-20-100-500-2500-5000 µg/plate **96-98 % pure sodium sulfite (anhydride)** (± S-9 mix)	*S. typhimurium:* TA 1535, TA 1537, TA 98, TA 100 Solvent: phosphate buffer, pH not reported	**Negative** **Cytotoxicity:** Only very slight bacteriotoxic effects were deduced from slight dose-related decrease in the number of revertants.	Missing tester strain: *S. typhymurium* TA 102 or *E. coli* WP2 uvrA (pKM101). Efficacy of S9-mix only tested with 2-aminoanthracene. It has to be noted that the test substance was dissolved in aqua dest. which results in alkaline conditions	Engelhardt, G. (1989), Project No.:40M0639/884492
Bacterial reverse mutation assay, similar to OECD 471 Non-GLP Rel. 2 (No positive control)	Preincubation test: Max. non-cytotoxic dose: 50 mg/plate 95 % pure **sodium metabisulfite (anhydride)** in phosphate buffer (± S-9 mix)	*S. typhimurium:* TA 92, TA 1535, TA 1537, TA 94, TA98, TA 100 Solvent: phosphate buffer, pH not reported	**Negative** Highest dose without observed cytotoxicity: 50 mg/plate. No results for cytotoxic doses presented.	Screening of various substances, no positive controls used but substances with positive results indicate functioning of test system. No negative controls (but many substances tested negative). No titer given. No individual number of colonies (mean, SD) or number of plates per dose given.	Ishidate et al. 1984; Fd Chem Toxic 22/8: 623-36
Bacterial reverse mutation assay, Non-guideline, non-GLP Rel. 2 (non-guideline study)	**Sodium bisulfite** ($NaHSO_3$) 0, 0.1, 0.5, 1.0, 1.5, 2.0 M	*S. typhimurium:* G46 (Target *his*G46), TA92, TA1950, TA2410, TS24, GW19	**Positive** for *S. typhymurium* strains carrying hisG46 allele, greater mutagenic response in strains with wild-type DNA repair capacity	Preincubation and pH<7 required for a positive test result. Very high concentrations used.	De Giovanni-Donnelly (1985), Teratogenesis, Carcinogenesis, and Mutagenesis 5: 195-203
Bacterial reverse mutation assay with various E. coli mutants, non-guideline, non-GLP Rel. 4 (experimental study)	**Sodium bisulfite** ($NaHSO_3$) 1 M in 0.2 M sodium acetate buffer, pH 5.2	*E. coli* strains K12 (TA mutant site) and 15 (CG mutant site)	**Positive** – specific mutagen for CG mutants, Frequency of revertants tester strains *vs.* control: 2- (min) – 31-fold (max) in strain 15 only	Optimal result after 30 min incubation at pH 5.2	Mukai et al. (1970), Biochem Biophys Res Commun. 39/5: 983-988

Method, Guideline, GLP status, Reliability	**Test substance, Doses**	**Relevant information about the study (e.g. cell type, strains)**	**Results**	**Remarks** (e.g. major deviations)	**Reference**
In vitro cytogenicity study in eucaryotic cells					
Gene mutation in vitro and host mediated assay; non-guideline, non-GLP Rel. 2 (non-guideline) Key study	**0.1 % sodium metabisulfite** host mediated assay: single and repeated dose (5 days): 30, 700, 1200 mg/kg bw	*Saccharomyces cerevisiae* D3, 5 x 10^7 cells/mL Host animals: random-bred Swiss-Webster male mice (28 – 30 g bw), 10 mice/treatment group	**Negative** *in vitro* and in host-mediated assay	Positive control; i.m.: 350 mg/kg w/v EMS (ethyl methane sulphonate), test result: positive	NTIS 1972 NTIS Report PB221825 Published as Maxwell and Newell; Mol. Environ. Aspects Mutagenesis, Proc. Publ., Rochester Int. Conf. Environ. Toxic. 6th, 223-252, 1974
In vitro cytogenicity study in mammalian cells					
Chromosomal aberration OECD 473 (1997) Non-GLP Rel. 1 Sister chromatide exchange in human lymphocytes, OECD 479 Key study	**Potassium metabisulfite (PMB)** (CAS No. 16731-55-8) in bidistilled water, pH controlled: no influence on medium pH (6.8 – 7.2)	Human peripheral blood lymphocytes (donors: 2 M, 2F, non-smokers, 22-23y)	**Positive** Reduction in MI to 56 – 60 – 45 – 42 % (for concentrations: 25 – 50 – 100 – 200 µg/mL) of concurrent negative control, positive control: MI: 43 % of neg. control; OECD 473 for PBLs: MI reduction to 45±5 % of controls **Slightly positive:** Concentration dependent significant increase in SCE but not twice as high as controls	All concentrations cytotoxic but MI is within OECD 473 (2014) proposal for cytotoxicity (human blood lymphocytes: 45±5 % of control), cytotoxicity not clearly dose related at 24 h.	Anonymous15
Chromosomal aberration, Sister Chromatid exchanges non-guideline, non-GLP Comparable to OECD 473 (1997) Rel. 2 (reporting deficiency, no historical data presented)	**Sodium metabisulfite** (CAS No. 7681-57-4) in bidistilled water 75, 150, 300 µg/mL pH controlled: no influence on medium pH (6.8 – 7.2)	Human peripheral blood lymphocytes (donors: 2 M, 2F, non-smokers, 18-19y) staining: fluorescence plus Giemsa technique	**Positive:** Conc. dependent increase of CA: aberrant cells (%) **24 h:** 2-fold; 2.4-fold; 2.8-fold over control, positive control: MMC: 6.2-fold; **48 h:** 1.8-fold; 2-fold, 4.8-fold; MMC: 12-fold	Reporting deficiencies: Lack of information on purity and stability (but substance purchased from Merck, identifiable with cat. no) of test substance. Conc.-dependent cytotoxicity (MI):	Rencüzogullari et al. 2001. Mutation Research 490: 107-112

Method, Guideline, GLP status, Reliability	**Test substance, Doses**	**Relevant information about the study (e.g. cell type, strains)**	**Results**	**Remarks** (e.g. major deviations)	**Reference**
			Weak positive at non-cytotoxic concentrations **SCE:** dose-dependent increase in SCE/cell: 1.5-; 1.9-; 2.7-fold	24 h: 87 %; 94 %, 78 %; MMI: 48 %; 48 h: 105 %, 76 %, 17 %; MMI: 28 %	
Chromosome aberration non-guideline, non-GLP Rel. 2 (well conducted but cytogenetic assay in anaphase)	**Sodium metabisulfite** 2.5; 25; 250 µg/mL; positive control: 0.05 µg/mL triethylenemelamine (TEM)	Anaphase analysis of diploid human embryonic lung cells (WI-38)	**Positive:** Dose-related sharp increase in the number of aberrant cells at low and intermediate dose, cytotoxic effect at high dose Positive control TEM produced positive results	Authors suggested that the test system may produce false positive results as positive results obtained in vitro with various compounds could not be confirmed in vivo.	NTIS 1972 NTIS Report PB221825 Published as Maxwell and Newell; Mol. Environ. Aspects Mutagenesis, Proc. Publ., Rochester Int. Conf. Environ. Toxic. 6th, 223-252, 1974
Micronucleus assay in human lymphocytes, Pre-OECD 487 Non-GLP Rel. 2	**$NaHSO_3$, Na_2SO_3**; 1:3 M/M 0, 0.05, 0.10, 0.50, 1.0, 2.0 mM in RPMI 1640 medium, pH: 7.0	Human lymphocytes obtained from 4 donors	**Positive**: Concentration dependent increase of MN from 16.5±2.87; 21±2.65; 27.75±1.7; 33±3.37; 38.75±1.31 MNPCE (mean ± SE), doubling of control values in 3 out of 4 donors	Reporting deficiencies: Lack of information on purity and stability of test substance (but sodium bisulfite solution freshly prepared).	Meng and Zhang 1992, Mutation Research 298: 63-69 (no robust study summary provided)
Chromosomal aberration Non-GLP Rel. 2 (No positive control, reporting deficiencies)	**Sodium metabisulfite (anhydride)** in physiol. saline	Chinese Hamster fibroblast cell line (CHL)	**Negative** Highest dose without observed cytotoxicity: 0.125 mg/mL. Highest tested dose caused 50 % cytotoxicity, but no details on dosing reported.	Screening of various substances, no positive controls used but substances with positive results indicate reliability of test system	Ishidate et al. 1984; Fd Chem Toxic 22/8: 623-36
Chromosome aberration and Sister chromatide exchange Non-guideline study, Non-GLP Rel. 2 (reporting deficiencies, no substance information) roughly OECD 479	**Sodium bisulfite** ($NaHSO_3$) 0, 10, 20, 40 mM	Hamster foetal cells (HFC),	Negative for chromosome aberration Statistically significant, dose-related increases in SCE: 17.00±1.09 (control) vs. 22.35±1.53 (40 mM; mean ± SEM) in arrested HFC; 9.65±0.67 (control) vs. 14.00±1.11 (40 mM) in exponentially growing HFC.	Reporting deficiencies: Lack of information on test substance (CAS no., purity lacking). Untypical cell line used. No positive control used. OECD TG479 deleted April 2014	Popescu and DiPaolo 1988 Cancer Researh 48:7746-7251
Chromosome aberration in mammalian oocytes	**Sodium sulfite** (Na_2SO_3) 0, 5, 50, 100, 150, 200, 250,	Oocytes from ewe, cow, and mouse	Chromosome aberration, meiotic inhibition	Positive *in vitro* effects were not confirmed *in vivo* in mice – but no positive control was	Jagiello et al. 1975 Environ Res. 9: 84-93

Method, Guideline, GLP status, Reliability	**Test substance, Doses**	**Relevant information about the study (e.g. cell type, strains)**	**Results**	**Remarks** (e.g. major deviations)	**Reference**
Non-GLP, non-guideline study Rel. 4 (experimental study)	350, 500, 1000, 10000 µg/mL		**Mouse oocytes:** inhibition of entry into chromosome damage from 25 µg/mL onwards meiosis at ≥ 10 µg/mL **Ewe and cow oocytes**: atresia and chromosome breaks at ≥ 250 µg/mL, inhibition of meiosis at ≥ 500 µg/mL in cow oocytes, no inhibition in ewe oocytes.	included.	
In vitro gene mutation study in mammalian cells					
Mouse lymphoma assay, hprt locus OECD 476 (1997) Rel. 1 Key study	**Sodium metabisulfite** **Experiment 1**: 200, 300, 400, 600, 800, 1200, 1600, and 1902 µg/mL (+/- S9 mix) **Experiment 2**: 100, 300, 600, 900, 1200, 1500, and 1902 µg/mL (+/- S9 mix) **Experiment 3:** 200, 400, 800, 1000, 1200, 1400, 1600, 1 700, 1800, and 1902 µg/mL (+S9 mix) **Positive controls:** -S9µg/mL: 0.10 and 0.15 itroquinoline 1-oxide (NQO) + S9: 2.00 and 3.00 µg/mL Benzo[a]pyrene (B[a]P)	Mouse lymphoma L5178Y tk $^{+/-}$ cells	**Negative** **Experiment 1: Positive** (at 1600 and 1902 µg/mL with metabolic activation, % relative survival: 74 % and 65 %, respectively). **Experiment 2:** Negative. **Experiment 3:** Negative**.**	Test was considered negative because positive results in Exp. 1 (+S9) were not reproducible	Stone, V. (2010) Covance Study Number: 8230958
Sulfur dioxide					
Bacterial reverse mutation assay, No- OECD TG Non-GLP Rel. 2 (only 1 tester strain, only one concentration)	50 ppm **SO_2**, exposure: 48 h, Coexposure: B(a)P in (0-5 µg/plate). Positive control: 5 µg 2-aminoanthracene	S. typhimurium TA 98 + metabolic activation (S9)	**Negative**, no increase in revertants when compared to negative controls. No increase of mutagenic activity while co-exposed to f benzo(a)pyrene.	in principle OECD 471 (1983), but only one tester strain used. Study was conducted to investigate coexposure of B(a)P and SO_2 + NO_x	Pool-Zobel, B.L. et al. 1990. Exp. Pathol. 39, 207-212

Method, Guideline, GLP status, Reliability	**Test substance, Doses**	**Relevant information about the study (e.g. cell type, strains)**	**Results**	**Remarks** (e.g. major deviations)	**Reference**
In vitro mammalian cell micronucleus test OECD 487 principle Rel. 2 (reporting deficiencies) Non-GLP	0.1, 0.5, 1.0 ppm **SO_2** (gas)	Human lymphocytes derived from 4 donors (no more details given) Positive control: Cyclophosphamide	**Positive** Frequency of micronuclei and SCE in human lymphocytes increased concentration-dependently at cytotoxic concentrations: 0, 0.1, 0.5, 1.0 ppm: MN median: 1.5, 2.0, 4.5, 5.5, pos. control: 9.5) (MI reduction vs. control: 65 %, 58 %, 31 %)	Reporting deficiencies. Not clear, whether metabolic activation was used. Positive control is for tests with metabolic activation	Üren et al. 2014 Toxicol Ind Health. 2014 30(4):311-5.

Method, Guideline, GLP status, Reliability	Test substance, Doses	Relevant information about the study (e.g. cell type, strains)	Results	Remarks (e.g. major deviations)	Reference
Table 15: Summary table of in vivo genotoxicity studies (Please refer to section 12 for further details (tables and figures) **on key studies.)** **Method, Guideline, GLP status, Reliability**	**Test substance, Doses**	**Relevant information about the study (e.g. species and strain, duration of exposure)**	**Observations**	**Remarks** (e.g. major deviations)	**Reference**
Sulfur dioxide					
In vivo Comet Assay Rel. 2 (reporting deficiencies, no positive control) Comparable to OECD 489 Non-GLP Key study	14, 28, 56, 112 mg/m^3 **SO_2** concentrations measured within the chambers by pararosaniline hydrochloride spectrophotometry every 30 min	Mouse, Kunming albino, 6 males and 6 females 6 hours/day for 7 days DNA damage measured as Olive tail moment (OTM): product of tail moment length and tail DNA %. Sampling time: immediately after last exposure Cell viability > 95 % shown with Trypan-blue dye-exclusion technique.	**Positive** Dose-dependent increase OTM from 14 mg/m^3 onwards in blood lymphocytes. Cells derived from brain, lung, liver, spleen, kidney, and intestine in both sexes and in testicles of males. No effects on food consumption and body weight gain; no deaths, morbidity or distinctive clinical signs.	No justification for dose selection, no positive control used or no information on historical positive control range, values expressed as mean ± SE instead SD, body weight gain and food consumption not reported.	Anonymous11 (2005)
In vivo Mouse Micronucleus test	1.00, 2.99, 10.26 and 30.55 ppm **SO_2**	Mouse, NMRI, 6 males and 6 females	**Negative** The number of micronuclei not increased; however not proven that the substance	Acceptability criteria for negative results according to OECD 474 not fulfilled[2]	Anonymous6, (2008) also published as:

[2] The study was designed as re-evaluation of the published data by Meng et al. (2002) using comparable doses. In consequence, only non-toxic doses were applied and the study does not meet the requirements of OECD 474.

The concentration of SO_2 in exposure chamber was not analysed, exposure via gas cylinders with certified SO_2/N_2 concentrations; control via flow rates.

Dose-dependent increases in malondialdehyde levels in erythrocytes of exposed mice in another study under the same conditions (statistically significant at 10 and 30 ppm).

Method, Guideline, GLP status, Reliability	**Test substance, Doses**	**Relevant information about the study (e.g. cell type, strains)**	**Results**	**Remarks** (e.g. major deviations)	**Reference**
Similar to OECD 474 GLP Rel. 2 Key study		4 hours/day for 7 days	reached the target organ. No signs of overall toxicity. PCE:NCE ratio unchanged.		Anonymous7, (2010)
In vivo Mouse Micronucleus test Similar to OECD 474 Non-GLP Rel. 2	14, 28, 56, 84 mg/m^3 (5, 10, 21, 32 ppm) **SO_2** concentrations measured within the chambers by pararosaniline hydrochloride spectrophotometry every 30 min	Mouse, Kunming albino, 10 males and 10 females 4 hours/day for 7 days Sampling time: 24 h	**Positive** Dose dependent increase in micronuclei in PCE, no sex differences. Increase statistically significant at 14 mg/m^3 SO_2 and higher.	PCE:NCE ratio was monitored but not reported. No positive control used, only 1000 PCE per animal scored, OECD 474 requires 2000 (4000 according to OECD 474, Sep 2014), no information on historical positive and negative control range, no justification for dose selection	Anonymous8, (2002)
In vivo Mouse Micronucleus test Conduction similar to OECD 474 Non-GLP Rel. 2	28 mg/m^3 **SO_2** concentrations measured within the chambers by pararosaniline hydrochloride spectrophotometry every hour	Mouse, Kunming albino, 12 males and 12 females 6 hours/day for 5 days Sampling time: 24 h following last exposure	**Positive** Significant increase in micronuclei (mono-, bi, and polymicronuclei) in PCE at 28 mg/m^3 compared to controls.	Reporting deficiencies, e.g. PCE:NCE ratio not reported. (Study was conducted to test the protective effect of seabuckthorn seed oil (i.p.) – only one test concentration chosen)	Anonymous10 (2003)
In vivo Mouse Chromosome aberration test Similar to OECD 475 Non-GLP Rel. 2	7, 14, 28, 56, mg/m^3 **SO_2** (nominal) concentrations measured within the chambers by pararosaniline hydrochloride spectrophotometry every 30 min	Mouse, Kunming albino, 10 males and 10 females 4 hours/day for 7 days Sampling time: 24 h following last exposure; 2 h after cholchicine injection	**Positive** Dose and duration dependent increase in aberrant cells, dose dependent decrease of mitotic index in both sexes Chromosome and chromatide breaks at 56 mg/m^3 SO_2; at lower concentrations chromatide breaks only sign. at ≥ 14 mg/m^3.	Short comings: Results of positive controls not documented, reporting deficiencies, sampling time after cholchecin 2 h (OECD: 3-5 h).	Anonymous9 (2002).

Method, Guideline, GLP status, Reliability	**Test substance, Doses**	**Relevant information about the study (e.g. cell type, strains)**	**Results**	**Remarks** (e.g. major deviations)	**Reference**
Sodium/potassium metabisulfite					
Chromosomal aberration OECD 474 (1997) Non-GLP Rel. 2 (i.p, insufficient no. of animals) Key study	**Potassium metabisulfite** (CAS No. 16731-55-8) in bidistilled water: 150, 300, 600, mg/kg bw, i.p. single dose	Albino rats 2M + 2 F per group, Sampling time: 12 h, 24 h	**Positive:** Dose related increase of aberrant cells	No 48 h sampling, only 2 animals per sex and group, pos. control: urethane	Anonymous15 2008;
In vivo Mouse Micronucleus test comparable to OECD 474 Rel 2 (only 1 sampling time for blood cells and bone marrow cells) Non-GLP Key study	Oral gavage single dose Pre-test and main tests: 0.5, 1.0, 2.0 g/kg bw Test substance: **sodium meta-bisulfite**	Mice CF1 outbred Pre-test for acute toxicity: 6/group (3F+3M) Main test: 10/group (5F+5M) Sampling time: 24 h	**Positive** Increased frequency of micronuclei in bone marrow and peripheral blood cells at 2g/kg (limit dose); significant reduction of PCE:NCE ratio at **2 g/kg**	Pre-test: no signs of toxicity; no mortalities, purity of test substance not reported but can be identified by catalogue no.) MN: PCE:NCE ratio in controls unusually high	Anonymous14 (2011)
Chromosome aberration Non-GLP; Pre- comparable to Guideline OECD 474 Reliability: 1 (pre-guideline but well conducted) Key study	**Sodium metabisulfite**; 0, 30, 700, 1200 mg/kg. Single or multiple oral dosing (5 d).	Rat (Albino, random-bred, 200 g bw) M	**Negative** Dose dependent decrease in mitotic index	Dose dependent increases in cytotoxicity	NTIS 1972 NTIS Report PB221825 Published as Maxwell and Newell; Mol. Environ. Aspects Mutagenesis, Proc. Publ., Rochester Int. Conf. Environ. Toxic. 6th, 223-252, 1974
Dominant Lethal Gene Test); Non-GLP; comparable to Guideline OECD 478 Reliability: 1 (pre-guideline but well conducted) Key study	**Sodium metabisulfite**; 0, 30, 700, 1200 mg/kg. p.o. Positive control: Triethylenmelamine (TEM) 0.2 mg/kg i.p. single dose	Rat (Albino) M; Single or multiple dosing (5 d). 10 M/treatment group	No consistent responses attributed to treatment, occasional statistical differences between control and sodium meta-bisulfite-dosed groups at $P < 0.01$; $P < 0.05$, and $P < 0.10$ without time or dose-response effect. At $P < 0.20$ indications of an effect	-	NTIS 1972 NTIS Report PB221825 Published as Maxwell and Newell; Mol. Environ. Aspects

Method, Guideline, GLP status, Reliability	**Test substance, Doses**	**Relevant information about the study (e.g. cell type, strains)**	**Results**	**Remarks** (e.g. major deviations)	**Reference**
			TEM: positive		Mutagenesis, Proc. Publ., Rochester Int. Conf. Environ. Toxic. 6th, 223-252, 1974
Comet assay comparable to OECD 489 Non-GLP Rel. 2 (no early sampling time, unusual scoring – see remarks, poor reporting)	Oral gavage single dose Pre-test and main tests: 0.5, 1.0, 2.0 g/kg bw Test substance: **sodium meta-bisulfite**	Mice CF1 outbred Pre-test for acute toxicity: 6/group (3F+3M) Main test: 10/group (5F+5M) Sampling time: 24 h	**Positive** <u>Comet assay</u> Dose dependent increase in DI and DF in % in blood, liver and bone marrow. Increase statically significant at **1 g/kg and 2 g/kg**.	Pre-test: no signs of toxicity; no mortalities, purity of test substance not reported but can be identified by catalogue no.) Damage Index (DI): cells were allocated into five classes according to tail size (0=no tails and 4 = maximum tail length). DI for maximum damage = 400 Damage Frequency (DF) = number of cells with tail in %.; Deviations: 1 sampling time only at 24h, no individual animal data – DI is an unusual scoring for Comet assay	Anonymous14 (2011)
Chromosome aberration, micronucleus assay and sister chromatid exchange assay; Non-GLP; non-guideline Reliability: 2 (reporting deficiencies, no MI, only two doses tested).	**Sodium metabisulfite**, calculated as SO_2.: Mouse: s.c.: 50 mg/kg , p.o.: 660 mg/kg (normal animal), 165 mg /kg in sulphit oxidase deficient animals) Hamster: .c.: 50 mg/kg , p.o.: 660 mg/kg (normal animal) 330 mg 165 mg SO_2/kg (SO deficient animals)	Chinese hamster, Charles River NMRI-mice) F + M In addition: up to 12 injections (subcutaneous).	**Negative.** No cytogenetic effect in all three assays in normal and SO deficient animals	No proof of proliferation (e.g. no mitotic index – MI – reported), not clear whether target organ was reached. Study cannot be regarded as key study as important information is lacking (see above)	Anonymous12 (1983)
Micronucleaus assay	**Sodium metabisulfite**,	Chinese hamster, Charles River	**Negative.** Frequency in micronucleated cells not increased in normal and SO	Not proven that target organ (bone marrow) was	Anonymous12

Method, Guideline, GLP status, Reliability	**Test substance, Doses**	**Relevant information about the study (e.g. cell type, strains)**	**Results**	**Remarks** (e.g. major deviations)	**Reference**
Non-GLP; Reliability: 2 (reporting deficiencies, no MI, only two doses tested).	calculated as SO_2.: Mouse: s.c.: 50 mg/kg , p.o.: 660 mg/kg (normal animal), 165 mg /kg in sulphit oxidase deficient animals) Hamster: s.c.: 50 mg/kg , p.o.: 660 mg/kg (normal animal) 330 mg 165 mg SO_2/kg (SO deficient animals)	NMRI-mice) F + M In addition: up to 12 injections (subcutaneous).	deficient animals	reached. Study cannot be regarded as key study as important information is missing. Treatment schedule does not comply with OECD 474 2014.	(1983)
Sodium sulfite					
In vivo mouse Micronucleus test OECD 474 GLP Rel. 2 (reporting and methodological deficiencies) Key study	Subcutane **Sodium sulfite** wasserfrei, food grade (E221) 250, 500, 1000 mg/kg bw in 10 mL/ kg bw Vehicle: purified water; Positive control: cyclophospha-mide (clastogenicity), vincristin sulphate (spindle poison),	Mice Crl:NMRI, M single dose	**Negative** Frequency of micronuclei in erythrocytes not increased compared to vehicle controls; PCE:NCE reduced at 1000 mg/kg bw at 48 h following administration	Dose selection after pretest, deaths observed at 1500 mg/kg bw, no information on number of animals used in pretest. No signs of toxicity although mortality occurred at 1500 mg/kg bw in pretest. Route of exposure: s.c. not recom-mended for reactive substances test substance not stable in water reporting deficiencies: route of exposure s.c. in text, p.o. in annex	Anonymous13 2008;
In vivo Comet Assay Non-GLP Reliability. 2 (sampling time, substance identification, missing purity, i.p. administration, sampling time) OECD 489	Intraperitoneal **Sodium sulfite**: sodium bisulfite (3:1 M/M) <u>Main test</u> 125, 250, 500 mg/kg bw	Mouse, Kunming albino, 6 males Daily for 7 days Sampling time: 24 h	**Positive** Dose dependent increase in OTM in cells from brain, lung, heart, liver, stomach, spleen, thymus, bone marrow and kidney ($p < 0.05$ one way ANOVA). Strongest increase in brain, lung and heart. Significant difference vs control in all tissues already at the lowest applied dose of 125 mg/kg bw ($p<0.05$; Dunnett test)	Reporting deficiencies: CAS No. missing, lack of information on stability Purity of test substance not reported (purchased from Sigma, ± identifiable from catalog). Sampling time 24 h after last dosing (recommended: 2- 6 h)	Anonymous16 (2004)

Method, Guideline, GLP status, Reliability	Test substance, Doses	Relevant information about the study (e.g. cell type, strains)	Results	Remarks (e.g. major deviations)	Reference
			At 125 mg/kg bw % of cells with DNA damage in all organs except for thymus and bone marrow ≥ 50 %. 50 % lethality at 1000 mg/kg bw observed in pretest		
Dominant-lethal and heritable translocation in mice Non-guideline, non-GLP Rel. 3 (no positive control, no proof that target organ was exposed)	**Sodium bisulfite i.p.**, vehicle (and neg. control): aqua dest. 300, 400 mg/kg bw/d, **repeated dose**: 300 mg/kg: 38 doses in 54 days; 400 mg/kg: 20 doses at 26 day; 550 mg/kg bw/d single dosed females; # mated females: 300 mg/kg: 60; controls: 76 400 mg/kg: 69, controls: 135 550 mg/kg: 29; controls: 32	Translocation study: male 101 x C3H F_1 mice mated with SEC x C57BL F_1 female mice immediately after last dosing Dominant lethal: mating with SEC x C57BL F_1 females up to 14.5 days after last injection Dominant lethal in females: C3H x 101 F_1 single dose i.p. of 550 mg/kg, mated to untreated males within 4.5 days after treatment	**Negative** No signs for induction of dominant lethal mutations or heritable translocation	No positive control used Dose selection on basis of pretest: 550 mg/kg as highest dose without mortality	Anonymous5, 1978;

Table 16: Summary table of human data relevant for germ cell mutagenicity(Please refer to section 12 (tables and figures) for further details on key studies.)

Summary table of human data on genotoxicity				
Type of data/ report, Reliability	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
Occupational study on clastogenicity of workers in a sulfite pulp factory Rel 2 (reporting deficiencies)	**SO_2**	Controls: 15 M (5 smokers) Test groups: SO_2 group: 7 M (1 smoker) Pulp bleaching group: 6 M (1 smoker) Paper mill group: 6 M (3 smokers) Chromosome aberrations in 100 cells/individual following 72 h of cell culture	SO_2 group: All types of aberrations were significantly increased in comparison to the control group with $p<0.01$ or $p<0.001$. Smoking was the only possible confounder recorded. Due to lack of evaluation/ matching for possible confounders and low number of participants, no final conclusion can be drawn from the study.	Nordenson et al (1980) Hereditas 93: 161-164. (published)

Summary table of human data on genotoxicity				
Type of data/ report, Reliability	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
		No data on exposure to SO_2 reported.		
Occupational study on clastogenicity Human Bio-Monitoring of workers in aluminium industry	**SO_2**	CA and SCE in high and low exposed workers (M; mean age: 47.9) (exposure not specified) Average daily SO_2 exposure estimated **0.2-3.0 ppm, individual mean exposure level 1.0 +- 0.85 ppm;**	Frequencies of CA and SCE were similar in all groups. However, due to lack of evaluation/ matching for possible confounders and low number of participants, no final conclusion can be drawn from the study. In addition, exposure towards SO_2 was very low.	Sorsa, M. *et al.* (1982). Hereditas 97: 159-161.
Occupational study on genotoxicity Human Bio-Monitoring on workers in a fertilizer factory	**SO_2**	MI, CA, SCE, satellite associations in workers (n = 42) and matched (age, sex, smoking, alcohol consumption) controls (n = 42) Average exposure reported to be **41.7 mg/m^3 (15.7 ppm;** 20°C, atmospheric pressure)	Exposed *vs*. controls ($p<0.05$): **MI:** 7.09 ± 0.79 *vs* . 4.34 ± 1.23 **SCE:** 7.27 ± 0.13 *vs*. 3.97 ± 0.12 **CA w/o gaps** (smokers): 3.52 ± 0.27 (n = 34) *vs.* 1.07 ± 0.16 (n = 27) **CA w/o gaps** (alcoholics): 3.24 ± 0.33 (n = 17) *vs.* 0.91 ± 013 (n = 23) **Satellite associations/cell:** 17.1 ± 1.2 *vs.* 8.1 ± 0.3 Exposure to high concentrations of SO_2 is associated with genotoxic effects in workers.	Yadav and Kaushik. (1996). Mutation Research 359:25-29.
Occupational study on genotoxicity (micronuclei formation) Human Bio-Monitoring of workers in a sulfuric acid factory	**SO_2**	Micronuclei formation in peripheral blood lymphocyte culture in workers (n = 40) and matched (age, sex, smoking) controls (n = 42, members/students of university)	Exposed *vs*. controls ($p<0.001$): **Lymphocytes with MN:** **w/o:** 0 % *vs* . 31 %	Meng and Zhang (1990). Environmental and Molecular Mutagenesis 15:218-220 (published)

Summary table of human data on genotoxicity				
Type of data/ report, Reliability	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
		Range of exposure reported to be **0.34 mg/m^3 to 11.97 mg/m^3 (0.13 ppm and 4.5 ppm;** 20°C, atmospheric pressure, respectively during the year)	**>0.1 %:** 72.5 % *vs* . 16.7 % **>0.2 %:** 17.5 % *vs.* 0 % Higher frequency of MN in smokers in both groups, but always higher in exposed workers whether smoking or not.	
Occupational study on genotoxicity (chromosome aberration, sister-chromatid exchange) Human Bio-Monitoring of workers in a sulfuric acid factory	**SO_2**	CA and SCE in peripheral blood lymphocyte culture in workers (n = 40) and matched (age, sex, smoking) controls (n = 42, members/students of university) Range of exposure reported to be **0.34 mg/m^3 to 11.97 mg/m^3 (0.13 ppm and 4.5 ppm; 20°C,** atmospheric pressure, respectively during the year)	Exposed *vs*. controls (p<0.01): **CA chromosome type:** 165 *vs* . 25 aberrant cells (2.1 ± 0.23 % vs. 0.3 ± 0.1 %) **CA chromatid type:** 77 vs. 24 aberrant cells (1.0 ± 0.2 % vs. 0.3 ± 0.1 %) **CA total number of cells:** 242 vs. 49 (3.0 ± 0.3 % vs. 0.6 ± 0.1 %) **SCE per cell:** 6.7 ± 0.2 vs. 2.7 ± 0.1 No difference of CA and SCE between smokers and non-smokers.	Meng and Zhang (1989). Mutation Research 241:15-20 (published) (same cohort as in the study above)

Summary table of human data on genotoxicity				
Type of data/ report, Reliability	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
Method: chromosomal aberrations in anaphase of human embryonic lung cells (WI-38); No data on GLP; Reliability: 2 (non-guideline study but well conducted)	**Sodium metabisulfite** test concentrations not specified (without metabolic activation).	Human embryonic lung cells (WI-38).	Positive in anaphase, negative in metaphase (without metabolic activation).	NTIS 1972 NTIS Report PB221825 Published as Maxwell and Newell; Mol. Environ. Aspects Mutagenesis, Proc. Publ., Rochester Int. Conf. Environ. Toxic. 6th, 223-252, 1974

Reliability of the human biomonitoring studies is "not assignable" according to Klimisch, because no OECD guideline was followed. However, all listed studies are considered reliable from the scientific point of view.

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

Several *in-vivo* studies confirmed a clastogenic effect observed *in vitro* with sulfur dioxide. All studies had short comings in testing protocols or – at least – reporting deficiencies. However, results derived from a recently performed micronucleus assay *in vivo* (Anonymous6 und Anonymous7, 2008/2010) are not regarded sufficient on a standalone basis to dismiss positive results from micronucleus and comet assays reported from several published studies (see table above). The conflicting results are in line with the observation that results are highly dependent on test conditions. Sulfur dioxide and bisulfite/metabisulfite participate in a large number of organic and inorganic reactions (e.g. Anonymous46, 1981), which is plausible as sulfur dioxide and sodium metabisulfite are reactive substances.

Gene mutation seen under special conditions *in vitro* (see chapter above) was not confirmed *in vivo* in a well-conducted dominant lethal gene test (NTIS 1972/Maxwell and Newell 1974). In conclusion, gene mutation effects seen *in vitro* were not confirmed *in vivo*.

Clastogenic effects *in vivo*:

Sulfur dioxide:

positive results:

Anonymous112005: Comet assay (regarded as key study)

Anonymous8 2002: Micronucleus

Anonymous10 2003: Micronucleus

Anonymous9 2002: Chromosome aberration

negative results:

Anonymous6 und Anonymous7, 2008/2010: Micronucleus (regarded as key study)

Conclusion: Equivocal results for sulfur dioxide *in vivo* – all studies reliability of 2.

Sodium/potassium metabisulfite:

positive results:

Anonymous15 2008: Chromosome aberration (regarded as key study, reliability 2)

Anonymous14 2011: Micronucleus (regarded as key study, reliability 2), Comet assay

negative results:

NTIS 1972/Maxwell and Newell 1974: Chromosome aberration (regarded as key study, reliability 1)

Anonymous12, 1983: Chromosome aberration, micronucleus

Conclusion: Equivocal results for sodium metabisulfite *in vivo* – negative key studies reliability 1, positive key studies reliability 2.

Sodium sulfite/sodium bisulfite:

positive results:

Anonymous16 2004: micronucleus i.p., comet assay i.p.

negative results:

Anonymous13: micronucleus, s.c. (regarded as key study, reliability 2)

Comet assays reported here (Anonymous14, Anonymous16) are difficult to interpret as essential information is lacking or at least not reported. In addition, sampling time was after 24 hours (instead of 2-6 hours following last treatment as recommended by OECD 489). Although this might be more important in negative test results, an indirect/cytotoxic effect cannot be excluded. No details or images were given on comets and cytotoxicity.

A bone-marrow micronucleus test in NMRI mice (m/f) according to OECD TG 474 following inhalation exposure to sulfur dioxide (Anonymous6 und 7 2008a,b, 2010) is available. Animals were exposed (whole-body) to 0 (clean air), 2.7, 8, 27, or 80 mg/m^3 (0, 1, 3, 10, or 30 ppm) SO_2 for 4h/day on 7 consecutive days. Exposure to SO_2 caused no acute toxicity, mortality, or reduction in body weight under test condition. Compared with the clean-air controls, haematological parameters such as haematocrit, haemoglobin, erythrocyte/platelet/total leukocyte counts, differential white blood cell counts, and indicators of blood formation (reticulocyte counts, ratio of polychromatic to normochromatic erythrocytes in the bone marrow) remained unchanged by SO_2 treatment. In contrast to various *in vivo* studies performed by Anonymous8and coworkers (see description below), SO_2 did not induce micronuclei in polychromatic erythrocytes of the bone marrow. SO_2 treatment significantly enhanced malondialdehyde levels in erythrocyte lysates (TBARS method), indicating SO_2-mediated oxidative stress. In the studies, dose dependent increases of chromosomal aberrations and micronuclei were shown *in vivo*. The dossier submitter evaluates the studies published by Anonymous8 and coworkers as reliable with restrictions (reliability 2). The studies were published in recognised peer review journals for toxicology. Anonymous7 performed the study under comparable conditions in order to refute or confirm the studies published by Anonymous8 and coworkers with the consequence that test concentrations were not chosen according to the requirements of OECD TG 474 (e.g. no observed toxicity, no indication that bone marrow was reached).

The group of Anonymous8 and coworkers conducted several *in vivo* studies on the genotoxic potential of inhalation exposure to sulfur dioxide in Kunming mice (micronucleus assay: Anonymous8, Anonymous10, chromosome aberration: Anonymous9, comet assay: Meng et al. 2005). In the chromosomal aberration test, male and female Kunming mice were exposed to concentrations of 0 to 56 mg/m^3 of SO_2 for 4 hours per day for a period of 7 days. A dose-dependent increase in chromatid-type aberrations at body weight concentrations (from 7 to 28 mg/m^3 – significant from 14 mg/m^3 onwards) and chromosome-type aberrations at higher concentrations (56 mg/m^3), were observed in a context of high cytotoxicity (reduced mitotic index) from 14 mg/m^3 onwards. Positive results with metabisulfites (sodium, potassium) in chromosome aberration assays were also reported in rats by other groups (Anonymous15, Anonymous14) as well as in lymphocytes of exposed workers (Yadav and Kaushik 1996).

In the micronucleus test (Anonymous8), animals of the same strain of mice were exposed to up to 84 mg/m^3 of SO_2 under comparable experimental conditions as in the chromosomal aberration test (Anonymous9). Anonymous10 investigated concentrations of 0 to 28 mg/m^3 of SO_2 tor 6 hours per day for a period of 5 days. A dose-dependent increase in the frequency of micronuclei in the polychromatic erythrocytes was observed in both studies. No information on the ratio of PCE/NCE was reported. However, as dose-dependent micronuclei formation was observed, the test substance must have reached the bone marrow but no information was given on cytotoxicity. In the chromosome aberration study cytotoxicity was seen at doses above 14 mg/m^3. Hence, it cannot be excluded that genotoxicity occurs at cytotoxic doses only.

In the comet assay (Anonymous11), male and female mice were treated with 14 - 112 mg/m^3 (5 – 40 ppm) SO_2 for 6 h/day for 7 days, while control groups were exposed to filtered air. SO_2 caused significant, dose-dependent increases in DNA damage (increased olive tail moment, OTM) in all the cell types derived from blood lymphocytes and cells from the brain, lung, liver, spleen, kidney, intestine, analysed from both sexes of mice and in testicles. Cell viability was high (>95 %) prior to exposure. In contrast to the high degree of cell viability in treated groups indicated by the trypan-blue assay, H & E staining and transmission electron microscopy showed cell toxicity induced by SO_2.

Studies with sulfites also indicated contradictive results. Anonymous14 conducted a micronucleus and a comet assay in order to evaluate the genotoxic potential of sodium metabisulfite on different tissues of the mouse. Positive results were only seen at the limit dose of 2000 mg/kg bw accompanied with indication for bone marrow toxicity (significant reduction in the ratio of polychromatic to normochromatic erythrocytes. In the comet assay positive results were obtained at 1000 and 2000 mg/kg bw in all tissues investigated (liver, bone marrow, blood). Negative findings in the micronucleus assay up to 1000 mg/kg bw (highest dose tested) were confirmed in an unpublished study with sodium sulfite (Anonymous13). The comet assay performed by Anonymous16 on the genotoxic potential of a mixture of sodium sulfite and sodium bisulfite, 3:1 M/M) in cells of various organs (brain, lung, heart, liver, stomach, spleen, thymus, bone marrow and kidney) of male mice showed dose-dependent increases in OTM from 125 mg/kg bw onwards. The dossier submitter regarded

the study as not reliable as important information on the test substance are lacking. 50 % lethality were already observed at 1000 mg/kg bw which could not be shown in any of the other studies.

In conclusion, a genotoxic potential of sulfur dioxide and sodium metabisulfite cannot be ruled out. The higher sensitivity of the comet assay following inhalation of SO_2 might be explained by formation of reactive oxygen species and, hence, an indirect genotoxic mechanism which might explain predominantly negative results *in* vitro. Concentration dependent increased levels of MDA, an indication for lipid peroxidation, were shown in erythrocytes at 10 and 30 ppm (Anonymous7).

Conclusions:

Currently, sulfur dioxide has no harmonised classification for mutagenicity, but the available data indicate a genotoxic potential. The higher sensitivity of the comet assay following inhalation of SO_2 might be explained by formation of reactive oxygen species and, hence, an indirect genotoxic mechanism may be postulated which might explain predominantly negative results *in vitro*. Concentration dependent increased levels of MDA, an indication for lipid peroxidation, were shown in erythrocytes at 10 and 30 ppm (Anonymous7).

Therefore, the proposal Muta. 2 for sulfur dioxide is based on positive evidence obtained from experiments in mammals supported by some *in vitro* findings. In addition, there is some indication for genotoxicity in lymphocytes of exposed workers. Also there was strand-breaking activity in testes in an in vivo comet assay and genotoxic effects in occupational studies.

California EPA[3] indicated that there was "considerable evidence that air pollution (with SO_2 used as an index measure in some studies) induces DNA damage in human sperm (…) as well as other cell types (…). The data from animal studies are also indicative of oxidative damage, including DNA damage in the testes caused by exposure to SO_2."

10.8.2 Comparison with the CLP criteria

The following table lists the criteria for germ cell mutagens required from CLP regulation:

CLP regulation
The classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans. The classification in Category 1B is based on: — positive result(s) from *in-vivo* heritable germ cell mutagenicity tests in mammals; or — positive result(s) from *in-vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells *in vivo*, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or — positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people. The classification in Category 2 is based on: — positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from: — somatic cell mutagenicity tests *in vivo*, in mammals; or — other *in vivo* somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.

[3] California Environmental Protection Agency, 2011: Evidence on the Developmental and Reproductive Toxicity of Sulfur Dioxide (Reproductive and Cancer Hazard Assessment Branch; Office of Environmental Health Hazard Assessment).

Note: Substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

Toxicological results and CLP classification

Muta. 2, based on positive evidence obtained from experiments in mammals *in vivo* supported *in vitro* findings. In addition, there indication for genotoxicity in lymphocytes of exposed workers. Also there was strand-breaking activity in testes in an in vivo comet assay and genotoxic effects in occupational studies.

The genotoxic potential of sulfur dioxide was dicussed in September 2018 for the biocide assesement procedure. The majority of the HH-WG members agreed that on the basis of the available information, sulfur dioxide is genotoxic.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

DS proposes to classify sulfur dioxide as Muta. 2.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

There is a large number of *in vitro* and *in vivo* studies both with SO_2 and its sulfite metabolites regarding genotoxicity. Although there are limitations and deficiencies in many of the studies evaluated, the DS considered the studies adequate to assess germ cell mutagenicity in a weight of evidence (WoE) approach.

In a series of *in vivo* mouse tests in Kunming albino mice, the genotoxic potential of inhalation exposure to SO_2 was studied in a micronucleus assay (Anonymous8 and Anonymous10), an assay for chromosome aberrations (Anonymous9), and a comet assay (Anonymous11). In the chromosomal aberration test, male and female Kunming mice were exposed to concentrations of 0 to 56 mg/m^3 of SO_2 for 4 hours per day for a period of 7 days. A dose-dependent increase in chromatid-type aberrations at concentrations from 7 to 28 mg/m^3 (statistically significant from 14 mg/m^3 onwards) and chromosome-type aberrations at higher concentrations (56 mg/m^3), were observed in association with high cytotoxicity (reduced mitotic index) from 14 mg/m^3 onwards.

In the micronucleus test (Anonymous8), Kunming albino mice were exposed to up to 84 mg/m^3 of SO_2 under comparable experimental conditions to those in the chromosomal aberration test (Anonymous9). Anonymous10 investigated concentrations of 0 to 28 mg/m^3 of SO_2 for 6 hours per day for a period of 5 days. A dose-dependent increase in the frequency of micronuclei in the polychromatic erythrocytes was observed in both studies. No information on the ratio of polychromatic to normochromatic erythrocytes (PCE/NCE) was reported. However, as dose-dependent micronuclei formation was observed, the test substance must have reached the bone marrow but no information was given on cytotoxicity. In the chromosome aberration study cytotoxicity was seen at doses above 14 mg/m^3. Hence, it cannot be excluded that genotoxicity occurs at cytotoxic doses only.

In the comet assay (Anonymous11), male and female mice were treated with 14 - 112 mg/m^3 (5 – 40 ppm) SO_2 for 6 h/day for 7 days, while control groups were exposed to

filtered air. SO_2 caused significant, dose-dependent increases in DNA damage (increased Olive tail moment, OTM) in all the cell types derived from blood lymphocytes and cells from the brain, lung, liver, spleen, kidney, intestine, analysed from both sexes of mice and in testicles.

In a similar and more recent study under comparable conditions to the micronucleus test described above, a bone-marrow micronucleus test in NMRI mice (m/f) conducted according to OECD TG 474 following inhalation exposure to SO_2 was performed (Anonymous6 and Anonymous7). The study was conducted in order to further investigate the studies published by the Meng group (Anonymous8) and co-workers with the consequence that test concentrations were not chosen according to the requirements of OECD TG 474 (e.g. no observed toxicity, no direct indication that bone marrow was reached). Animals were exposed (whole-body) to 0 (clean air), 2.7, 8, 27, or 80 mg/m^3 (0, 1, 3, 10, or 30 ppm) SO_2 for 4h/day on 7 consecutive days. Exposure to SO_2 caused no acute toxicity, mortality, or reduction in body weight under the test conditions. Compared with the clean-air controls, haematological parameters such as haematocrit, haemoglobin, erythrocyte/platelet/total leukocyte counts, differential white blood cell counts, and indicators of blood formation (reticulocyte counts, PCE/NCE ratio in the bone marrow) remained unchanged by SO_2 treatment. In contrast to the *in vivo* studies mentioned above and performed by the Meng group, SO_2 did not induce micronuclei in polychromatic erythrocytes of the bone marrow.

Contradictory results were also reported when considering all the studies with sulfites. Studies with sulfites also indicated contradictive results. Anonymous14 conducted a micronucleus study and a comet assay in order to evaluate the genotoxic potential of sodium metabisulfite on different tissues of the mouse. In the micronucleus test, positive results were only seen at the limit dose of 2000 mg/kg bw and were accompanied by indications of bone marrow toxicity (a significant reduction in the ratio of PCE/NCE). In the comet assay, positive results were obtained at 1000 and 2000 mg/kg bw in all tissues investigated (liver, bone marrow, blood), expressed as significant increases in damage index and damage frequency values. Negative findings in the micronucleus assay up to 1000 mg/kg bw (highest dose tested) were confirmed in an unpublished study with sodium sulfite (Anonymous13). The comet assay performed by Anonymous16 on the genotoxic potential of a mixture of sodium sulfite and sodium bisulfite, 3:1 M/M) in cells of various organs (brain, lung, heart, liver, stomach, spleen, thymus, bone marrow and kidney) of male mice showed dose-dependent increases in OTM from 125 mg/kg bw onwards. The DS regarded the study as not reliable since important information on the test substance was lacking. In addition, 50% lethality was observed at 1000 mg/kg bw, which is data that could not be verified in any of the other studies.

In summary, the DS argued that the available data provided evidence for the genotoxic potential of SO_2. Several *in-vivo* studies confirmed the clastogenic effect observed *in vitro* (see table 15 in CLH report) with SO_2. All studies had shortcomings in testing protocols and/or reporting deficiencies. However, results derived from a recently performed micronucleus assay *in vivo* (Anonymous6 and Anonymous7) were not regarded sufficient on on their own to dismiss positive results from micronucleus and comet assays reported from several published studies. The conflicting results are in line with the observation that results are highly dependent on test conditions. SO_2 and bisulfite/metabisulfite are known to participate in a large number of organic and inorganic reactions, which is expected as SO_2 and sodium metabisulfite are reactive substances.

Moreover, the observed higher sensitivity of the comet assay following inhalation of SO_2 might be explained by the formation of reactive oxygen species and hence an indirect genotoxic mechanism may be postulated, which might explain the predominantly negative results *in vitro*. Concentration dependent increased levels of MDA (malondialdehyde), the end product of lipid peroxidation and an indication of lipid peroxidation, were shown in erythrocytes at 10 and 30 ppm (Anonymous7).

In conclusion, the DS proposed classification for SO_2 as **Muta. 2, H341: Suspected of causing genetic defects**, based on positive evidence obtained from experiments in mammals, supported by a few *in vitro* findings. In addition, there is indication for genotoxicity in lymphocytes of exposed workers. Moreover, there was strand-breaking activity in testes in an *in vivo* comet assay and genotoxic effects in occupational studies.

Comments received during consultation

There were four comments from industry/industry associations addressing the genotoxic properties of SO_2 and the corresponding evaluation by the DS. The general consensus from the industry comments was the disagreement with the proposed classification. The industry comments and the reasoning for the different conclusions arising from the available data, concern both SO_2 and its metabolites and are summarized below:

- There was no evidence for mutagenicity from *in vitro* studies in bacteria
- Equivocal *in vitro* evidence for clastogenicity/aneugenicity in a large number of literature references, which were considered unreliable
- There was no evidence for mutagenicity from *in vitro* studies in mammalian cells
- There was no evidence for clastogenicity from *in vivo* studies. The positive findings originated largely from unreliable studies via unphysiological routes of exposure
- Positive findings were largely obtained from studies published by one research group, whose study design and reporting shows recurring deficiencies (such as using a mouse strain with questionable suitability for genetic toxicity testing)
- The most reliable study among the various tests to assess genotoxicity is the mouse bone marrow micronucleus test (Ziemann, 2010) which clearly shows that SO_2 is not genotoxic.
- Several studies considered by the DS do not satisfy OECD guidelines and the reliability of the studies was wrongly assessed by the DS.
- In the occupational studies where an increase in the incidence of chromosomal aberrations (CA) and sister chromatid exchanges (SCE) in lymphocytes of exposed workers was observed, the potentially relevant co-exposure to other chemical agents as described in the occupational settings, does not allow for a firm conclusion about the genotoxicity of SO_2 in exposed workers.
- The genotoxicity data base has already been recently reviewed by several other reputable scientific organizations (including EFSA), all concluding on an absence of concern for genotoxicity.

In conclusion, the most essential comment by industry focuses on the Zeimann micronucleus study and the fact this specific research group was not able to reproduce the results from the Meng group.

The DS responded that:

- There are several studies with positive *in vitro* genotoxicity results both in bacteria and mammalian cells. However, the DS noted that there are limitations and deficiencies in several of the studies.
- The study reliability was assessed individually study-by-study and the outcome of one study should not be taken as evidence for lack of reliability of another study.
- Little of the available data has been acquired and reported in a way which complies with current OECD and EU guidelines for the testing of chemicals. Therefore, the DS had to adopt a WoE-based approach based on a large number of studies with a range of individual limitations. Nevertheless, the data package provides the information required for an assessment of the human health effects of SO_2.
- The studies which failed to show genotoxic responses are not considered sufficiently reliable to refute the findings from positive genotoxicity studies *in vitro* and *in vivo*.
- In occupational studies there were indications for genotoxicity in lymphocytes of exposed workers.
- In the EFSA opinion from 2016, it is pointed out that there are "[…] several uncertainties and limitations in the database." It was therefore concluded by EFSA that the current group acceptable daily intake (ADI) should "[…] be considered temporary while the database was improved." As stated in the EFSA conclusion, the Panel recommended that the database and the temporary group ADI should be re-evaluated.

Regarding the available contradictory micronucleus tests, the DS noted that in the CLH report there were deficiencies in both the more recent and reliable Ziemann study and the older Meng study. However, the dose-dependent increase in micronuclei in the latter study cannot be ruled out by the negative outcome of the Ziemann study. In addition, the observed ROS (reactive oxygen species) generation indicated by Ziemann, Meng and Etlik is one of the key indirect mechanisms leading to genotoxicity and ultimately to mutagenic responses. This is of particular importance if detoxification and repair mechanisms are saturated.

Further analysis by the DS can be found in the "Summary of the Dossier Submitter's proposal" section above.

In addition to the industry comments, there were two comments from MSCAs, both supporting the DS' proposal. The reasoning was as follows:

- Positive evidence for mutagenicity is found in *in vitro* studies in bacteria (at pH < 7, physiologically less relevant) and mammalian cells
- Genotoxicity was demonstrated in *in vivo* studies, though noting the limitations of some of the studies.
- The negative results of the *in vivo* micronucleus study of the Ziemann group (Anonymous6 and Anonymous7) cannot be used to disregard the positive effects observed in other studies.
- SO_2 induces the production of ROS, which in turn can interact with macromolecules (DNA, proteins and lipids). It is also possible that DNA adducts with aldehydes are formed as a result of lipid peroxidation, as revealed by the presence of MDA. These phenomena could therefore partly explain the negative

results obtained in the *in vitro* studies and the uniformly positive response observed in the comet assay study via systemic exposure to reactive oxygen species.

- Indications for genotoxicity were also observed in multiple epidemiological studies related to occupational exposure. Furthermore, no confounding effect for smoking was found on SO_2-induced genotoxicity in workers exposed to SO_2 by Meng *et al.* (1989).

In conclusion, the main comments from all parties involved focused on the contradictory micronucleus studies, the SO_2 induced production of reactive oxygen species, the genotoxic effects observed in the occupational studies and the equivocal *in vitro* results.

Assessment and comparison with the classification criteria

In order to evaluate mutagenicity/genotoxicity, the studies from Tables 14-16 of the CLH report, were assessed by RAC.

Mutagenicity/Genotoxicity tests in vitro

Regarding bacterial gene mutation assays with SO_2 and its metabolites, inconsistent results from studies with deficiencies and differences among them were observed. Positive results were obtained in 3/8 studies (Pagano and Zeiger 1987; De Giovanni-Donnelly 1985; Mukai et al. 1970) with bacteria in various strains. The most important factor for the outcome of the testing proved to be the pH (positive results were seen at pH = 5-6) as shown by Pagano and Zeiger (1987) with sodium metabisulfite. A non-physiological pH can not only influence the mutagenicity of many compounds but can be mutagenic per se, leading to false positive results. However, in the aforementioned studies negative controls were used, which showed no false positives. In general, inconsistencies with the purity/stability of the test substance, the use of negative/positive controls, the tester strains, the range of concentrations used, the study design and the reporting of the findings were noted.

Out of the 10 cytogenicity studies in eukaryotic (1 study *Saccharomyces cerevisiae*) / mammalian cells, positive results were reported in 7 studies both with SO_2 (1/1 studies Uren *et al.*, 2014) and sulfites (chromosomal aberration, micronucleus assay and sister chromatid exchange). The same shortcomings as above were observed. The most reliable study was an *in vitro* chromosomal aberration test (chromosome aberration, sister chromatid exchange and micronuclei formation in human lymphocytes) with potassium metabisulfite (Anonymous15), which was considered by the DS to be an important study. Positive results were also observed with sodium metabisulfite in a chromosome aberration and sister chromatide exchange study in human peripheral blood lymphocytes and a chromosome aberration study in human embryonic lung cells (Rencüzogullari *et al.* 2001; NTIS, 1972 respectively).

In the mouse lymphoma gene mutation study (Stone, 2010), on the other hand, equivocal results were obtained (positive at the two higher doses with metabolic activation in the first experiment, but negative in the other two experimental branches of the study at similar concentrations).

Mutagenicity/Genotoxicity tests in vivo

There are 15 *in vivo* studies available, 5 with SO_2 and 10 with sulfites. In the *in vivo* studies with SO_2, positive results were observed in two micronucleus assays, a chromosomal aberration test in mouse bone marrow and a comet assay, all in the Kunming mouse strain and by the same research group (the Meng *et al.* group) in China (Anonymous8, 9, 10, 11). In fact, increased OTM in testicles reported by Anonymous11 could be regarded as a major adverse effect. The clear positive results in this comet assay in all organs studied in Kunming mice raise clear concern on the genotoxic potential of the substance. Cytotoxicity seems acceptable (cell viability > 95%), although trypan blue may have underestimated cytotoxicity. A genotoxic MoA related to the formation of reactive species, which can interact with DNA, might explain the similar results in all organs.

On the other hand, in a micronucleus study conducted in 2010 using similar SO_2 concentrations as in the Anonymous8 study, but with a different mouse strain (NMRI), the number of micronuclei did not increase (Anonymous7). Nevertheless, in this latter study there are only indirect indications of target tissue exposure and no signs of overall toxicity. Furthermore, the top dose tested in this study is 30.55 ppm (80 mg/m^3) SO_2. The reported dose dependent increase in MDA indicates that some oxidative stress was induced in this strain and could be one of the prominent mechanisms of SO_2 toxicity affecting DNA. To this end the reported negative results could be due to insufficient dosing for this specific strain of mice.

There are two major differences between the Meng and the Ziemann studies. Firstly, a different strain of mice was used. It is possible that Kunming mice are more prone to DNA damage than NMRI mice, e.g., due to a reduced DNA-repair capacity. Unfortunately, no positive control substance was used in the study by Meng to allow a direct comparison of Kunming and NMRI mice to SO_2. In addition, a higher sensitivity to SO_2 could be related to a lower activity of sulfite oxidase (SOX) in Kunming mice. However, neither of these hypotheses are supported by data. The demonstrated, unexpectedly nearly equal, concentration-dependent DNA-damage induction from inhaled SO_2 in all organs/tissues/cells tested (brain, lung, heart, liver, spleen, kidney, intestine, testicles, blood lymphocytes) in the comet assay (Meng *et al.* 2005) may point to a general SOX deficiency in the test animals but could also be due to a greater sensitivity to inhaled SO_2 for the specific strain of mice used in these studies.

The second difference between the Meng and the Ziemann studies is in the way the SO_2 atmospheres were generated. The atmospheres were also homogenized differently in the exposure chambers (fan at the top vs. laminators at both sides). Unfortunately, there is no information with respect to flow rates, air-exchange rates, homogeneity (potential gradients in the exposure chamber), temperature, and humidity of the exposure atmospheres and separated or "combined" exposure of the animals in the older study. However, despite the limited reporting in the Meng studies, the concentration of SO_2 in the chamber was measured every 30 mins. Due to the mentioned limitations, it is difficult to compare the animal exposure to SO_2 and the effect this uncertainty may have on the micronucleus induction.

Overall, the Ziemann study is considered to be more reliable according to the evaluation by EFSA but on the other hand, both studies are published in peer reviewed journals and evaluated with reliability 2 in the Klimisch scale by the DS. RAC notes that the main issue with the Meng *et al.* group studies is the reporting, since only the published results in

scientific journals are available and not the actual study reports, definite evaluation and firm conclusions cannot be drawn.

In conclusion, it is noted that both the Meng and the Ziemann studies have inconsistencies, while the former has significant deficiencies especially in reporting. Due to the limited information, it is only possible to speculate on the reasons for the contradictory results. The potentially higher sensitivity of Kunming mice to SO_2 and/or the very different means by which the SO_2 exposure atmospheres were generated could be possible explanations. However, the contradictory *in vivo* studies do not unequivocally show that SO_2 does or does not possess genotoxic properties.

In the 10 available *in vivo* studies with the SO_2 derivatives (various sulfites), 4 reported positive results: (1) a study showing chromosomal aberrations in a bone marrow assay in albino rats, shortcomings of which included i.p. administration, only 2 animals per sex per group (Anonymous15); (2) a mouse micronucleus study in CF1 outbred mice (peripheral blood and bone marrow), shortcomings of which included lack of purity of the test substance, unusually high MN and PCE/NCE ratio in controls and shorter exposure time for peripheral blood (24 instead of 36 h, Anonymous14); (3) a comet assay in CF1 outbred, shortcomings of which included possibly insufficient dosing, unusual scoring for a comet assay (damage index is an unusual scoring for Comet assay, Anonymous14); and (4) a comet assay in the Kunming mouse (DNA-damage induction in brain, lung, heart, liver, stomach, spleen, thymus, bone marrow, kidney), shortcomings of which included an uncommon mouse strain, purity/stability of the substance and long sampling time after the last dose (Anonymous16).

In the rest of the available studies, with reported negative results, there were issues with the dosing scheme used (Anonymous13, Anonymous5) and whether the target tissues were reached (Anonymous12). Finally, in a negative chromosome aberration study of high reliability in albino rats, a dose dependent decrease in mitotic index (MI) along with increased cytotoxicity (NTIS, 1972) were observed. In the same study, sodium metabisulfite was negative in a dominant lethal assay test showing no mutagenic effects in germ cells. However, the authors suggest that this substance should be tested again using greater number of animals due to nearly statistically significant findings.

Human data relevant for germ cell mutagenicity

The results in the available occupational studies are also contradictory with two main limitations: the very small number of participants (min 7, max 42) and the lack of statistical evaluation regarding confounding factors. A significantly increased frequency of chromosomal aberrations in lymphocyte cultures was found among workers at a sulfite pulp factory in northern Sweden. This increase was found to be associated mainly with exposure to SO_2 (boiling of sulfite pulp and handling of sulfuric acid), n=7, and not with exposure to chlorine (n=6) and dust (n=6) in other workplaces within the factory (Nordenson *et al.,* 1980). Similarly, in a study by the Meng group (Meng and Zhang, 1990a), a statistically significant increase in the frequency of chromosomal aberrations in peripheral blood lymphocytes of SO_2 exposed workers (n=40) in a sulfuric acid factory was observed. In the same study, it was shown that the mean SCEs/cell of the same SO_2 exposed workers also increased significantly. The same group (Meng and Zhang, 1990b), in a study with the same

population (same factory/exposure) observed a significant increase in the micronuclei frequency in peripheral blood lymphocytes of SO_2 exposed workers.

In a more recent study (Yadav *et al.,* 1996), workers (n=42) in a fertilizer factory exposed to SO_2 showed significant increases in mitotic index, chromosomal aberrations, sister-chromatid exchanges and satellite associations.

In contrast, no effects on chromosomal aberrations and sister chromatid exchanges were observed in workers (n=8) exposed to SO_2 in the aluminium industry (Sorsa *et al.,* 1982). RAC notes the rather low average exposure of 1 ppm/2.62 mg/m^3 in this specific study.

Overall, in the occupational studies there may be an association of SO_2 exposure and genotoxic effects on workers. However, serious limitations are noted including the very small number of participants, possible co-exposure to other carcinogenic substances in the industrial settings, co-exposure to lifetime cofounders (smoking, alcohol), as well as uncertainties about the concentrations of SO_2 to which the subjects were exposed.

In conclusion, the following key points are relevant:

- The *in vitro* data provide evidence for the possible genotoxic (clastogenic/ aneugenic) properties of SO_2 and its metabolites, stemming mainly from the cytogenicity studies in mammalian cells.
- In the *in vivo* studies, a series of shortcomings have been observed in those reporting positive as well as negative findings.
- The positive *in vivo* results from the Meng group studies were not reproduced by the Ziemann study, possibly due to the strain specificity to SO_2 exposure.
- The positive findings *in vivo* with sulphites, although rather inconclusive, could support the possible *in vivo* mutagenic properties of SO_2. The fact that human organ tissues are continuously exposed to endogenous levels of sulfites and that detoxification process exist is not sufficient to disregard the results of the genotoxicity studies (hormesis).
- There is only 1 study with positive findings assessing germ cell related tissue, while ADME data show that SO_2 could reach the germ cells. A dominant lethal assay with sulphites was reported to be negative but with inconsistencies (dose selection, no positive control).
- Worker exposure to SO_2 in three different occupational settings showed a potential association between SO_2 exposure and genotoxicity in humans. However, RAC notes that there are serious limitations as explained above that reduce the weight of the supporting evidence of the occupational studies for classification.

Considering all the above, RAC notes that the available data set for the evaluation of the genotoxic properties for SO_2 is quite extensive but the quality of the studies is not sufficient to provide unequivocal evidence for the mutagenicity classification of SO_2. Although there are indications for the possible genotoxic properties of SO_2, the evidence is not strong enough to support classification and therefore, **no classification due to inconclusive data for mutagenicity is warranted.**

10.9 Carcinogenicity

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON SULFUR DIOXIDE

Following data and information are available on sulfur dioxide and related relevant compounds. For justification of read-across from metabisulfite, please refer to section 10.

Table 17: Summary table of *in vivo* carcinogencity studies in animals

Summary table of carcinogenicity studies in animals						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance Dose levels, Route of exposure, Duration of exposure**	**NOAEL, LOAEL**	**Results (***Please indicate any results that might suggest carcinogenic effects, as well as other toxic effects.)*	**Remarks (e.g. major deviations)**	**Reference**
Metabisulphites						
Pre-guideline -carcino-genicity study cannot be evaluated with respect to requirements of OECD due to poor study reporting Rel. 2 (poor reporting)	Mouse ICR/JCL mice, 50 M/ 50 F per group	1 and 2 % **potassium metabisulphite** solutions ad libitum for 24 months, corresponding to 2500 – 3000 mg/kg bw $K_2S_2O_5$ or 1450 – 1730 mg/kg bw/d SO_2 equivalents	No data	No evidence for carcinogenicity, Number of lung tumours higher in 2 % group, but statistically not significant	Calculation in mg/kg bw based on mice body weight of 20 - 25 g and a daily water intake of 3 - 5 ml. Only number of tumors and no data on other endpoints reported.	Anonymous64.
Non-guideline study Rel. 3 (well conducted study but carcinogenicity part not reliable: high tumour incidences in control)	Rat Wistar-derived F0-generation: 20 males /20 females F1-generation: 10 males/ 10 females F2-generation: 10 males/15 females	0, 0.125, 0.25, 0.5, 1.0, or 2.0 **% sodium metabisulphite** in the diet (considering sulphite loss corresponding to approx. 49, 108, 220, 460, 955 mg/kg bw/d) Exposure of F_0 and F_1 rats: 104 weeks, F_2 rats: 30 weeks)	Local: NOAEC: 0.25 % (0.215 when considering sulphite losses) Systemic: reduced bw: >0.25 % (108 mg/kg bw/d $Na_2S_2O_5$, 72 mg /kg bw/d SO_2) LOAEC: 0.5 % (gastric lesions) LOAEL: systemic: 0.5 % (220mg/kg bw/d $Na_2S_2O_5$, 147 mg /kg bw/d SO_2)	Local effects: ≥0.5 %: Lesions and inflammatory infiltration in forestomach in F2 generation , ≥1 %: Occult blood in faeces, hyperplasia and inflammation in fore- and glandular stomach, reduced thiamine content in liver 2 %: Haematological effects Systemic effects: >0.25 % Reduced bw in F2 animals (-9 %) considered adverse at 0.5 % (-11 %) No compound-related tumour incidence was reported. The number of lymphoreticular pulmonary tumours in males decreased with increasing levels of sulphite in the diet. Incidence of thyroid and pituitary	Losses of sulphite in the diet: 22; 14; 12; 8, 4.5 % respectively. Local effects in the forestomach of rats are considered of minor relevance for human RC. Number of rats with tumours very high (10/24 malignant lymphoreticular tumour in controls, lower in treatment groups)	Anonymous62

Summary table of carcinogenicity studies in animals						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance Dose levels, Route of exposure, Duration of exposure**	**NOAEL, LOAEL**	**Results (***Please indicate any results that might suggest carcinogenic effects, as well as other toxic effects.)*	**Remarks (e.g. major deviations)**	**Reference**
				tumours in control group very low; higher values in various test groups in random manner corresponding to historical controls – no relationship between number, location or type of tumours and treatment		
Sulphur dioxide						
Carcinogenicity, Pre-guideline study, does not follow OECD 451 principles Rel. 2 (untypical study design)	Mouse (LX colony), Group 1) control: 41 M/39 F; 2) free radical group: 30M/30F, 3) SO_2: 35M/30 F	Group 1: Untreated controls Group 2: Free radical inhalation Group 3: **SO_2** inhalation: **500 ppm (1330 mg/m^3)** /5min, 5 days/week, life long exposure. Examination of mice that survived ≥ 300 days (authors stated that no primary tumours of the lung were seen in LX mice below this age)	No data	**Adenoma** : Group 1 (Control): M: 11/35 (31 %) F: 5/30 (17 %) Group 2 (free radical inhalation): M: 12/29 (41 %) F: 7/30 (23 %) Group 3 (SO_2 inhalation): M: 15/28 (54 %) F: 13/30 (45 %) **Primary carcinoma:** Group 1 (Control): M: 2/35 (6 %) F: 0/30 Group 2 (free radical inhalation): M: 3/29 (10 %) F: 0/30 Group 3 (SO_2 inhalation): M: 2/28 (7 %) F: 4/30 (18 %)	Authors used LX mice because they are highly susceptible to the induction of lung adenoma in response to urethane. Adenoma in this study: all primary tumours of the lung are counted as adenomas, primary carcinoma: tumours which invade blood vessels. (primary carcinoma were also listed under adenoma) Assessment only in mice that survived at least 300 days.	Anonymous65
Carcinogenicity Non-guideline study, does not follow OECD 451 principles Rel. 2 (no typical	Rat SD C.D. M; no. per group: 1)43 2)26	Group 1: Control (filtered air) Group 2: Control (filtered air + intratracheal instillation of gelatine	SO_2: 30/>30 ppm:	**Negative:** No malignant tumours observed in control and SO_2 groups; high frequency of tumours in all B(a)P groups. No influence of SO_2 on tumour	Treatment duration too short for a guideline conform carcinogenicity study High incidence of tumours in B(a)P – treated groups	Anonymous60

Summary table of carcinogenicity studies in animals						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance Dose levels, Route of exposure, Duration of exposure**	**NOAEL, LOAEL**	**Results (***Please indicate any results that might suggest carcinogenic effects, as well as other toxic effects.)*	**Remarks (e.g. major deviations)**	**Reference**
carcinogenicity study)	3)20 4)18 5)72 6)72 7)74	vehicle) Group 3: 10 ppm **SO_2** Group 4: 30 ppm **SO_2** Group 5: B(a)P Group 6: B(a)P + 10 ppm SO_2 Group 7: B(a)P + 30 ppm SO_2 6h/d; 5d/wk; 21 weeks (treatment); observation period: 105 weeks		frequency in B(a)P groups.	precluded detection of tumour enhancing effect of SO_2. However, no tumours observed in groups exposed to SO_2 alone.	
Carcinogenicity Non-guideline study, does not follow OECD 451 principles Rel. 3	Rat strain and sex not specified. 45 – 48 / group; 20 / control group	**Sulphur dioxide** Carcinogenicity part of study: exposure: 500 ppm Exposure induced mortality – inhalation of irritants: 10, 51, 105, 567 ppm (corr. 26, 134, 276, 1488 mg/m^3 – 24 °C) 6h/d; 5d/wk; 12 – 113 days (lowest to highest concentration)	10/105 ppm (from cumulative mortality study)	Neoplastic effects: no effects (No neoplastic effects were observed in the groups solely exposed to sulphur dioxide.) Non-neoplastic findings: Exposure-induced cumulative mortality (%): 10 ppm vs. control: 5 vs. 15 (day 113) 51 ppm vs. control: 18 vs. 10 (day 113) 105 ppm vs. control: 40 vs. 5 (day 22) 567 ppm vs. control: 87 vs. 10 (day 12), 105 and 567 high incidences of bronchitis, congestion, and pneumonia, regenerative hyperplasia and early metaplasia 4 d after exposure at 105 ppm	Major deficiencies when compared with guideline conform carcinogenicity study: duration too short, group size, major reporting deficiencies, study design	Anonymous61

Table 18: Summary table of human data relevant for carcinogenicty

Summary table of human carcinogenicity data			
Kind of study (e.g. case reports)	**Examination methods, number of individuals examined**	**Results**	**References**
Cohort study on mortality due to cancer in workers of a paper company	Standardised mortality ratios (SMR) of selected causes of death; 883 subjects	460 workers were still alive, 414 were death, and 9 were lost to follow up. Employment in pulp or paper mills is associated with excess mortality due to digestive (SMR = 152, pancreatic cancer: SMR = 305) and lymphopoietic cancers (SMR = 241). Findings are not clearly **SO_2** related as workers might have been exposed towards other compounds (hydrogen sulfide, methyl mercaptan, chlorine, chlorine dioxide esp. palp mill workers).	Henneberger, P.K. *et al.* (1989) Brit. J. Ind. Med. 46: 658-664. (published)
Cohort study on mortality due to cancer in workers of pulp and paper workers in Finland	Mortality (SMR) compared to national mortality rates 3520 subjects, six subcohorts compared to 1290 sawmill workers (control group)	Higher mortality from ischaemic heart disease in workers in sulphite, sulphate, and paper mills, maintenance department, and power plants compared to sawmills (SMR 121). Finding generally for occupational exposure in pulp and paper workers but cannot be related to **SO_2**.	Jäppinen, P. (1987). Brit. J. Ind. Med. 44: 580-587. (published)
Cohort study on mortality due to cancer in workers of pulp and paper workers in the USA	Mortality (SMR) compared to national mortality rates 3572 subjects	No increased cancer mortality or any mortality was observed in the cohort. Cohort of **sulphite** mill workers: Risk for stomach cancer was elevated for workers employed for 20 years in sulphite mills but did not increase with duration of employment.	Robinson, C.F. *et al.* (1986). Scand. J. Work Environ. Health 12: 552-560. (published)
Cohort study on cancer incidence among pulp and paper mill workers in British columbia	SIR (Standardised incidence ratios) in comparison to cancer incidence in the cohort 1756 cancer cases Cohort: 28278 workers; 475787 person-years; years worked (mean): 11.6 years	Excess risks of prostate and stomach cancers, leukemias in kraft and sulphite processes, rectal cancer for work in sulphite process only. Mesotheliomas associated with asbestos. Pulp and paper workers may have been exposed to asbestos, biocides, formaldehyde, hypochlorite (Band et al. 1997)	Band et al. (2001). Scand J Work Environ Health. 27/2:113-119
Cohort study on male pulp and paper workers in Norway	SIR Cohort: 23780 workers at least one year exposure between 1920 and 1993 in Norway	Excess incidence of lung cancer among short- and long-term employees: SIR for sulphite mill workers 1.5, 95 % CI 1.09-1.99). Lung cancer can be attributed to smoking and asbestos exposures. Other work-related exposures: sulphur and chloride compounds, wood dust).	Langseth and Andersen (2000) Scand J Work Environ Health. 26/2: 99-105
Cohort study on workers in pulp and paper industry in 12 countries (Brazil, Denmark, Finland, France,	SMR based on age-specific and calendar period-specific national mortality rates and cancer mortality risk.	Positive relationship between weighted cumulative SO_2 exposure and lung cancer mortality (p-value of test for linear trend = 0.009 among all exposed workers; p = 0.3 among workers with high exposure. Mortality from non-Hodgkin lymphoma and	Lee et al. (2002) Environ Health Perspect. 110:991-995

Summary table of human carcinogenicity data			
Kind of study (e.g. case reports)	**Examination methods, number of individuals examined**	**Results**	**References**
Japan, New Zealand, Norway, Poland, South Africa, Spain, Sweden, USA). Data from Brazil and South Africa not included in analysis	Cohort: 57 613 workers ≥ 1 year employed in pulp and paper industry	from leukaemia increased among workers with high sulphur dioxide exposure, dose–response relationship with cumulative sulphur dioxide exposure suggested for non-Hodgkin lymphoma. Conclusion: exposure with high concentrations of SO_2 in pulp and paper industry may be associated with increased lung cancer risk. SO_2 may have a cancer promoting effect in combination with other carcinogens. Residual confounding may have occurred. (e.g. Smoking was not considered as possible confounder, asbestos only assessed at level of department). Controlled possible co-exposure: asbestos, combustion products, welding fumes.	

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

Takening into account all available information including genotoxicity, there is sufficient evidence that genotoxic effects occur at cytotoxic concentrations. The lung is the primary target organ following inhalation exposure to SO_2 but also following oral exposure to sulphites (bisulphites, metabisulphites). SO_2 and sulphite toxicity predominantly occur in tissues with lower sulphite oxidase activity (e.g. lung). As sulphite is a reactive substance, a carcinogenic effect mediated by binding to biomolecules (DNA, proteins) is principally possible, especially in tissues with low activity of sulphite oxidase. However, no clear evidence can be retrieved from the literature. A potential cytotoxic effect on chromosome aberration was postulated by Popescu and DiPaolo (1988). Bisulphite inhibition of DNA replication might be involved in the observed occurrence of abnormal chromosomes. Neoplastically transformed cells exhibit persistent chromosome rearrangements. This observation is in accordance with in vitro chromosome aberrations, especially at cytotoxic concentrations.

No classification for carcinogenicity is proposed. IARC (1992) came to the following conclusion: Sulphur dioxide, sulphites, bisulphites, and metabisulphites are not classifiable as to their carcinogenicity to humans (Group 3). No carcinogenicity study has been published since then. A comprehensive cohort study (Lee et al. 2002) concluded that exposure to sulphur dioxide of employees in pulp and paper industry may be associated with increased cancer risk, especially for lung cancer. Results were adjusted for some confounders. Controlled possible co-exposure: asbestos, combustion products, welding fumes. Increased relative risk (RR) for coexposure with asbestos and high SO_2 exposure as well as co-exposure of welding fumes and high SO_2. Exposure with sulphur dioxide was not measured but estimated by using international industrial hygiene measurement data from mills included in the study and from nonparticipating European and North American mills. Misclassification to exposure groups cannot be ruled out, completely. The authors further discussed the lack of potential lifestyle confounders (e.g. smoking) as important limitation of the study but considered the possible confounding effect of smoking habits not outside the range of 0.5 - 1.5 (smoking habits of not exposed and exposed population may not differ substantially higher). The authors identified as main result of the analysis an association between SO_2 exposure and mortality from all neoplastic diseases and lung cancer. The well-designed analysis comprises cohorts of several other publications. Apart from stated limitations by the authors, there is also uncertainty from other confounding factors in the paper processing (which may include, according to Band et al. 1997, chloroform, arsenic, formaldehyde, chlorophenols).

10.9.2 Comparison with the CLP criteria

In conclusion, there is evidence for an increased cancer risk especially associated with high sulphite exposure in exposed workers of the pulp and paper industry. However, due to potential co-exposure to other substances, information is considered not sufficient for classification for carcinogenicity category 1A.

Some animal experiments with sulfur dioxide or sulfur dioxide releasing compounds are available. However these had limitaions regarding study design or reporting when compared to OECD TG recommendations. There are some results indicating carcinogenic effects in non-standard assays.

In summary, taking into account the limitations of the available data on carcinogenicity, DS does not see sufficient evidence to propose classification for carcinogenic hazards, even though sulfur dioxide is proposed to be a genotoxic compound.

10.9.3 Conclusion on classification and labelling for carcinogenicity

No classification for carcinogenicity is proposed.

RAC evaluation of carcinogenicity
Summary of the Dossier Submitter's proposal

The DS evaluated SO_2 as a genotoxic substance and noted that there is sufficient evidence that genotoxic effects occur at cytotoxic concentrations. The lung is the primary target organ following inhalation exposure to SO_2 but also following oral exposure to sulfites (bisulfites, metabisulfites). SO_2 and sulfite toxicity predominantly occur in tissues with lower sulfite oxidase activity (e.g. lung). As sulfite is a reactive substance, a carcinogenic effect mediated by binding to biomolecules (DNA, proteins) is in principle possible, especially in tissues with low activity of sulfite oxidase. However, no clear evidence for this could be retrieved from the literature. A potential cytotoxic effect on chromosome aberration was postulated by Popescu and DiPaolo (1988). Bisulfite inhibition of DNA replication might be involved in the observed occurrence of abnormal chromosomes. Neoplastically transformed cells exhibit persistent chromosome rearrangements. This observation is consistent with the *in vitro* chromosome aberration data discussed under "germ cell mutagenicity", especially at cytotoxic concentrations. In conclusion, some animal experiments with SO_2 or SO_2 releasing compounds are available. However, these had limitations in study design or reporting when compared to OECD TG recommendations. There are some results indicating carcinogenic effects but in non-standard assays with limitations.

In occupational studies, a comprehensive cohort study (Lee *et al.*, 2002) concluded that exposure to SO_2 of employees in pulp and paper industry may be associated with increased cancer risk, especially for lung cancer. However, due to potential co-exposure to other substances in the working environment as well as to potential lifestyle confounders (e.g. smoking), the available data is not considered robust enough for classification by the DS.

In summary, taking into account the limitations of the available data on carcinogenicity, the DS does not see sufficient evidence to propose classification for carcinogenic hazards, even though SO_2 is proposed to be a genotoxic compound.

Comments received during consultation

During the consultation there were two comments received (both by MSCAs).

The first MSCA agreed that the animal data do not warrant classification for carcinogenicity. Results of carcinogenicity of metabisulfites and SO_2 in *in vivo* animal studies are contradictory. Multiple *in vivo* animal studies show negative results for carcinogenicity for SO_2 and metabisulfites, administered via the inhalation or oral routes, respectively. Some studies were not reliable because of high tumour incidence observed in control groups and limitations with respect to study design. Furthermore, no dose-related tumour incidence was observed, or no formation of malignant tumours was demonstrated upon exposure to SO_2 or metabisulfites. Thus, *in vivo* studies supporting a classification for SO_2-induced carcinogenicity are clearly lacking.

The same MSCA noted that a positive correlation between tumour formation and exposure to SO_2 in workers had been demonstrated in various occupational studies. In addition, a dose-related correlation of SO_2 exposure and lung cancer was found in workers (Lee *et al.,* 2002). Confounders (e.g. smoking) could not be excluded with confidence in these studies, but this is not *per se* an obstacle to warrant classification for carcinogenicity. Furthermore, smoking was not found to be a confounder in a human genotoxicity study by Meng *et al.* (1989), as discussed in the CLH report. Therefore, the carcinogenic potential of SO_2 for human is suspected, based upon limited evidence of SO_2-induced carcinogenicity in humans.

The MSCA asked the DS to reflect on the need to classify in category 2 for carcinogenicity (H351: suspected of causing cancer).

The second MSCA supported the DS SO_2 evaluation as non-carcinogenic, based on the experimental studies not being of adequate quality to properly conclude on classification for this endpoint (low duration, one tested concentration, inadequate control group, inadequate assessment of tumours etc). Moreover, the excess risks of cancers reported in workers are not consistent and the excess risk may be attributable to confounding factors.

Assessment and comparison with the classification criteria

The animal carcinogenicity data assessed by RAC are summarised in Table 17 of the CLH report.

There are five animal carcinogenicity studies included in the CLH report, three with SO_2 (mouse LX, rat SD C.D., rat strain not specified) and two with metabisulfite (oral exposure, mouse ICR/JCL, rat Wistar) (Table 17 of the CLH report). In addition, another carcinogenicity study with SO_2 nose-only exposure was found in the literature with Syrian golden Hamsters designed to demonstrate that SO_2 enhances the tumour formation in the respiratory tract caused by benzo(a)pyrene inhalation (Pauluhn *et al.*, 1985). One of the major limitations in all the SO_2 carcinogenicity studies of the CLH report is the short duration of exposure (5 min daily, 5 days per week, life-time exposure [Anonymous65]; 6h daily, 5 days per week, 21 weeks treatment and 105 weeks observation [Anonymous60]; 6h daily, 5 days per week, 12-113 days [Anonymous61]) compared to OECD guidelines (6 hours daily, 5 or 7 days/week, 104 weeks). Furthermore, the metabilsufites studies are of low reliability, either due to poor data reporting (Anonymous64) or to high tumour incidences (lymphoreticular pulmonary tumours) in the control group (Anonymous62). Nevertheless, none of these metabisulfite studies provide evidence for compound-related carcinogenicity. The same applies also to two of the three studies with SO_2 (Gunnison *et al.,* 1988; Laskin *et al.,* 1970).

In the Peacock study, pulmonary adenomas were significantly ($p = 0.02$) increased in female LX mice compared to controls (13/30 compared with 5/30), while the incidence of pulmonary primary carcinomas was not significantly increased (4/30 compared with 0/30). In male animals the incidence of pulmonary neoplasms was not significantly increased (15/28 – 54% compared to 11/35 – 31% in controls), while the incidence of pulmonary carcinomas remains practically unchanged (2/28 compared with 2/35). In this study, a deficiency was noticed in the tumour characterisation and allocation, with primary carcinomas, defined as tumours which invade blood vessels, being also listed under adenomas. Peacock *et al.* (1967) concluded in their publication that the increased incidence of primary lung tumours in LX mice of both sexes is a consequence of the initial essentially inflammatory reaction to SO_2, and "does not justify the classification of SO_2 as a chemical carcinogen as generally understood". This latter explanation is also supported by the non-neoplastic findings of the Laskin (1970) study (bronchitis, congestion, and pneumonia, regenerative hyperplasia and early metaplasia).

***Table**: Summary table of human data relevant for carcinogenicity*

Kind of study (e.g. case reports)	Examination methods, number of individuals examined	Results	References
Cohort study on mortality due to cancer in workers of a paper company	Standardised mortality ratios (SMR) of selected causes of death; **883** subjects	460 workers were still alive, 414 were dead, and 9 were lost to follow up. Employment in pulp or paper mills is associated with excess mortality due to digestive (SMR = 152), pancreatic cancer (SMR = 305) and lymphopoietic cancers (SMR = 241). Findings were not clearly **SO_2** related as workers might have been exposed towards other compounds (hydrogen sulfide, methyl mercaptan, chlorine, chlorine dioxide esp. pulp mill workers).	Henneberger, *et al.* 1989
Cohort study on mortality due to cancer in workers of pulp and paper workers in Finland	Mortality (SMR) compared to national mortality rates 3520 subjects, six subcohorts compared to **1290** sawmill workers (control group)	Higher mortality from ischaemic heart disease in workers in sulfite, sulfate, and paper mills, maintenance department, and power plants compared to sawmills (SMR = 121). Finding generally for occupational exposure in pulp and paper workers but cannot be related to **SO_2**.	Jäppinen, P. (1987). Brit. J. Ind. Med. 44: 580-587. (published)
Cohort study on mortality due to cancer in workers of pulp and paper workers in the USA	Mortality (SMR) compared to national mortality rates **3572** subjects	No increased cancer mortality or any mortality was observed in the cohort. Cohort of **sulfite** mill workers: Risk for stomach cancer was elevated for workers employed for 20 years in sulfite mills but did not increase with duration of employment.	Robinson, C.F. *et al.* (1986). Scand. J. Work Environ. Health 12: 552-560. (published)
Cohort study on cancer incidence among pulp and paper mill workers in British Columbia	SIR (Standardised incidence ratios) in comparison to cancer incidence in the cohort 1756 cancer cases Cohort: **28278** workers; 475787 person-years; years worked (mean): 11.6 years	Excess risks of prostate and stomach cancers, leukaemias in kraft and sulfite processes, rectal cancer for work in sulfite process only. Mesotheliomas associated with asbestos. Pulp and paper workers may have been exposed to asbestos, biocides, formaldehyde, hypochlorite (Band *et al.* 1997)	Band *et al.* (2001). Scand J Work Environ Health. 27/2:113-119
Cohort study on male pulp and	SIR	Excess incidence of lung cancer among short- and long-term	Langseth and Andersen, 2000

paper workers in Norway	Cohort: **23780** workers at least one year exposure between 1920 and 1993 in Norway	employees: SIR for sulfite mill workers 1.5, 95% CI 1.09-1.99). Lung cancer can be attributed to smoking and asbestos exposures. Other work-related exposures: sulfur and chloride compounds, wood dust).	
Cohort study on workers in pulp and paper industry in 12 countries (Brazil, Denmark, Finland, France, Japan, New Zealand, Norway, Poland, South Africa, Spain, Sweden, USA). Data from Brazil and South Africa not included in analysis	SMR based on age-specific and calendar period-specific national mortality rates and cancer mortality risk. Cohort: **57 613** workers ≥ 1 year employed in pulp and paper industry	Positive relationship between weighted cumulative SO_2 exposure and lung cancer mortality (p-value of test for linear trend = 0.009 among all exposed workers; p = 0.3 among workers with high exposure. Mortality from non-Hodgkin lymphoma and from leukaemia increased among workers with high SO_2 exposure, dose–response relationship with cumulative SO_2 exposure suggested for non-Hodgkin lymphoma. Conclusion: exposure with high concentrations of SO_2 in pulp and paper industry may be associated with increased lung cancer risk. SO_2 may have a cancer promoting effect in combination with other carcinogens. Residual confounding may have occurred (e.g. smoking was not considered as possible confounder, asbestos only assessed at level of department). Controlled possible co-exposure: asbestos, combustion products, welding fumes.	Lee *et al.,* 2002
Retrospective epidemiological study on cancer cases in Taiwan	Investigation of possible correlations between air pollutants and cancer cases in Taiwan.	Positive correlations for SO_2, was found, but not after Bonferroni correction. Additional studies are required to confirm or refute these findings	Su *et al.,* 2019, Associations between ambient air pollution and cancer incidence in Taiwan: an ecological study of geographical variations. BMC Public Health 19, 1496.
Cohort study on cancer cases in Tianjin, China with regards to air pollutants	One thousand five hundred patients across 27 districts in Tianjin were studied for lung cancer incidences. The air pollutant compositions ($PM_{2:5}$, PM_{10}, SO_2, NO_2, CO, and O_3) of environments the patients lived in were	– When SO_2 concentrations are high, lung cancer incidences are high; – When SO_2 concentrations are high and CO concentrations are near the average value, incidences of lung cancer increase substantially; and – When SO_2 concentrations decrease, incidences of lung cancer decrease	Yue *et al.,* 2017

	determined using the nearest air monitoring station to the patient		

Exposure to SO_2 occurs in different occupational environments (Table 18 of the CLH report). The epidemiological studies have been conducted primarily in smelter workers and in pulp and paper workers, where exposure to SO_2 is rather high. In IARC (1992) and MAK (1998) reviews, numerous studies are available in which workers employed in the smelting of copper and other non-ferrous metals were also exposed to SO_2. Correlations were found between an increased incidence of lung cancer and exposure to arsenic or smoking. However, SO_2 alone was not found to have any effects. Nevertheless, in a key epidemiological study (Lee *et al.*, 2002; meta-analysis including cohorts from Henneberger *et al.*, 1989; Langseth and Andersen, 2000; Band *et al.*, 2001; Jäppinen, 1987; Robinson *et al.,* 1986) conducted on a cohort of 57613 workers exposed to SO_2 in the pulp and paper industry from 12 countries, lung cancer mortality increased only marginally in exposed workers (SMR = 1.08; 95% CI = 0.98–1.18). Mortality from non-Hodgkin lymphoma and from leukaemia also increased among workers with high SO_2 exposure, and a dose–response relationship with cumulative SO_2 exposure was suggested for non-Hodgkin lymphoma. The authors of the study concluded that occupational exposure to SO_2 in the pulp and paper industry may be associated with an increased risk of lung cancer and non-Hodgkin lymphoma. The statistical analysis of the study did not account for confounding demographical factors, such as smoking. Similarly, while Su *et al.*, 2019 (study provided by the DS during the consultation) reported an association between increased environmental SO_2 exposure and cancer incidence, after a Bonferroni correction for multiple testing (a total of 70 correlations were tested), this association was no longer significant. Thus, the authors concluded that further data would be necessary in order to confirm a positive correlation of increased incidences of cancers and SO_2 exposure. In addition, it should be noted that in general, the workers in the pulp and paper manufacturing occupational setting are exposed to numerous other substances such as hydrogen sulfide, methyl mercaptan, asbestos and various chlorinated compounds. The results of the Lee study could be regarded as compatible with the results in some animal studies demonstrating that SO_2 may have a cancer promoting effect when it occurs in combination with other carcinogens. In a study by Yue *et al.* (2017), provided by the DS during the consultation, lung cancer incidence and environmental concentrations for various pollutants in Tianjin districts in China were correlated. The conclusion of the study was that when SO_2 concentrations are high, lung cancer incidences are high and that SO_2 concentrations have a strong impact on lung cancer incidences. Finally, in a study by Guo *et al.* (2021) also provided by the DS during the consultation, association between SO_2 and the incidence rate of male lung cancer was found to be stronger in Chinese counties with low education levels than in those with high education levels.

Considering all of the above, RAC concludes that the available animal data set for the SO_2 classification is rather limited and the quality of the studies is not high enough to provide unequivocal evidence for the carcinogenicity classification of SO_2. In addition, occupational reports on workers exposure and on general public environmental exposure to SO_2, indicate a positive correlation between SO_2 exposure and carcinogenicity, but fail to demonstrate a causal relationship. Serious limitations are noted concerning possible co-exposure to other

carcinogenic substances in the industrial settings, co-exposure to lifetime cofounders (smoking, alcohol), as well as uncertainties about the concentrations of SO_2 exposure.

Overall and in a weight of evidence approach, RAC concludes that based on the existing evidence **SO2 does not warrant classification as a carcinogen.**

10.10 Reproductive toxicity

Endpoint not addressed.

10.10.1 Adverse effects on sexual function and fertility

Endpoint not addressed.

10.10.2 Adverse effects on development

Endpoint not addressed.

10.10.3 Adverse effects on or via lactation

Endpoint not addressed.

10.11 Specific target organ toxicity-single exposure

The selected published studies were used to evaluate the ability of sulfur dioxide to induce bronchoconstriction. Main pulmonary function parameters amongst studies were SRaw (specific airway resistance) and $FEV_{1.0}$ (forced expiratory volume in one second). According to the American Thoracic Society (ATS), reductions in $FEV_{1.0}$, of <10, 10-20 %, and >20 % were graded as mild, moderate, or severe, respectively (Samet et al. 2000). Another useful assessment of airflow limitations is the ratio of FEV1.0 to FVC (forced vital capacity). The FEV_1/FVC ratio is normally greater than 0.75 to 0.8, and possibly greater than 0.90 in children. Any values less than these suggest airflow limitation (GINA Report 2012). As the majority of studies with sulfur dioxide did not provide data on FEV_1/FVC ratio, reductions in $FEV_{1.0}$ and/or SRaw were used instead (criteria as described below). Changes in lung function parameters were identified at concentrations of 0.4 ppm with asthmatics being the most vulnerable group. Increases in SRaw of ≥ 100 % (according to criteria of the "German Society for Pneumology") and moderate decreases of $FEV_{1.0}$ of ≥ 10 % were used as criterion to define an adverse effect indicating airflow restriction following short-term exposure.

Table 19 Summary table of other studies relevant for respiratory sensitisation/ irritation

Summary of controlled human exposure studies with asthmatics and healthy volunteers exposed to SO_2							
Reference / study characteristics	**SO2 exposure**			**(Lung) function parameters**	**Venti-lation rate**	**Results**	**Others/ Remarks**
	Conc. mg/m^3	**Conc.** ppm	**Duration** min				
Linn et al. 1987 / 85 volunteers in 4 clinical groups: 1: normal subjects (15M 9F; age: 18 - 37) 2: atopic subjects (12M 9F, age: 18 - 32) 3: subjects with minimal or mild asthma (10M 6F, age: 20 - 33) 4: subjects with moderate to severe asthma (10M 14 F; age: 18 – 35, 1x 46) Key study	0.5 1.1 1.6	0.2 0.4 0.6	60	PD_{20} in $FEV_{1.0}$, SRaw at FRC, FVC, PEF, EKG, maximal mid expiratory flow, symptom score	40 L/min (during exercise)	Changes in pulmonary function $FEV_{1.0}$ - in clinical groups (results of 1. round) Group 1: none Group 2: none Group 3: **0.4 ppm**: $FEV_{1.0}$: ↓ (-6 %), SRaw: ↑ (129 %) **0.6 ppm**: $FEV_{1.0}$: ↓ (-11 %), SRaw: ↑ (153 %) Increases control SRaw: 29 % Group 4: **0.4 ppm**: $FEV_{1.0}$: ↓ (-13 %), SRaw: ↑ (108 %) **0.6 ppm**: $FEV_{1.0}$: ↓ (-24 %), SRaw: ↑ (200 %) Increases control SRaw: 73 % Significant increase ($p<0.0001$) of symptom score with increasing SO_2 concentrations **LOAEL: 0.4 ppm** **NOAEL: 0.2 ppm**	Alternate 10 min exercise and resting periods within a 1-h-exposure cycle. Pulmonary function prior to, early and late in exposure; cross-over study
Roger et al. 1985 / 28 non-smoking male asthmatics currently receiving no corticosteroid, cromolyn sodium or desensitization therapy, baseline SRaw: 2.2 – 12.8 cm H_2O x sec.; $FEV_{1.0}$/FVC = 56 – 89 %, age: 19 – 34	0 0.65 1.3 2.6	0 0.25 0.5 1.0	75	SRaw, $FEV_{1.0}$, FVC, $FEV_{1.0}$/FVC, V_{tg}, FEF_{25-75}, Exercise at a)0, b)25, and c)50 min after entering the chamber SRaw in a, b, c: 3, 5, 7, and 9 min post-exercise Symptome questionaire	normalised to body surface: 21.4±0.4 L/m^2/min	Significant increase ($p \leq 0.005$) following exercise at 0, 25, and 50 min at 0.5 ppm SO_2 (93 %, 63 %; 52 % compared to pre-exposure; 39 %, 28 %, 24 % compared to clean air exposure) and 1.0 ppm (191 %, 147 %, 116 % compared to pre-exposure; 100 %, 86 %, 68 % compared to clean air exposure) **LOAEL: 0.5 ppm** **NOAEL: 0.25 ppm**	Chamber: 4 x 6 x 3.2 m 26.1±0.3°C, no pre-selection of SO_2 sensitive asthmatics

Summary of controlled human exposure studies with asthmatics and healthy volunteers exposed to SO_2							
Reference / study characteristics	**SO2 exposure**			**(Lung) function parameters**	**Venti-lation rate**	**Results**	**Others/ Remarks**
	Conc. mg/m^3	**Conc.** ppm	**Duration** min				
moderate exercise: V_E = 42 L/min, double-blind Key study							
Linn et al. 1982 24 volunteers (13 M, 11 F), moderate exercise mild to moderate asthmatics ($FEV_{1.0}$/FVC: 1x 59 %; 2x 66-68 %, 21 normal), non-smokers, mean age: 23±4 y exposure: 2 exercise phases: 1) 0-10 min followed by body plethysmography 40-50 min followed by body plethysmography, rests in between Key study	0 0.7 1.3	0 0.25 0.5	60	FVC, $FEV_{1.0}$ (D_{10}), V_{tg}, SRaw, Raw	27±6 L/min during exercise	**0.25 ppm**: no significant changes observed **0.5 ppm**: no significant changes observed **LOAEL: >0.5 ppm** **NOAEL: 0.5 ppm**	Chamber Temperature 23°C
Schachter et al. 1984, 10 healthy (4M 6F) and 10 asthmatic (5M 5F) volunteers age (healthy): 26.1±6.3 asthmatics: 27.3±5.1 $FEV_{1.0}$ asthmatics: 2.66±0.52,	0 0.66 1.3 2.0 2.6	0 0.25 0.5 0.75 1.0	40	SRaw, $FEV_{1.0}$, $V_{max50\%}$, $MEF_{40\%}$,		No statistically significant differences (P<0.05) in parameters examined without exercise. Changes with exercise (significant changes from baseline at resp. SO_2 concentration, asthmatics): **$FEV_{1.0}$ (L):** 1 min post exercise: 0.75 ppm: -8 % 1.00 ppm: -14 %	Chamber: 3x3.7x2.4 m All group changes in pulmonary function were transient, with values returning to near baseline within 10 min after cessation of exercise despite

Summary of controlled human exposure studies with asthmatics and healthy volunteers exposed to SO_2							
Reference / study characteristics	**SO2 exposure**			**(Lung) function parameters**	**Venti-lation rate**	**Results**	**Others/ Remarks**
	Conc. mg/m^3	**Conc.** ppm	**Duration** min				
SRaw: 5.01±1.5 $cmH_2O/L*sec$. Key study						5 min post exercise: 1.00 ppm: -11 % **SRaw (cm H_2O/L x sec)** 1 min post exercise: 1.00 ppm: + 54 % 5 min post exercise: 0.75 ppm: + 30 % 1.00 ppm: + 68 % **$MEF_{40\%}$ (L/s):** 1 min post exercise: 0.75 ppm: - 22 % 1.00 ppm: - 27 % 5 min post exercise: 0.75 ppm: - 16 % 1.00 ppm: - 16 % **$V_{max50\%}$ (L/s)** 1 min post exercise: 0.25 ppm: - 5 % 0.75 ppm: - 11 % 1.00 ppm: - 22 % 5 min post exercise: 0.50 ppm: - 6 % **LOAEL: 0.75 ppm** **NOAEL: 0. 5 ppm**	continued presence of SO_2. In healthy subjects, upper airway complaints predominated in the absence of pulmonary functional changes.
Sandström et al. 1988 / 8 healthy nonsmoking subjects, 21 – 29 y, normal lung function	1 5 10	0.4 2 4	20	Heart rate, breathing pattern, frequency of eye blinks, standardized questionnaire, spirometry: FVC, $FEV_{1.0}$, FEF_{25-75}, MTT	n.r.	Increase in nasal and throat irritation at 10 mg/m^3 in 5/8 subjects, no difference in spirometry parameters 90-100 heart beats/min, 18-23 breaths/min – no changes while exposed **LOAEL: 4 ppm (throat irritation)** **NOAEL: 2 ppm**	Chamber: 3.2x2.0x2.2 m Air volume: 14.1 m^3, air exchange ca. every 2 min last 15 min on bicycle ergo-meter (75 W)
Sandström et al. 1989 / 12 healthy nonsmoking subjects, 22 – 30	0 10 20	0 4 8	20	Bronchoscopy; BAL Lung function	n.r.	**4 ppm**: Normal endobronchial findings and normal lung function, activation of	Chamber: 3.2x2.0x2.2 m Air volume: 14.1 m^3, air

Summary of controlled human exposure studies with asthmatics and healthy volunteers exposed to SO_2							
Reference / study characteristics	**SO2 exposure**			**(Lung) function parameters**	**Venti-lation rate**	**Results**	**Others/ Remarks**
	Conc. mg/m^3	**Conc.** ppm	**Duration** min				
y, normal lung function; 4 subjects/group						alveolar macrophages; mild symptoms from eye and nose (no details reported) **8 ppm**: mucosal erythaema in the distal part of trachea and proximal main bronchi; normal lung function, mild lymphocytosis, mild symptoms from eye and nose (no details reported) **LOAEL: 8 ppm (mucosal erythaema)** **NOAEL: 4 ppm**	exchange ca. every 2 min
Sandström et al. 1989 / 22 healthy nonsmoking male subjects, 22 – 37 y, mean: 27 y; normal lung function	20	8	20	BAL, spirometry: $FEV_{1.0}$	n.r.	**8 ppm:** 4h following exposure: mucosal erythema in trachea and proximal main bronchi of all subjects (dis-appeared 72h after exposure) total lymphocytes ↑, mast cells ↑, 8h following exposure: total cell number ↑ peak at 24h (alveolar macrophages / monocytes, lymphocytes, mast cells ↑; eosinophils and neutrophils unaffected)., non-significant decrease in $FEV_{1.0}$ **LOAEL: 8 ppm (mucosal erythema)** **NOAEL: <8 ppm**	Chamber: 3.2x2.0x2.2 m Air volume: 14.1 m^3, air exchange ca. every 2 min, last 15 min on bicycle ergo-meter (75 W)
Bedi et al. 1984 / 9 + 14 healthy (M) nonsmoking subjects, 19 – 28 y (mean: 21.8), normal lung function	0 2.6 5.8	0 1 2	120	FRC, FVC, $FEV_{1.0,2.0,3.0}$, SRaw, MMV, V_{tg}, ERV, IC, FEF_{50}, FEF_{25-75}, FEF_{75}	40 L/min	No significant changes in lung function parameters observed. **LOAEL: >2 ppm** **NOAEL: 2 ppm**	Chamber: 1.8x2.4.2.6 m air exchange ca. every 5 min; 22 °C; 40 % rel. humidity

Summary of controlled human exposure studies with asthmatics and healthy volunteers exposed to SO_2							
Reference / study characteristics	**SO2 exposure**			**(Lung) function parameters**	**Venti-lation rate**	**Results**	**Others/ Remarks**
	Conc. mg/m^3	**Conc.** ppm	**Duration** min				
Andersen et al. 1974; 15 healthy male volunteers; age: 20 – 28 y; 4 smoker; 11 non-smoker	0 2.6 13.2 65.8	0 1 5 25	360	Nasal mucus flow rate, cross-sectional nasal airway, rhinomanometry, $FEV_{1.0}$, FEF_{25-75},	n.r.	**1 ppm:** cross-sectional nasal airway significantly ↓ (more pronounced after 1-3 hours than after 4-6 hours exposure), FEF_{25-75} significantly ↓ **5 ppm:** mucus flow rate significantly ↓, cross-sectional nasal airway ↓, FEF_{25-75} significantly ↓ **25 ppm:** mucus flow rate (significantly) ↓ up to mucostasis; cross-sectional nasal airway ↓ (29 %), $FEV_{1.0}$ significantly ↓ (4 %), FEF_{25-75} significantly ↓ concentration dependent increase in severity of all parameters investigated **LOAEL: 25 ppm** **NOAEL: 5 ppm**	Climate chamber, all volunteers were exposed together, no exercise; no information on air change; 23 ± 0.3°C; 50±5 % rel. humidity; clean air exposure day 0; 1.0; 5.0; 25.0 ppm at days 1 – 3 respectively. Study design different from majority of other studies
Van Thriel et al. 2010; 16 healthy, non-smoking volunteers (8M/8F); age: F: 24.3±5.2 y; M: 28.4±3.9; $FEV_{1.0}$/FVC: 71 – 96 %; $FEV_{1.0}$: F: 3.85±0.6 L M: 4.56±0.54 L	0 1.3 2.6 5,2	0 0.5 1.0 2.0	240	$FEV_{1.0}$, FVC, $FEV_{1.0}$/FVC, PWC130, nasal airway resistance, eye blink frequency	n.r.	$FEV_{1.0}$/FVC: no effect observed. $\Delta FEV_{1.0}$: all ≤ 10 %, majority ≤ 5 %, no significant changes in parameters investigated were observed in healthy volunteers. **LOAEL: >2 ppm** **NOAEL: 2 ppm**	Chamber: 4. x 2.65 x 2.27 m; 23.9 °C No single or tabulated data reported
Linn et al. 1985 24 volunteers (15 M, 9 F) with COPD, exposure included 2x 15 min exercise, age: mean: 60 (49 – 68 y)	0 1.05 2.1	0 0.4 0.8	60	V_{tg}, SRaw, FVC, $FEV_{1.0}$, S_{aO2}, MMFR, VE and heart rate at S_{aO2}, symptom questionnaire		SRaw: 0.0; 0.4; 0.8 ppm: 20.2; 17.8; 17.4 8 cm H_2O x sec, respectively $FEV_{1.0}$: no significant differences between groups; No evidence for a clinical or physiological effects of	Influence of COPD higher than of SO_2 exposure

Summary of controlled human exposure studies with asthmatics and healthy volunteers exposed to SO_2							
Reference / study characteristics	**SO2 exposure**			**(Lung) function parameters**	**Venti-lation rate**	**Results**	**Others/ Remarks**
	Conc. mg/m^3	**Conc.** ppm	**Duration** min				
$FEV_{1.0}$/FVC = 47 % (1x 70 %), 17 former heavy smoker, 6 smoker, 1 non-smoker						SO_2 exposure in this patient collective. **LOAEL: >0.8 ppm** **NOAEL: 0.8 ppm**	
Linn et al. 1983 23 asthmatic volunteers (13 M, 10 F), heavy exercise $FEV_{1.0}$/FVC = 67 – 100 %, patients hyperreactive to metacholine	0 0.53 1.06 1.6	0 0.2 0.4 0.6	5	Electrocardiogram FVC V_{tg}, SRaw, , $FEV_{1.0,2.0,3.0}$, S_{aO2}, PEFR, FVC, $V_{max75,50,25}$	Mean: 48 L/min	**0.2 ppm**: no significant changes **0.4 ppm**: SRaw: ↑ (69 %) $V_{max75/50/25}$: significant decrease at 0.4 (-8 %/ -10 %/-12 %) **0.6 ppm:** SRaw: ↑ (129 %), $FEV_{1.0}$: ↓ (-13 %), $V_{max75/50/25}$: ↓ (-21 %/ -25 %/- 31 %) PEFR: ↓ (-14 %) All compared to pre-exposure values; control: SRaw: ↑ 36 %. **LOAEL: 0.6 ppm** **NOAEL: 0.4 ppm**	Exposure temperature 23°C, rel. humiditiy: 85 %
Linn et al. 1984 24 volunteers (13 M, 11 F), heavy exercise mild to moderate asthmatics, non-smokers	0 0.8 1.6	0 0.3 0.6	Ca. 8 min 3x/week; 3 weeks	V_{tg}, SRaw, SG_{aw}	50 L/min during exercise	**0.3 ppm**: SRaw: ↑ (< 100 % compared to changes in controls); **0.6 ppm**: SRaw: ↑ (> 100 % compared to changes in controls) **LOAEL: 0.6 ppm** **NOAEL: 0.3 ppm**	Values for mg/m^3 at 21°C, only SO_2 responder participated, exposure investigated at 21°C, 7°C, -6°C, additive effects on temperature observed, but temperature influence lower at higher SO_2 concentrations
Linn et al. 1988 20 volunteers (13 M, 7 F), heavy exercise ($FEV_{1.0}$/FVC: 69 – 90 %), non-smokers,	0 0.8 1.6	0 0.3 0.6	10	FVC, $FEV_{1.0}$, SRaw, symptom score	50	**0.3 ppm**: SRaw ↑ (< 100 %), $FEV_{1.0}$ ↓ (changes > 20 %) **0.6 ppm**: SRaw ↑ (>100 %), $FEV_{1.0}$ ↓ (changes > 20 %)	No information on chamber size and conditions

Summary of controlled human exposure studies with asthmatics and healthy volunteers exposed to SO_2							
Reference / study characteristics	**SO2 exposure**			**(Lung) function parameters**	**Venti-lation rate**	**Results**	**Others/ Remarks**
	Conc. mg/m^3	**Conc.** ppm	**Duration** min				
age: 19-36 y Volunteers with minimal – moderate asthma (9 on medication)						**LOAEL: 0.3 ppm (decrease** $FEV_{1.0}$ **)** **NOAEL: <0.3 ppm**	Study was conducted to test influence of pre-treat-ment on SO_2-induced bronchoconstr iction. Values without medication were used in this table.
Horstman et al. 1986 / 27 non-smoking male asthmatics currently receiving no corticosteroid, cromolyn sodium or desensitization therapy, baseline SRaw: 6.8 cm H_2O x sec.; $FEV_{1.0}$/FVC = 72 %, age: 18 - 35	0 0.6 5 1.3 2.6 5.2	0 0.2 5 0.5 1.0 2.0 #	10	SRaw, $FEV_{1.0}$/FVC	normalis ed to body surface: V_E: 21 $L/m^{2/}$mi n	SO_2 concentration required to induce an increase of 100 % of SRaw: 0.28 – 1.9 ppm (23 subjects) >2 ppm (4 subjects) median: 0.75 ppm, for 6 subjects: <0.5 ppm **LOAEL: 0.28 ppm** **NOAEL: <0.28 ppm**	Chamber: 4 x 6 x 3.2 m 26.1±0.3°C, no pre-selection of SO_2 sensitive asthmatics ± the same cohort as in Roger et al. 1985
Gong et al. 1995 14 unmedicated SO_2 sensitive asthmatics, non-smoker, age: 27±11; 19-50 y	0 1.3 2.6	0 0.5 1.0	10	Psychophysical measurements: BS; VAS, $FEV_{1.0}$	Exercise light: 30 medium: 36 heavy: 43 L/min	10 min SO_2 exposure > 0.5 ppm and ventilation > 30 L/min can cause or intense asthma manifestations comparable to those usually expected from everyday stress. (Study with reporting deficiency, no tabulated results)	Chamber: 2.2 m^3, pre-selection of SO_2 sensitive asthmatics: ≥ 75 % SRaw increase at 1 ppm SO_2 and heavy exercise
Study with workers at an apricot farm							
Koksal et al. 2003 / 69 volunteer male workers at 15 apricot farms, mean age: 31.29±14.66, 15 – 69 y, duration of work in sulfurization	285 – 1920	107 – 722* mean 342 ±	≤ 60	Symptom score, FVC, $FEV_{1.0}$, $FEV_{1.0}$/FVC%, FEF_{25-75}, PEFR, $V_{max25/50/75}$	n.r.	Asthma-like symptoms such as acute mucosal irritation, decrease in pulmonary functions, dyspnoea (80 %), cough (78 %), itchy or scratchy throat (36 %), eye and nose irritation (83/70 %)	Exclusion criteria: history of allergy or known pulmonary or systemic diseases (23

Summary of controlled human exposure studies with asthmatics and healthy volunteers exposed to SO_2							
Reference / study characteristics	**SO2 exposure**			**(Lung) function parameters**	**Venti-lation rate**	**Results**	**Others/ Remarks**
	Conc. mg/m^3	**Conc.** ppm	**Duration** min				
process: 10.98 ± 10.83 y, 1 – 45 y, 45 smoker/24 non-smoker		195				$FEV_{1.0}$↓ (1-40 % in 88 % of workers); significant decrease ($p<0.05$) in FVC%; $p<0.001$: $FEV_{1.0}$, $FEV_{1.0}$/FVC%, PEFR, $V_{max25/50/75}$, **LOAEL/ NOAEL: n.d.**	subjects excluded)
Results derived from Reviews							
Goodman et al. 2010, data from 13 controlled clinical exposure studies with a total of 274 asthmatic volunteers	0 0.5-2.7	0 0.2 - 1.0	n.r.	SRaw, $FEV_{1.0}$	30-90	**≥ 0.4 ppm** SRaw ↑ (≥ 100 %) and/or $FEV_{1.0}$ ↓ (≥ 10 %) **LOAEL: 0.4 ppm** **NOAEL: 0.2 ppm**	Some studies already included as single studies in this table.
Johns and Linn 2011, data from 55 controlled clinical exposure studies with a total of 948 asthmatic volunteers	0 0.27 – 21.3	0 0.1 - 8	1-240?	SRaw, $FEV_{1.0}$	≤ 1	Increase in bronchomotor response with increasing SO_2 concentrations with significant interindividual variability in response. **LOAEL/NOAEL: n.d.** Only few studies reported effects with SO_2 concentrations < 0.4 ppm.	Some studies already included as single studies in this table.

BAL: bronchoalveolar lavage, BS: Borg scale, COPD: chronic obstructive pulmonary disease, EKG: electrocardiogram, ERV: Expiratory reserve volume; $FEV_{1.0}$: forced expiratory volume in 1 sec, FEF_{25-75}: mean expiratory flow during the middle half of FVC; FEF_{max}: maximal expiratory flow during FVC; $FEF_{50/75}$: instantaneous expiratory flows after 50 and 75 %of the FVC are exhaled, FRC: functional residual capacity, FVC: forced vital capacity, IC: inspiratory capacity; MEFV: maximal flow volume, MMFR: maximal midexpiratory flow rate, MTT: mean transit time, MV: minute ventilation, n.r. not reported, PC_8: Provocative concentration causing a SRaw increase of 8 units (L x cm H_2O/L/s), PEFR: peak expiratory flowrate, PEFV: partial flow volume, MVV: maximal voluntary ventilation; PFT: pulmonary function tests, S_{aO2}, PWC130: physical capacity at a heart rate of 130 bpm; SG_{aw}: specific conductance in cm $H2O^{-1}sec^{-1}$ (reciprocal of sRaw), sRaw: specific airway resistance, VAS: visual analog scale, V_E: minute ventilation, $V_{max25/50/75}$: flows at 25/50/75 % of vital capacity, V_{tg}: thoracic gas volume

**: volume of sulfurization chambers and amount of sulfur differed between apricot farms*

#: Subjects whose SRaw increase was < 100 % at 1.0 ppm were also exposed to 2.0 ppm;

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity- single exposure

Sulfur dioxide is a corrosive substance with irritating properties at lower concentration. Irritation at lower concentrations is covered by the derived reference value for inhalation exposure. In animal studies there is some indication for respiratory tract irritation that is supported by human data. There are numerous data available on respiratory tract irritation of sulfur dioxide in humans. The studies are mainly of short-term durations in occupationally exposed workers, volunteers or represent medical surveillance data. Exposure of

volunteers or occupationally exposed workers to sulfur dioxide at concentrations higher than 1 ppm caused complains of dryness in the throat, nose, eyes and upper respiratory passages. Reductions in clearance rates and symptoms of discomfort as well as inflammatory reactions in the human lung were observed. Relative air humidity had no influence on effects at low exposure concentrations (until 6 ppmV). Generally, all pulmonary changes were reversible. However, significant changes in pulmonary function, dyspnoea, pain on deep breathing, severe conjunctivitis and airway obstruction were reported in people who survived after acute accidental exposure to extremely high concentrations of sulfur dioxide. Some changes were partially irreversible (e.g. damage of the ciliated epithelium with impairment of pulmonary clearance, increased sensibility to external irritants and infections). They showed also symptoms of chronic bronchitis. In dead persons, lung oedema, emphysematous changes with fundamental lesions of extensive peribronchiolar fibrosis and bronchiolitis obliterans were observed.

No statistically significant changes in physiology or symptoms could be attributed to sulfur dioxide exposure at concentrations of 1 ppm and lower in healthy subjects including smokers and volunteers with chronic obstructive pulmonary disease (COPD). Nevertheless, a wide range of sensitivities to sulfur dioxide was found among the asthmatic subjects (see Table 11 above).

Indication for respiratory tract irritation such as nasal and throat irritation was observed in healthy humans following exposure to 4 ppm sulfur dioxide (Sandström et al. 1988). Sulfur dioxide is classified as corrosive and classification for respiratory tract irritation is considered required. Also based on the broad, well documented human experience on irritating effect to respiratory system, sulfur dioxide is used as an example of respiratory tract irritant substance in the Guidance on the Application of the CLP Criteria (2017, section 3.8.5.1.3., page 456).

10.11.2 Comparison with the CLP criteria

Toxicological results	**CLP criteria**
Sulfur dioxide: - Broad, well documented human experience on irritating effect to respiratory system. - Corrosive substance with irritating properties at lower concentration - In animal studies there is some indication for respiratory tract irritation that is supported by human data - Transient effects observed Proposed classification as STOT-SE3 (Respiratory Tract Irritant)	Category 1 (H370): Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure. Substances are classified in Category 1 for specific target organ toxicity (single exposure) on the basis of: (a) reliable and good quality evidence from human cases or epidemiological studies; or (b) observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations. Guidance dose/concentration values are provided below to be used as part of weight-of-evidence evaluation. Equivalent guidance value ranges for single dose exposures: Oral (rat): C ≤ 300 mg/kg bw
	Category 2 (H371): Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following single exposure. Substances are classified in Category 2 for specific target

Toxicological results	**CLP criteria**
	organ toxicity (single exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided below in order to help in classification. In exceptional cases, human evidence can also be used to place a substance in Category 2. Equivalent guidance value ranges for single dose exposures: Oral (rat): 2000<C ≤ 300 mg/kg bw
	Category 3 (H335): Transient target organ effects This category only includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2 indicated above. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function. Substances are classified specifically for these effects as laid down in 3.8.2.2. **Annex I:** *3.8.2.2.1 Criteria for respiratory tract irritation* *The criteria for classifying substances as Category 3 for respiratory tract irritation are:* *(a) respiratory irritant effects (characterized by localized redness, oedema, pruritis and/or pain) that impair function with symptoms such as cough, pain, choking, and breathing difficulties are included. This evaluation will be based primarily on human data.* *(b) subjective human observations could be supported by objective measurements of clear respiratory tract irritation (RTI) (such as electrophysiological responses, biomarkers of inflammation in nasal or bronchoalveolar lavage fluids).* *(c) he symptoms observed in humans shall also be typical of those that would be produced in the exposed population rather than being an isolated idiosyncratic reaction or response triggered only in individuals with hypersensitive airways. Ambiguous reports simply of "irritation" shall be excluded as this term is commonly used to describe a wide range of sensations including those such as smell, unpleasant taste, a tickling sensation, and dryness, which are outside the scope of classification for respiratory irritation.* *(d) there are currently no validated animal tests that deal specifically with RTI, however, useful information may be obtained from the single and repeated inhalation toxicity tests. For example, animal studies may provide useful information in terms of clinical signs of toxicity (dyspnoea, rhinitis etc) and histopathology (e.g. hyperemia, edema, minimal inflammation, thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above. Such animal studies can be used as part of weight of evidence evaluation.*

Toxicological results	CLP criteria
	(e) *this special classification would occur only when more severe organ effects including in the respiratory system are not observed.*

10.11.3 Conclusion on classification and labelling for STOT SE

DS proposes classification in STOT-SE Category 3, Respiratory tract irritant, H335 May cause respiratory irritation.

The classification criteria for Category 3 (Respiratory Tract Irritation) is fulfilled based on well documented experience in humans. RAC may also consider Categorie 1 of STOT-SE as significant (asthmatic effects on humans) for sulfur dioxide classification.

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

Summary of the Dossier Submitter's proposal

In the CLH report (table 19, pages 76-83), the DS presented a selection of studies in humans which have been published in the literature, including one occupational study, in order to evaluate the ability of SO_2 to induce bronchoconstriction. The main pulmonary function parameters monitored amongst studies were specific airway resistance (SRaw)) and forced expiratory volume in one second (FEV1.0) (). According to the American Thoracic Society, reductions in FEV1.0 of < 10, 10-20%, and > 20% were graded as mild, moderate, or severe, respectively (Samet *et al.,* 2000). Another useful assessment of airflow limitations is the ratio of FEV1.0 to forced vital capacity (FVC). The FEV1/FVC ratio is normally greater than 0.75 to 0.8, and possibly greater than 0.90 in children. Any values less than these suggest airflow limitation. As the majority of studies with SO_2 did not provide data on FEV1/FVC ratio, reductions in FEV1.0 and/or SRaw were used instead. Changes in lung function parameters were identified at concentrations of 0.4 ppm with asthmatics being the most vulnerable group. Increases in SRaw (according to criteria of the "German Society for Pneumology") and moderate decreases of FEV1.0 of 10% were used as criterion to define an adverse effect indicating airflow restriction following short-term exposure.

The DS stated in the CLH report that "*SO_2 is a corrosive substance with irritating properties at lower concentrations, which is covered by the derived reference value for inhalation exposure*". The DS recognised that in animal studies there is some indication for respiratory tract irritation (without providing direct reference to animal studies) that is supported by human data. There are numerous data available on respiratory tract irritation of SO_2 in humans. The studies are mainly of short-term durations in occupationally exposed workers, volunteers or represent medical surveillance data. Exposure of volunteers or occupationally exposed workers to SO_2 at concentrations higher than 1 ppm caused complains of dryness in the throat, nose, eyes and upper respiratory passages. Reductions in clearance rates and symptoms of discomfort as well as inflammatory reactions in the human lung were observed. Relative air humidity had no influence on effects at low exposure concentrations (until 6 ppmV). Generally, all pulmonary changes were reversible. However, significant changes in pulmonary function, dyspnoea, pain on deep breathing, severe conjunctivitis

and airway obstruction were reported in people who survived after acute accidental exposure to extremely high concentrations of SO_2. Some changes were partially irreversible (e.g. damage of the ciliated epithelium with impairment of pulmonary clearance, increased sensibility to external irritants and infections). They also showed symptoms of chronic bronchitis. In dead persons, lung oedema, emphysematous changes with fundamental lesions of extensive peribronchiolar fibrosis and bronchiolitis obliterans were observed.

No statistically significant changes in physiology or symptoms could be attributed to SO_2 exposure at concentrations of 1 ppm and lower in healthy subjects including smokers and volunteers with chronic obstructive pulmonary disease. Nevertheless, a wide range of sensitivities to SO_2 was found among the asthmatic subjects.

Indications of respiratory tract irritation such as nasal and throat irritation was observed in healthy humans following exposure to 4 ppm SO_2 (Sandström *et al.,* 1988). SO_2 is classified as corrosive and classification for respiratory tract irritation is considered required. Also based on the broad, well documented human experience on irritating effect to respiratory system, SO_2 is used as an example of respiratory tract irritant substance in the Guidance on the Application of the CLP Criteria (2017, section 3.8.5.1.3., page 456).

Therefore, the DS proposed classification in STOT SE Category 3, H335: May cause respiratory irritation for SO_2. The DS also states that RAC may consider STOT SE Category 1 as significant effects on asthmatic humans are observed after SO_2 exposure.

Comments received during consultation

There was one comment by Industry submitted for this endpoint during the consultation supporting the classification of SO_2 as STOT SE 3 and the reasoning proposed by the DS.

Assessment and comparison with the classification criteria

For the evaluation of specific target organ toxicity after single exposure, RAC retrieved results for some animal studies included in the CLH report under the Section of acute inhalation toxicity endpoint (table 9 of the CLH report, pages 24-30), as summarised in the following table:

***Table**: Summary table of respiratory effects of SO_2 exposure in animal studies*

Study	Species/ strain/ Sex/ per No group	SO_2 concentration/ exposure	Effects
Anonymous18	Dogs/ Beagle/ (M+F) 8 animals in total/ 4 per group, control and treated	400 ppm/ 2 hours	An immediate increase of bronchial responsiveness to histamine that lasted for about 2 hours post-exposure. Cell numbers in bronchoalveolar lavage (BAL) were increased up to 1 hour for epithelial cells and from 1-4 hours for neutrophils. There was no significant change of lymphocytes, macrophages, eosinophils, goblet cells, or mast cells in lavages.
Anonymous19	Rats/ Wistar / no data on sex/ 7	41-751 ppm/ 2 hours	*General effects*: sneezing, coughing and lachrymation, intermittent burst

	groups of 10 rats (plus control group)		of quick and deep inspirations and expirations **0 and 40 ppm** no adverse histological changes of lungs **64-231 ppm** 10-30% of the lungs showed pulmonary oedema **426-751 ppm** 70-80% of the lungs showed pulmonary oedema **750 ppm** animals became grievously laboured A positive correlation between the frequency of occurrence of pulmonary damage and the concentration of SO_2 was shown.
Anonymous20	Dogs/ Beagle)/ (F+M)/ 7 animals	200 ppm/ 2 hours endotracheally intubated	Airway hyperreactivity to histamine induced in dogs after a 2 hour inhalation period of 200 ppm SO_2 was associated with significant inflammatory changes lasting up to the end of the observation period of 22 h
Anonymous21	Mice/ dd strain/ no data on sex/ 4 mice per test concentration, 7 test groups (including controls)	0, 23, 38, 75, 128, 250, 500 ppm / 10 min whole body	Sensory irritation, decrease of respiratory rate from 23 ppm
Anonymous22	Mice/ Ha/ICR)/ Male/ 3 DF-mice and 2 CO-mice/time point of sacrifice; controls: 9 DF-mice, 7 CO-mice	10 ppm/ 4, 24, 48, 72 hours continuously whole body(gas): whole body	Severe injury of respiratory and olfactory epithelium of the nasal cavity (oedema, necrosis and desquamation) from 24 hours exposure and on
Anonymous27	Rats/ Sprague Dawley)/ Male/ 15 animals (pre-treated with tracer particles), divided into 3 groups: control, SO_2, HCHO after exposure	20.1 ppm/ 4 hours exposure (SO_2 gas after inhalation of radioactive tracer particles), nose only	Delayed upper respiratory tract particle clearance Clearance from the deep lung not affected
Anonymous28	Rats/ Wistar / Male/ 5 gnotobiotic and 5 controls	800 ppm/ 8 hours whole body	Upper trachea represented the most affected region of epithelial damage Gradient of decreasing cellular damage was observed in the tracheobronchial tree in peripheral direction accompanied by decreasing mitotic and metabolic activity of surviving cells
Anonymous29	Mice/ ICR/ Female/ 56 healthy mice/ 44 mice were exposed to SO_2, 12 as controls	20 ppm/ whole body 30, 60 and 120 min	Severe injury of respiratory and olfactory epithelium of the nasal cavity (depending on exposure/observation time) The changes were primarily degenerative rather than inflammatory

In addition, RAC noted the reported effects from SO_2 exposure of healthy individuals from the studies mentioned in table 19 of the CLH report, pages 76-83, as summarised in the following table.

***Table**: Summary table of respiratory effects of SO_2 exposure on healthy subjects*

Study	**Number of healthy subjects**	**SO_2 concentration (mg/m^3)/ Duration**	**Effects**
Linn *et al.* 1987	15M, 9F, control group healthy individuals	0.5, 1.1, 1.6/ 60 min	No changes in pulmonary functions as assessed in the study
Schachter *et al.* 1984	10 healthy (4M 6F)	0, 0.66, 1.3, 2.0, 2.6/ 40 min	Upper airway complaints predominated in the absence of pulmonary functional changes
Sandström *et al.* 1988	8 healthy non-smoking subjects, age 21 – 29, normal lung function	1, 5, 10/ 20 min	Increase in nasal and throat irritation at 10 mg/m^3 in 5/8 subjects, no difference in spirometry parameters 90-100 heart beats/min, 18-23 breaths/min – no changes while exposed from 10 ppm
Sandström *et al.* 1989	12 healthy non-smoking subjects, age 22 – 30, normal lung function; 4 subjects/group	0, 10, 20/ 20 min	**10 mg/m^3** Normal endobronchial findings and normal lung function, activation of alveolar macrophages; mild symptoms from eye and nose (no details reported) **20 mg/m^3** Mucosal erythema in the distal part of trachea and proximal main bronchi; normal lung function, mild lymphocytosis, mild symptoms from eye and nose (no details reported)
Sandström *et al.* 1989	22 healthy non-smoking male subjects, age 22 – 37; normal lung function	20/ 40 min	Delayed (4-8 hours after exposure): mucosal erythema in trachea and proximal main bronchi of all subjects (reversible 72h after exposure), total lymphocytes ↑, mast cells ↑, total cell number ↑ peak at 24h (alveolar macrophages / monocytes, lymphocytes, mast cells ↑; eosinophils and neutrophils unaffected) Non-significant decrease in FEV1.0
Bedi *et al.* 1984	9 + 14 healthy (M) non-smoking subjects, age 19 – 28, normal lung function	0, 2.6, 5, 8/ 120 min	No significant changes in lung function parameters observed
Andersen *et al.* 1974	15 healthy male volunteers; age: 20 – 28; 4 smokers, 11 non-smokers	0, 2.6, 13.2, 65.8/ 360 min	**2.6 mg/m^3** cross-sectional nasal airway significantly ↓ (more pronounced after 1-3 hours than after 4-6 hours exposure), FEF_{25-75} significantly ↓ **13.2 mg/m^3** mucus flow rate significantly ↓, cross-sectional nasal airway ↓, FEF_{25-75} significantly ↓

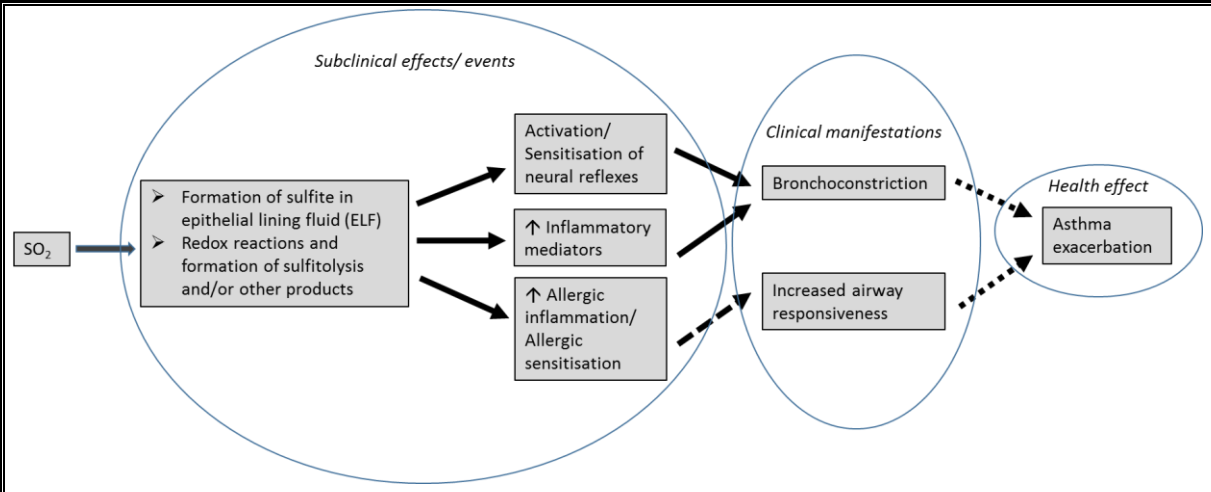
			65.8 mg/m³ mucus flow rate (significantly) ↓ up to mucostasis; cross-sectional nasal airway ↓ (29%), $FEV_{1.0}$ significantly ↓ (4%), FEF_{25-75} significantly ↓
Van Thriel *et al.* 2010	16 healthy, non-smoking volunteers (8M/8F)	0, 1.3, 2.6, 5.2/ 240 min	FEV1.0/FVC: no effect observed. No significant changes in parameters investigated were observed in healthy volunteers
Linn *et al.* 1988	20 volunteers (13 M, 7 F), heavy exercise (FEV1.0/FVC: 69 – 90%), non-smokers, age: 19 - 36	0, 0.8, 1.6/ 10 min	0.3 ppm: SRaw ↑ (< 100%), $FEV_{1.0}$ ↓ (changes > 20%) 0.6 ppm: SRaw ↑ (> 100%), $FEV_{1.0}$ ↓ (changes > 20%)

A selection of 21 human studies is presented by the DS in table 19 of the CLH report, pages 76-83, concerning either asthmatics (8 studies, in total 1222 asthmatics volunteers) or healthy individuals (10 studies), 1 occupational study on 69 apricot farm workers and 2 review studies summarising.

Additionally, literature reports on reactive airway dysfunction syndrome (RADS) caused by SO_2, mainly on workers were retrieved. More specifically, RADS, also called irritant-induced asthma, is a type of occupational asthma that can occur after accidental peak exposure to airborne irritant chemicals within a very short period of latency. RADS is characterized clinically by asthma-like symptoms including cough, wheezing, chest tightness, and breathlessness. The symptoms of RADS usually occur within 24 h after exposure to high amounts of harmful gases and may cause a three-fold increase in the risk of asthma. RADS shares no features of immunology and allergy, which is distinct from classic asthma. However, clinical manifestations of both RADS and asthma are very similar and both share common characteristics, especially airway hyperresponsiveness. Therefore, RADS is thought as a type of occupational asthma, or an adult-onset asthma and accounts for 5%–18% of all occupational asthma cases. The exact cause of RADS is not yet known, but the syndrome is considered to be uncommon and recognized in less than one-fifth of workers with "occupational asthma" (Lindstrom *et al.* 2021; Chai *et al.* 2018; Shakeri *et al.* 2008). In a 13-year follow-up of 9 men exposed to SO_2 after an explosion in a pyrite mine, acute inflammatory obstruction caused by the said exposure left, as sequelae, obstructive impairment of ventilatory function and permanent bronchial hyperreactivity. The clinical picture displayed was recognized as RADS in 1985. Four of the patients also showed symptoms of chronic bronchitis (Piirila *et al.* 1996). In addition, results from an animal study performed to elucidate the mechanism of RADS, reveal that inhalation of a high concentration of SO_2 reduces CD19 expression and causes structural change of the nasal septum in rats. CD19 deficiency causes hyporesponsiveness to transmembrane signals, and weak T cell-dependent humoral responses (Chai *et al.* 2018).

Mode of action

Evidence was gathered both from animal and human studies that support the presence of at least 3 different mechanisms, as described in the Integrated Science Assessment for Sulfur Oxides – Health Criteria of the United States Environmental Protection Agency, 2017 (US EPA, 2017) and are summarised in the Figure below:



The propensity for airways to narrow following inhalation of some stimuli is termed airway responsiveness – bronchoconstriction. Different kinds of stimuli can elicit bronchoconstriction, but in general they act on airway smooth muscle receptors (direct stimuli, e.g., methacholine) or act via the release of inflammatory mediators (indirect stimuli, e.g., allergens) (O'Byrne *et al.*, 2009). SO_2 is a non-specific bronchoconstrictive stimulus that cannot be easily classified as a direct or indirect stimulus. Because inhalation of SO_2 results in chemical reactions in the epithelial lining fluid, the initiating event in the development of respiratory effects is the formation of sulfite, sulfitolysis products, hydrogen ion, and/or other products. Both sulfite and S-sulfonates have been measured in tracheal and bronchial tissue as well as in tracheal washings of experimental animals exposed to SO_2. Reactive products formed as a result of SO_2 inhalation are responsible for a variety of downstream key events, which may include activation or sensitization of sensory nerves in the respiratory tract resulting in neural reflex responses, release of inflammatory mediators, and modulation of allergic inflammation or sensitization. These key events may collectively lead to several clinical manifestations, including bronchoconstriction and increased airway responsiveness. Bronchoconstriction is characteristic of an asthma attack. However, individuals who are not asthmatic may also experience bronchoconstriction in response to SO_2 inhalation; generally, this occurs at higher concentrations than in an individual who is asthmatic. Additionally, SO_2 exposure may increase airway responsiveness to subsequent exposures of other stimuli such as allergens or methacholine. These pathways may be linked to the epidemiologic outcome of asthma exacerbation (US EPA, 2017).

In adults without asthma, respiratory response to SO_2 exposure occurred primarily as a result of activation of sensory nerves in the respiratory tract resulting in neural reflex responses mediated by cholinergic parasympathetic pathways involving the vagus nerve. However, in adults with asthma, evidence indicates that the response is only partially due to vagal pathways and that inflammatory mediators such as histamine and leukotrienes also play an important role. Activation of sensory nerves in the respiratory tract, which result in neural reflex responses, has been studied in humans exposed to occupationally relevant concentrations of SO_2 (up to 2 ppm). Responses measured in these studies included increased respiratory rate and decreased tidal volume, which involves the vagus nerve, and increased nasal air-flow resistance, which involves the trigeminal nerve. These responses are not a part of the mode of action described here but are mentioned because they are known irritant effects of SO_2. Studies in experimental animals demonstrated that

SO_2 exposure activates reflexes that are mediated by cholinergic parasympathetic pathways involving the vagus nerve. However, non-cholinergic mechanisms may also play a role because some studies demonstrate that a local axon reflex resulting in C-fibre secretion of neuropeptides (i.e., neurogenic inflammation) is responsible for the effects of SO_2 (US EPA, 2017).

Finally, evidence demonstrates that SO_2 exposure enhances allergic inflammatory responses in humans and animals. Experimental findings comprise leukotriene-mediated increases in numbers of sputum eosinophils in humans and increased numbers of BAL fluid (BALF) inflammatory cells, levels of BALF cytokines, histopathology, activation of the NFκB pathway, and upregulation of intra-cellular adhesion molecules, mucin, and cytokines, in lung tissue in animals. In naive animals, SO_2 exposure as low as 0.1 ppm over several days promoted allergic sensitization (allergen-specific IgG levels) and enhanced allergen-induced bronchial obstruction (an indicator of increased airway responsiveness) and inflammation (airway fluid eosinophils and histopathology), when animals were subsequently sensitized and challenged with an allergen. These changes in allergic inflammation may enhance airway responsiveness and promote bronchoconstriction in response to a trigger. Thus, allergic inflammation and increased airway responsiveness may link short-term SO_2 exposure to asthma exacerbation (US EPA, 2017).

Summarising the above, it was noted that:

- human data indicate that the respiratory system as a whole is the target organ of SO_2 when subjects are exposed via inhalation. Dryness in the throat, nose, eyes and upper respiratory passages were reported). In addition, reduction in clearance rates and symptoms of discomfort, as well as inflammatory reactions in the human lung were observed. No statistically significant changes in physiology or symptoms could be attributed to sulfur dioxide exposure at concentrations of 1 ppm and lower in healthy subjects including smokers. Generally, all pulmonary changes were reversible.

- for asthmatics, however, exposure both to SO_2 and to sulfites can lead to severe asthma exacerbation and affected lung function parameters, as already discussed under the Respiratory Sensitisation endpoint.
- rather low concentrations of SO_2 were tested in healthy humans, probably due to its irritant properties, with rather serious pulmonary effects (e.g. obstruction of air escaping from the lungs–$FEV_{1.0}$, obstructive peripheral airflow–FEF_{25-75} Andersen *et al.*, 1974), as well as effects in the upper respiratory system (e.g. nose)
- acute accidental exposure to relatively high concentrations of SO_2 leads to RADS with long-lasting pulmonary effects mainly due to the corrosive/ irritating properties of SO_2
- animal data support observations in humans. Following SO_2 exposure of animals, indications are provided for respiratory tract irritation, along with inflammation and tissue degeneration and hyperreactivity to histamine from doses well below the LC_{50}. These doses correspond to STOT SE Category 1 guidance values according to the Guidance for the Application of CLP criteria (version 5.0, 2017, Annex I 3.8.2.1.9.3).

- The doses applied in animal testing, according to the CLP Regulation, could justify classification even in category 1. Nevertheless, according the CLP Regulation, Annex I, table 3.8.2 note a, the guidance values are intended only for guidance purposes, to be used as part of the weight of evidence approach, and are not intended as strict demarcation values.
- the effects observed in both the human and animal studies are considered 'significant' because they clearly show functional disturbance and morphological changes in the respiratory tract as a whole. For the cluster of effects observed, respiratory irritation (category 3) seems less appropriate
- a mode of action is described and is substantiated by experimental findings
- the effects caused by SO_2 single exposure are always fully reversible, thus reducing the concern, and no other hard endpoints are observed (e.g. mortality)

Therefore, RAC proposes, mainly based on the animal studies, on the severity of the RADS effects and on the human data set as a whole, that SO_2 should be classified as **STOT-SE category 1, H370 Causes damage to the respiratory system by inhalation**.

10.12 Aspiration hazard

No data available.

11 REFERENCES

CHEMSAFE (2016): Database that contains safety characteristic data for fire and explosion prevention, evaluated and recommended by experts at BAM and PTB. CHEMSAFE is a joint project between BAM (Federal Institute for Materials Research and Testing, Berlin), PTB (Physikalisch-Technische Bundesanstalt, Braunschweig) and DECHEMA (Gesellschaft für Chemische Technik und Biotechnologie e.V., Frankfurt am Main); http://dechema.de/en/chemsafe.html

ISO 10156:2010: Gases and gas mixtures - Determination of fire potential and oxidizing ability for the selection of cylinder valve outlets; http://www.iso.org/iso/catalogue_detail.htm?csnumber=44817

Overall reference list (including data owner and confidentiality claim)

Part of the dossier

Section No / Reference No/ ESR in IUCLID	Substance	Author(s)	Year	Title Source, Report No. GLP / (Un)Published	Data Protection Claimed (Yes/No)	Owner
8. TOXICOLOGICAL PROFILE FOR HUMAN AND ANIMAL INCLUDING METABOLISM						
8.1_01 Summary: Toxicological profile for humans and animals	Sulphites	Chemservice S.A.	2015	Read-across concept for sulphur dioxide, sulphites, hydrogensulphites and metabisulphites in aqueous solution Not GLP / Unpublished	Yes	Micro-Pak B.V.
8.1_02 Summary: Toxicological profile for humans and animals	Sulfites	Betts, R.H. and Voss, R.H.	1970	The kinetics of oxygen exchange between the sulphite ion and water	No	Published
8.3_02 Skin sensitisation	Sodium metabisulfite	Anonymous1	2010		Yes	SDIOC (Micro-Pak B.V. has LoA)
8.3_02 Skin sensitisation	Sodium metabisulfite	Haferkorn, J.	2010	Attachment of summary table (source indicated above for 8.3_02). GLP / Unpublished	Yes	SDIOC (Micro-Pak B.V. has LoA)
8.3_03 Skin sensitisation	Potassium metabisulfite	Gillman, S.A.	1982	Metabisulfite Sensitivity as a Cause of Asthma. West J Med. 1982; 137 (29): 120-1. Not GLP / Published	No	Published
8.3_04 Skin sensitisation	Sodium metabisulfite	Belchi-Hernandez, J., Florido-Lopez, F., Estrada-Rodriguez, J.L., Martinez-Alzamora, F., Lopez-Serrano, C.	1993	Sulphite-induced urticaria. Annals of Allergy, 71, 230-232. Not GLP / Published	No	Published

Section No / Reference No/ ESR in IUCLID	Substance	Author(s)	Year	Title Source, Report No. GLP / (Un)Published	Data Protection Claimed (Yes/No)	Owner
		and J.A. Ojeda-Casas				
8.3_05 Skin sensitisation	Sodium metabisulfite	Jiménez-Aranda, G.S., Flores-Sandoval, G., Gómez-Vera, J. and M. Orea-Solano	1996	Prevalencia de urticaria crónica posterior a la ingestión de aditivos alimentarios en un hospital de tercer nivel. Revista Alergia México, Vol. XLIII, Num. 6, noviembre-deciembre 1996, 152-156. Not GLP / Published	No	Published
8.3_06 Skin sensitisation	Potassium metabisulfite	Cifuentes, L., Ring, J., Brockow, K.	2013	Clonal Mast Cell Activation Syndrome with Anaphylaxis to Sulphites. Int Arch Allergy Immunol: 2013; 162: 94–96. Not GLP / Published	No	Published
8.3_07 Skin sensitisation	Sodium metabisulfite	Cussans, A., McFadden, J. and L. Ostlere	2015	Sytemic sodium metabisulfite allergy. Contact Dermatitis, Contact Points, pp. 1-2. Not GLP / Published	No	Published
8.4_01 Respiratory sensitisation	Sulfur dioxide	Park, J.K. et al.	2001	Repeated exposure to low levels of sulfur dioxide (SO2) enhances the development of ovalbumin-induced asthmatic reactions in guinea pigs. Ann. Allergy. Asthma Immunol. 86: 62-67. Not GLP / Published	No	Published
8.4_02 Respiratory sensitisation	Sulfur dioxide	Anonymous2	1988		No	Published
8.4_03 Respiratory sensitisation	Sulfur dioxide	Anonymous3	1992		No	Published
8.4_04 Respiratory sensitisation	Sulfur dioxide	Anonymous4	1995		No	Published
8.4_05 Respiratory sensitisation	Sodium metabisulfite	Twarog, F.J., Leung D.Y.	1982	Anaphylaxis to a Component of Isoetharine (Sodium Bisulphite). JAMA, 1982: 22: 248 (16): 2030-1. Not GLP / Published	No	Published
8.4_06 Respiratory sensitisation	Sodium metabisulfite	Delohery, J. et al.	1984	The relationship of inhaled sulphur dioxide reactivity to ingested metabisulphite sensitivity in patients with asthma. Am. Rev. Respir. Dis. 130: 1027-1030 Not GLP / Published	No	Published
8.4_07 Respiratory sensitisation	Potassium metabisulfite	Schwartz, H.J., Chester, E.H.	2000	Bronchospastic responses to aerosolized metabisulphite in asthmatic subjects: Potential mechanisms and clinical implications. J Allergy Clin Immunol. 1984: 74 (4) Pt 19: 511-513. Not GLP / Published	No	Published

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8.4_08 Respiratory sensitisation	Sodium metabisulfite	Mansour, E, Ahmed, A., Cortes, A., Caplan, J., Burch, R.M., Abraham, W.M.	1992	Mechanisms of metabisulphite-induced bronchoconstriction: evidence for bradykinin B,-receptor stimulation. Journal of Applied Physiology (Bethesda, Md: 1985): 1992; 72 (5): pp. 1831-1837. Not GLP / Published	No	Published
8.4_09 Respiratory sensitisation	Sodium metabisulfite	Vallon C., Sainte-Laudy, J. and Nasr, M.	1995	Allergie et exposition professionnelle aux composes soufres:questions posees. Allerg Immunol (Paris), 27, 83-87 Not GLP / Published	No	Published
8.4_10 Respiratory sensitisation	Sodium metabisulfite	Hein, H., Kirsten, D., Jörres, R.A. and H. Magnussen	1996	Die orale Testung auf Sulfitasthma. Pneumologie, Vol. 50, 394-398. Not GLP / Published	No	Published
8.4_11 Respiratory sensitisation	Sodium metabisulfite	Boner AL, Guarise A, Vallone G, Fornari A, Piacentini F, Sette L.	1990	Metabisulphite oral challenge: incidence of adverse responses in chronic childhood asthma and its relationship with bronchial hyperreactivity J Allergy Clin Immunol. 1990 Feb;85(2):479-83 Not GLP / Published	No	Published
8.4_12 Respiratory sensitisation	Sulfur dioxide and Sodium metabisulfite	Bush RK, Taylor SL, Holden K, Nordlee JA, Busse WW	1986	Prevalence of sensitivity to sulfiting agents in asthmatic patients Am J Med. 1986 Nov;81(5):816-20 Not GLP / Published	No	Published
8.5.1_01 Mutagenicity	Sulfur dioxide	Pool-Zobel, B.L. et al.	1990	In vitro and ex vivo effects of the air pollutants SO2 and NOx on benzo(a)pyrene activating enzymes of the rat liver. Exp. Pathol. 39, 207-212. Not GLP / Published	No	Published
8.5.1_02 Mutagenicity	Sodium metabisulfite	Ishidate, M., Sofuni, T., Yoshikawa, K. et al.	1984	Primary mutagenicity screening of food additives used in Japan Food Chem. Toxicol. 22: 623-636 Not GLP / Published	No	Published
8.5.1_03 Mutagenicity	Sodium metabisulfite	Simmon, V.F. and Eckford, S.L.	1978	Microbial mutagenesis testing of substances: compound report: F76-004, sodium meta-bisulphite Published report: PB89-193684, SRI Project LSU-6909. Report No: FDA/CFSAN-89/83. Report date: 1978-04-01 Not GLP / Published	No	Published
8.5.1_04a Mutagenicity	Sodium metabisulfite	Simmon, V.F. and Eckford, S.L.	1978	Microbial mutagenesis testing of substances: compound report: F76-004, sodium meta-bisulphite Published report: PB89-193684, SRI Project LSU-6909. Report No: FDA/CFSAN-89/83. Report date: 1978-04-01 Not GLP / Published	No	Published

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8.5.1_04b Mutagenicity	Sodium metabisulfite	Prival, M.J., Simmon,V.F. , Mortelmans, K.E.	1991	Bacterial mutagenicity testing of 49 food ingredients gives very few positive results. Mutat. Res. 260 (4), 321-329 Not GLP / Published	No	Published
8.5.1_05 Mutagenicity	Sodium metabisulfite	Green, S. J.	1977	Present and future uses of mutagenicity tests for assessment of the safety of food additives. Environ. Pathol. Toxicol. 1, pp. 49-54 Not GLP / Published	No	Published
8.5.1_06 Mutagenicity	Sodium metabisulfite	Green, S. J.	1977	Present and future uses of mutagenicity tests for assessment of the safety of food additives. Environ. Pathol. Toxicol. 1, pp. 49-54 Not GLP / Published	No	Published
8.5.1_07a Mutagenicity	Sodium metabisulfite	National Technical Information Service U.S. Department of Commerce (NTIS)	1972	Study of the Mutagenic Effect of Sodium Meta- Bisulphite (71-22), Report No: PB-221 825 (July 1972). Report date: 1972-07-01. Not GLP / Published	No	Published
8.5.1_07b Mutagenicity	Sodium metabisulfite	Maxwell, W.A. Newell, G.W.	1974	Screening Techniques for Environmental Mutagens. Mol. Environ. Aspects Mutagenesis, Proc. Publ., Rochester Int. Conf. Environ. Toxic. 6th, 223-252, 1974. Not GLP / Published	No	Published
8.5.1_08 Mutagenicity	Sodium sulfite	Engelhardt, G	1989	Report on the Study of Natriumsulfit wasserfrei A in the Ames test (standard plate test and preincubation test with Salmonella typhimurium). BASF Department of Toxicology, Ludwigshafen, Germany. Report No. 40M0639/884492. Report date: 1989-12-20. Not GLP / Unpublished	Yes	AFEPASA (Micro-Pak B.V. has LoA)
8.5.1_09 Mutagenicity	Sodium bisulfite	De Giovanni-Donnelly, R.	1985	The Mutagenicity of Sodium Bisulphite on Base-Substitution Strains of Salmonella typhimurium. Teratogenesis, Carcinogenesis, and Mutagenesis 5: 195-203. Not GLP / Published	No	Published
8.5.1_10 Mutagenicity	Sodium metabisulfite	Pagano, D.A., Zeiger, E.	1987	Conditions affecting the mutagenicity of sodium bisulphite in Salmonella typhimurium. Mutation Research, 179 (1987) 159-166. Not GLP / Published	No	Published
8.5.1_11 Mutagenicity	Sulfur dioxide and Sodium metabisulfite	Shapiro R	1977	Genetic effects of bisulphite (sulphur dioxide). Mutat Res. 1977;39(2):149-75 Not GLP / Published	No	Published
8.5.1_12 Mutagenicity	Sodium metabisulfite	Hayatsu H	2008	Discovery of bisulphite-mediated cytosine conversion to uracil, the key reaction for DNA methylation analysis--a personal account.	No	Published

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				Proc Jpn Acad Ser B Phys Biol Sci. 2008;84(8):321-30 Not GLP / Published		
8.5.1_13 Mutagenicity	Sulfur dioxide and Sodium metabisulfite	Jagiello GM, Lin JS, Ducayen MB	1975	SO2 and its metabolite: effects on mammalian egg chromosomes. Environ Res. 9: 84-93 Not GLP / Published	No	Published
8.5.1_14 Mutagenicity	Sodium metabisulfite	Mukai, F Hawryluk I, Shapiro R.	1970	The mutagenic specificity of sodium bisulphite. Biochem Biophys Res Commun. 39/5: 983-988 Not GLP / Published	No	Published
8.5.2_02 Mutagenicity	Sodium metabisulfite	Green, S. J.	1977	Present and future uses of mutagenicity tests for assessment of the safety of food additives. Environ. Pathol. Toxicol. 1, pp. 49-54 Not GLP / Published	No	Published
8.5.2_03 Mutagenicity	Sulfur dioxide	Uren, N., Yuksel, S. and Onal, Y.	2014	Genotoxic effects of sulphur dioxide in human lymphocytes. Toxicol Ind Health 30(4), pp. 311-315. Not GLP / Published	No	Published
8.5.2_04a Mutagenicity	Sodium metabisulfite	National Technical Information Service U.S. Department of Commerce (NTIS)	1972	Study of the Mutagenic Effect of Sodium Meta- Bisulphite (71-22), Report No: PB-221 825 (July 1972). Report date: 1972-07-01. Not GLP / Published	No	Published
8.5.2_04b Mutagenicity	Sodium metabisulfite	Maxwell, W.A. Newell, G.W.	1974	Screening Techniques for Environmental Mutagens. Mol. Environ. Aspects Mutagenesis, Proc. Publ., Rochester Int. Conf. Environ. Toxic. 6th, 223-252, 1974. Not GLP / Published	No	Published
8.5.2_05 Mutagenicity	Potassium metabisulfite	Anonymous15	2008		No	Published
8.5.2_06 Mutagenicity	Sodium metabisulfite	Rencüzogullari, E., Basri H.I., Kayraldiz, A., Topaktas, M.	2001	Chromosome aberrations and sister chromatid exchanges in cultured human lymphocytes treated with sodium metabisulphite, a food preservative. Mutation Research 490 (2001) 107–112. Not GLP / Published	No	Published
8.5.2_07 Mutagenicity	Sodium bisulfite	Meng, Z., Zhang, L.	1992	Cytogenetic damage induced in human lymphocytes by sodium bisulphite. Mutation Research, 298 (1992) 63-69. Not GLP / Published	No	Published
8.5.2_08 Mutagenicity	Sodium bisulfite	Popescu, N.C., DiPaolo J.A.	1988	Chromosome Alterations in Syrian Hamster Cells Transformed in Vitro by Sodium Bisulphite, a Nonclastogenic Carcinogen. Cancer Research 48, 7246-7251, December	No	Published

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				15, 1988. Not GLP / Published		
8.5.3_02 Mutagenicity	Sodium metabisulfite	Stone, V.	2010	Mutation at the hprt locus of mouse lymphoma L5178Y cells using the Microtitre fluctuation technique: Sodium metabisulphite. Report No: 8230958. Report date: 2010-12-06. GLP / Unpublished	yes	AFEPASA (Micro-Pak B.V. has LoA)
8.5.3_02 Mutagenicity (Attachment of tables)	Sodium metabisulfite	Stone, V.	2010	Attachment of tables as contained in reference above (section 8.5.3_02). Not GLP / Unpublished	yes	AFEPASA (Micro-Pak B.V. has LoA)
8.6_01 Mutagenicity	Sulfur dioxide and Sodium metabisulfite	Anonymous5	1978		No	Published
8.6_01a In vivo genotoxicity study	Sulfur dioxide	Anonymous6	2008		yes	AFEPASA (Micro-Pak B.V. has LoA)
8.6_01b In vivo genotoxicity study	Sulfur dioxide	Anonymous7	2010		yes	AFEPASA (Micro-Pak B.V. has LoA)
8.6_02 In vivo genotoxicity study	Sulfur dioxide	Anonymous8	2002		No	Published
8.6_03 In vivo genotoxicity study	Sulfur dioxide	Anonymous9	2002		No	Published
8.6_04 In vivo genotoxicty study	Sulfur dioxide	Anonymous10	2003		No	Published
8.6_05 In vivo genotoxicty study	Sulfur dioxide	Anonymous11	2005		No	Published
8.6_08a In vivo genotoxicty study	Sodium metabisulfite	National Technical Information Service U.S.	1972	Study of the Mutagenic Effect of Sodium Meta- Bisulphite (71-22), Report No: PB-221 825 (July 1972). Report date: 1972-07-01. No GLP / Published	No	Published

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		Department of Commerce(NTIS)				
8.6_08b In vivo genotoxicty study	Sodium metabisulfite	Maxwell, W.A. Newell, G.W.	1974	Screening Techniques for Environmental Mutagens. Mol. Environ. Aspects Mutagenesis, Proc. Publ., Rochester Int. Conf. Environ. Toxic. 6th, 223-252, 1974. Not GLP / Published	No	Published
8.6_09 In vivo genotoxicty study / SCE	Sodium metabisulfite	Anonymous12	1983		No	Published
8.6_10 In vivo genotoxicty study / Chromosom aberration	Sodium metabisulfite	Anonymous12	1983		No	Published
8.6_11 In vivo genotoxicty study / Micronucleus	Sodium metabisulfite	Anonymous12.	1983		No	Published
8.6_12 In vivo genotoxicity study	Sodium metabisulfite	National Technical Information Service U.S. Department of Commerce (NTIS), (1979)	1979	Study of the Mutagenic Effect of Sodium Meta-Bisulphite (76-73) by Dominant Lethal Test in Rats, Report No: PB-299 836. Report date: 1979-05-18. Not GLP / Published	No	Published
8.6_13 In vivo genotoxicity study	Sodium sulfite	Anonymous13	2008		Yes	AFEPASA (Micro-Pak B.V. has LoA)
8.6_14a In vivo genotoxicity study	Sodium metabisulfite	National Technical Information Service U.S. Department of Commerce(NTIS)	1972	Study of the Mutagenic Effect of Sodium Meta- Bisulphite (71-22), Report No: PB-221 825 (July 1972). Report date: 1972-07-01. No GLP / Published	No	Published
8.6_14a In vivo genotoxicity study	Sodium metabisulfite	Maxwell, W.A. Newell, G.W.	1974	Screening Techniques for Environmental Mutagens. Mol. Environ. Aspects Mutagenesis, Proc. Publ., Rochester Int. Conf. Environ. Toxic. 6th, 223-252, 1974. Not GLP / Published	No	Published
8.6_15 In vivo	Sodium metabisulfite	Anonymous14	2011		No	Published

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genotoxicity study						
8.6_16 In vivo genotoxicity study	Potassium metabisulfite	Anonymous15	2008		No	Published
8.6_17 In vivo genotoxicity study	Mixture of sodium sulfite and sodium bisulfite	Anonymous16	2004		No	Published
8.7.2_01 Acute toxicity: inhalation	Sulfur dioxide	Anonymous17	1973		No	Published
8.7.2_02 Acute toxicity: inhalation	Sulfur dioxide	Anonymous18	1988		No	Published
8.7.2_03 Acute toxicity: inhalation	Sulfur dioxide	Anonymous19	1965		No	Published
8.7.2_04 Acute toxicity: inhalation	Sulfur dioxide	Anonymous20	1989		No	Published
8.7.2_05 Acute toxicity: inhalation	Sulfur dioxide	Anonymous21	1975		No	Published
8.7.2_06 Acute toxicity: inhalation	Sulfur dioxide	Anonymous22	1972		No	Published
8.7.2_07 Acute toxicity: inhalation / rats	Sulfur dioxide	Anonymous23	1961		No	Published
8.7.2_08 Acute toxicity: inhalation / mice	Sulfur dioxide	Anonymous23	1961		No	Published
8.7.2_09 Acute toxicity: inhalation / guinea pigs	Sulfur dioxide	Anonymous23	1961		No	Published

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8.7.2_10 Acute toxicity: inhalation	Sulfur dioxide	Anonymous24	1973		No	Published
8.7.2_11 Acute toxicity: inhalation	Sulfur dioxide	Anonymous25	1961		No	Published
8.7.2_12 Acute toxicity: inhalation	Sulfur dioxide	Anonymous26	1988		No	Published
8.7.2_15 Acute toxicity: inhalation	Sulfur dioxide	Anonymous27	1983		No	Published
8.7.2_16 Acute toxicity: inhalation	Sulfur dioxide	Anonymous28	1991		No	Published
8.7.2_17 Acute toxicity: inhalation	Sulfur dioxide	Anonymous29	1994		No	Published
8.7.2_18 Acute toxicity: inhalation	Sulfur dioxide	Anonymous30	1990		No	Published
8.7.2_19 Acute toxicity: inhalation	Sulfur dioxide	Anonymous31	2003		No	Published
8.7.2_20 Acute toxicity: inhalation	Sulfur dioxide	Anonymous32	1977		No	Published
8.7.2_21 Acute toxicity: inhalation	Sodium metabisulfite	Anonymous33	1973		No	Published
8.7.2_22 Acute toxicity: inhalation	Sodium sulfite	Anonymous34	1976		No	Published
8.7.2_23 Acute toxicity: inhalation	Sodium sulfite	Anonymous35	1980		No	Published
8.7.2_24 Acute toxicity: inhalation	Sodium sulfite	Anonymous36	1987		No	Published

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8.8 Toxicokinetics and metabolism studies in mammals (Attachment)	Sodium metabisulfite	Chemservice S.A.	2013	Toxicokinetics, metabolism and distribution of Sodium metabisulphite Not GLP / Unpublished	Yes	Micro-Pak B.V.
8.8a Toxicokinetics and metabolism studies in mammals (Attachment - Reference for TK statement)	Sulfur species	EFSA	2008	Peer review of the pesticide risk assessment of the active substance sulphur. Question No EFSA-Q-2008-393. Issued on 19 December 2008. Not GLP / Published	No	Published
8.8b Toxicokinetics and metabolism studies in mammals (Attachment - Reference for TK statement)	Sulfur species	TGD	2003	Technical Guidance Document on Risk Assessment. European commission. Joint research centre. Part I. 2003. Not GLP / Published	No	Published
8.8c Toxicokinetics and metabolism studies in mammals (Attachment - Reference for TK statement)	Sulfur species	WHO	1986	Sulphur dioxide and sulphites (WHO Food Additives Series 21). http://www.inchem.org/documents/jecfa/jecmono/v21je15.htm. Not GLP / Published	No	Published
8.8_01 Summary of available metabolism studies in mammals	Sulfur dioxide	Chemservice S.A.	2014	Metabolism studies in mammals Not GLP / Unpublished	Yes	Micro-Pak B.V.
8.8.1_01 Further toxicokinetics and metabolism	Sulfur dioxide	Anonymous37	1971		No	Published

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studies in mammals						
8.8.1_02 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous38	1969		No	Published
8.8.1_03 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous39	1967		No	Published
8.8.1_04 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous19	1965		No	Published
8.8.1_05 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous25	1961		No	Published
8.8.1_06 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous40	1987		No	Published
8.8.1_07 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Gunnison, A.F. and Benton, A.W.	1971	Sulphur dioxide: sulphite. Interaction with mammalian serum and plasma. Arch. Environ. Health 22: 381-388. Not GLP / Published	No	Published
8.8.1_08 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous41	1983		No	Published

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8.8.1_09 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous42	1973		No	Published
8.8.1_10 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Tejnorová, I.	1978	Sulphite Oxidase Activity in Liver and Kidney Tissue in Five Laboratory Animals Species. Toxicology and Applied Pharmacology 44: 251-256. Not GLP / Published	No	Published
8.8.1_11 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Cohen, H.J. and Fridovich, I.	1971	Hepatic Sulphite Oxidase. The Journal of Biological Chemistry 246(2):359-366. Not GLP / Published	No	Published
8.8.1_12 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Yargicoglu, A. et al.	1999	Age-Related Alterations in Antioxidant Enzyms, Lipid Peroxide Levels, and Somatosensory-Evoked Potentials: Effect of Sulphur Dioxide. Arch. Environ. Contam. Toxicol. 37: 554-560. Not GLP / Published	No	Published
8.8.1_13 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous43	1982		No	Published
8.8.1_14 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Cabré, F. et al.	1990	Occurrence and comparison of sulphite oxidase activity in mammalian tissues. Biochem. Med. Metabol. Biol. 43: 159-162. Not GLP / Published	No	Published
8.8.1_15 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Johnson, J.L. and Rajagopalan, K.V.	1976	Human sulphite oxidase deficiency. Characterization of the molecular defect in a multicomponent system. J. Clin. Invest. 58: 551-556. Not GLP / Published	No	Published
8.8.1_16 Further toxicokinetics and metabolism	Sulfur dioxide	Anonymous44	1996		No	Published

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studies in mammals						
8.8.1_17 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Constantin, D. et al.	1994	Alternative pathways of sulphite oxidation in human polymorphonuclear leukocytes. Pharmacol. Toxicol. 74: 136-140. Not GLP / Published	No	Published
8.8.1_18 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous45 ,	2005		No	Published
8.8.1_19 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous46	1981		No	Published
8.8.1_20 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous47	2000		No	Published
8.8.1_21 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous48	1981		No	Published
8.8.1_22 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous49	2003		No	Published
8.8.1_23 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous50	2003		No	Published

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8.8.1_24 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous51	2003		No	Published
8.8.1_25 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous52	2004		No	Published
8.8.1_26 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous53	2003		No	Published
8.8.1_27 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous54	1985		No	Published
8.8.1_28 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	MacLeod, R.M. et al.	1961	Purification and properties of hepatic sulphite oxidase. J. Biol. Chem. 236: 1841-1846. Not GLP / Published	No	Published
8.8.1_29 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Kilic, D.	2003	The effects of ageing and sulphur dioxide inhalation exposure on visual-evoked potentials, antioxidant enzyme systems, and lipid-peroxidation levels of the brain and eye. Neurotoxicol. Teratol. 25: 587-598. Not GLP / Published	No	Published
8.8.1_30 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Petering, D.H.	1977	Sulphur dioxide: A view of its reactions with biomolecules. Lee S (Ed.) Biochemical effects of environmental pollutants. Ann Arbour Science Publishers, p. 293-306. Not GLP / Published	No	Published
8.8.1_31 Further toxicokinetics and metabolism	Sulfur dioxide	Gunnison, A.F. and Jacobsen, D.W.	1987	Sulphite hypersensitivity. A critical review. Crit. Rev. Toxicol. 17: 185-214. Not GLP / Published	No	Published

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studies in mammals						
8.8.1_32 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Gunnison, A.F.	1981	Sulphite toxicity: a critical review of in vitro and in vivo data. Food Cosmet. Toxicol. 19: 667-682. Not GLP / Published	No	Published
8.8.1_33a Further toxicokinetics and metabolism studies in mammals	Sodium metabisulfite	Anonymous12	1983	Attempts to induce cytogenetic effects with sulphite in sulphite oxidasedeficient chinese Hamsters and mize. Food Chem. Toxic. 21(2), 123-127 Not GLP / Published	No	Published
8.8.1_33b Further toxicokinetics and metabolism studies in mammals	Sodium metabisulfite	OECD SIDS	2001	SIDS Initial Assessment Report for for 13th SIAM (Disodium disulphite, CAS 7681-57-4; Bern, 6-9 November 2001) Not GLP / Published	No	Published
8.8.1_34a Further toxicokinetics and metabolism studies in mammals	Sodium metabisulfite	Rost, E.	1933	Handbuch der Lebensmittelchemie Band 1, S. 993, Springer-Verlag (1993) cited in : Toxicological Evaluation of Certain Food additives and contaminants. WHO Food Addiives Series 21, 1986 Not GLP / Published	No	Published
8.8.1_34b Further toxicokinetics and metabolism studies in mammals	Sodium metabisulfite	OECD	2001	SIDS Initial Assessment Report for for 13th SIAM (Disodium disulphite, CAS 7681-57-4; Bern, 6-9 November 2001) Not GLP / Published	No	Published
8.8.1_34c Further toxicokinetics and metabolism studies in mammals	Sodium metabisulfite	WHO	1987	Toxicological Evaluation of Certain Food Additives and Contaminantes. WHO Food Additives Series 21, 30th Meeting of the Joint FAO/WHO Expert Committee on Food Additives, 1987. Not GLP / Published	No	Published
8.8.1_35 Further toxicokinetics and metabolism studies in mammals	Sodium metabisulfite	Gunnison, A.F., Bresnahan, C.A. and E.D. Palmes	1977	Comparative Sulphite Metabolism in the Rat, Rabbit, and Rhesus Monkey. Toxicology and Applied Pharmacology, 42, pp. 99-109. Not GLP / Published	No	Published

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8.8.1_36 Further toxicokinetics and metabolism studies in mammals	Sodium metabisulfite	Gunnison, A.F., Palmes, E.D.	1976	A Model for the Metabolism of Sulphite in Mammal. Toxicology and Applied Pharmacology 38, 111-126 (1976). Not GLP / Published	No	Published
8.8.1_37 Further toxicokinetics and metabolism studies in mammals	Sodium metabisulfite	Anonymous55	1960		No	Published
8.8.1_38 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous56, O.J., Dybicki, J. and G.R. Meneely	1960	The dynamics of sulphur dioxide inhalation. Absorption, distribution, and retention. A.M.A. Archives of Industrial Health, Vol. 21 (June 1960), 564-569. Department of Medicine, University of Southern California, School of Medicine, Los Angeles, USA. Not GLP / Published	No	Published
8.8.1_38 Further toxicokinetics and metabolism studies in mammals (Attachment of tables)	Sulfur dioxide	Balchum, O.J., Dybicki, J. and G.R. Meneely	1960	Attachment of tables as contained in reference above for section 8.8.1_38. Not GLP / Published	No	Published
8.8.1_39 Further toxicokinetics and metabolism studies in mammals	Sodium sulphate	Cocchetto, D.M. and G. Levy	1981	Absorption of Orally Administered Sodium Sulphate in Humans. Journal of Pharmaceutical Sciences, 70, pp. 331-333. Department of Pharmaceutics, School of Pharmacy, State University of New York, Buffalo, NY, USA. Not GLP / Published	No	Published
8.8.1_40 Further toxicokinetics and metabolism studies in mammals	Sulfur	Bauer, J.H.	1976	Oral administration of radioactive sulphate to measure extracellular fluid space in man. Journal of Applied Physiology, 40, pp. 648-650. Department of Medicine, Indiana University Medical Center, Indianapolis, Indiana, USA. Not GLP / Published	No	Published
8.8.1_41 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous57	1960		No	Published

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8.8.1_41 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Balchum, O.J., Dybicki, J., Meneely, G.R.	1960	Attachment of tables as contained in reference above for section 8.8.1_41. Not GLP / Published	No	Published
8.8.1_42 Further toxicokinetics and metabolism studies in mammals	Sulphate	Florin, T., Neale, G., Gibson, G.R., Christl, S.U., Cummings, J.H.	1991	Metabolism of dietary sulphate: absorption and excretion in humans. Gut, 1991,32,766-773. Not GLP / Published	No	Published
8.8.1_42 Further toxicokinetics and metabolism studies in mammals	Sulphate	Florin, T., Neale, G., Gibson, G.R., Christl, S.U., Cummings, J.H.	1991	Attachment of tables as contained in reference above for section 8.8.1_42. Not GLP / Published	No	Published
8.8.1_43 Further toxicokinetics and metabolism studies in mammals	Sulfuric acid	Walser, M., Seldin, D.W., Grollman, A.	1953	An Evaluation of Radiosulphate for the Determination of the Volume of Extracellular Fluid in Man and Dogs. J Clin Invest; 1953; 32: 299-311. Not GLP / Published	No	Published
8.8.1_44 Further toxicokinetics and metabolism studies in mammals	Sodium sulphate	Ryan, R.J., Pascal, L.R., Inoye, T., Bernstein, L.	1956	Experiences with Radiosulphate in the Estimation of Physiologic Extracellular Water in Healthy and Abnormal Man. J Clin Invest. 1956: 35 (10): 1119-30. Not GLP / Published	No	Published
8.8.1_45 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide and Sodium metabisulfite	Beck-Speier I, Hinze H, Holzer H.	1985	Effect of sulphite on the energy metabolism of mammalian Biochim Biophys Acta. 1985 Jul 26;841(1):81-9.tissues in correlation to sulphite oxidase activity. Not GLP / Published	No	Published
8.8.1_46 Further toxicokinetics and metabolism studies in mammals	Sodium metabisulfite	Cocchetto DM, Levy G.	1981	Absorption of orally administered sodium sulphate in humans. J Pharm Sci. 1981 Mar;70(3):331-3. Not GLP / Published	No	Published
8.8.1_47 Further toxicokinetics and metabolism	Sulfur dioxide and Sodium	Constantin D, Bini A, Meletti E, Moldeus P,	1996	Age-related differences in the metabolism of sulphite to sulphate and in the identification of sulphur trioxide radical in human polymorphonuclear leukocytes. Mech Ageing Dev. 1996 Jul 5;88(1-2):95-109.	No	Published

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studies in mammals	metabisulfite	Monti D, Tomasi A.		Not GLP / Published		
8.8.1_48 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide and Sodium metabisulfite	Feng C, Tollin G, Enemark JH	2007	Sulphite oxidizing enzymes. Biochim Biophys Acta. 2007 May;1774(5):527-39 Not GLP / Published	No	Published
8.8.1_48 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide and Sodium metabisulfite	Gregory RE, Gunnison AF.	1984	Identification of plasma proteins containing sulphite-reactive disulfide bonds. Chem Biol Interact. 1984 Apr;49(1-2):55-69 Not GLP / Published	No	Published
8.9_01 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous58	1986		No	Published
8.10_01 Further toxicokinetics and metabolism studies in mammals	Sulfur dioxide	Anonymous59	2013		No	Published
8.11_01 Carcinogenicity	Sulphur dioxide	Anonymous60	1988		No	Published
8.11_02 Carcinogenicity	Sulphur dioxide	Anonymous61	1970		No	Published
8.11_04 Carcinogenicity	Sodium metabisulphite	Anonymous62	1972		No	Published
8.11_05a Carcinogenicity	Sodium metabisulphite	Anonymous63	1960		No	Published
8.11_06 Carcinogenicity	Potassium metabisulphite	Anonymous64	1979		No	Published
8.11_07 Carcinogenicity	Sulphur dioxide	Anonymous65	1967		No	Published

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8.12.1_01 Medical surveillance data on manufacturing plant personnel	Sulfur dioxide	BAuA	2011	Begründung zu Schwefeldioxid in TRGS 900. Bundesanstalt für Arbeitsschutz und Arbeitsmedizin (BAuA). October 2011 Not GLP / Published	No	Published
8.12.1_02 Medical surveillance data on manufacturing plant personnel	Sulfur dioxide	SCOEL	2009	Recommendation from the Scientific Committee on Occupational Exposure limits for Sulphur dioxide. SCOEL/SUM/137, Updated December 2009 Not GLP / Published	No	Published
8.12.1_03 Medical surveillance data on manufacturing plant personnel	Sulfur dioxide	EPA	2008	Integrated Sciences Assessment for Sulphur Oxides – Health Criteria. United States Environmental Protection, EPA/600/R-08/047F. Not GLP / Published	No	Published
8.12.1_04 Medical surveillance data on manufacturing plant personnel	Sulfur dioxide	EPA	2010	621. Sulphur dioxide and sulphites (WHO Food Additives Series 21). Not GLP / Published	No	Published
8.12.1_05 Medical surveillance data on manufacturing plant personnel	Sulfur dioxide	IARC	1992	Occupational exposure to mists and vapours from strong inorganic acids; and other industrial chemicals – Summary of data reported and evaluation. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans vol. 54; therein Section : Sulphur dioxide and some sulphites, bisulphites and metabisulphites" (pp. 131-188), Lyon, France, ISBN 92 832 1254-1 Not GLP / Published	No	Published
8.12.1_06 Medical surveillance data on manufacturing plant personnel	Sulfur dioxide	Nordenson, I. et al.	1980	Is exposure to sulphur dioxide clstogenic? Hereditas 93: 161-164. Not GLP / Published	No	Published
8.12.1_07 Medical surveillance data on manufacturing plant personnel	Sulfur dioxide	Sorsa, M. et al.	1982	No effect of sulphur dioxide exposure, in aluminium industry, on chromosomal aberrations or sister chromatid exchanges. Hereditas 97: 159-161. Not GLP / Published	No	Published
8.12.1_08 Medical surveillance	Sulfur dioxide	Yadav, J.S. and Kaushik, V.K.	1996	Effect of sulphur dioxide exposure on humen chromosomes. Mutat. Res. 359: 25-29. Not GLP / Published	No	Published

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data on manufacturing plant personnel						
8.12.1_09 Medical surveillance data on manufacturing plant personnel	Sulfur dioxide	Meng, Z. and Zhang, L.	1990	Observation of frequencies of lymphocytes with micronuclei in human peripheral blood cultures from workers in a sulphuric acid factory. Environ. Mol. Mutagen. 15: 218-220. Not GLP / Published	No	Published
8.12.1_10 Medical surveillance data on manufacturing plant personnel	Sulfur dioxide	Meng, Z. and Zhang, L.	1990	Chromosomal aberrations and sister-chromatid exchanges in lymphocytes of workers exposed to sulphur dioxide. Mutat. Res. 241: 15-20. Not GLP / Published	No	Published
8.12.1_11 Medical surveillance data on manufacturing plant personnel	Sulfur dioxide	Henneberger, P.K. et al.	1989	Mortality among pulp and paper workers in Berlin, New Hampshire. Brit. J. Ind. Med. 46: 658-664. Not GLP / Published	No	Published
8.12.1_12 Medical surveillance data on manufacturing plant personnel	Sulfur dioxide	Jäpinnen, P.	1987	A mortality study of Finnish pulp and paper workers. Brit. J. Ind. Med. 44: 580-587. Not GLP / Published	No	Published
8.12.1_13 Medical surveillance data on manufacturing plant personnel	Sulfur dioxide	Robinson, C.F. et al.	1986	Mortality among production workers in pulp and paper mills. Scand. J. Work Environ. Health 12: 552-560. Not GLP / Published	No	Published
8.12.1_14 Medical surveillance data on manufacturing plant personnel	Sodium metabisulfite	Confidential Business Information (please refer to separate reference list)				
8.12.2_01 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Amdur, M.O. et al.	1953	Effects of inhalation of sulphur dioxide by man. The Lancet, Oct. 10, 1953: 758 Not GLP / Published	No	Published

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8.12.2_02 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Balmes, J.R. et al.	1987	Symptomatic bronchoconstriction after short-term inhalation of sulphur dioxide. Am. Rev. Respir. Dis. 136: 1117-1121 Not GLP / Published	No	Published
8.12.2_03 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Bedi, J.F. et al.	1979	Human exposure to sulphur dioxide and ozone: absence of a synergistic effect. Arch. Environ. Health 32: 233-239 Not GLP / Published	No	Published
8.12.2_04 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Hackney, J.D. et al.	1984	Time course of exercise-induced bronchoconstriction in asthmatics exposed to sulphur dioxide. Environ. Res. 34: 321-327 Not GLP / Published	No	Published
8.12.2_05 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Horstman, D. et al.	1986	Airway sensitivity of asthmatics to sulphur dioxide. Toxicol. Ind. Health 2: 289-298 Not GLP / Published	No	Published
8.12.2_06 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Horstman, D.H. et al.	1988	The relationship between exposure duration and sulphur dioxide-induced bronchoconstriction in asthmatic subjects. Am. Ind. Hyg. Assoc. J. 49: 38-47 Not GLP / Published	No	Published
8.12.2_07 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Koenig, J.Q. et al.	1983	A comparison of the pulmonary effects of 0.5 ppm versus 1.0 ppm sulphur dioxide plus sodium chloride droplets in asthmatic adolescents. J. Toxicol. Environ. Health 11: 129-139 Not GLP / Published	No	Published
8.12.2_08 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Kreisman, H. et al.	1976	Effect of low concentrations of sulphur dioxide on respiratory function in man. Lung. 154: 25-34 Not GLP / Published	No	Published

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8.12.2_09 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Lawther, P.J. and Lond, M.B.	1955	Effects of inhalation of sulphur dioxide on respiration and pulse-rate in normal subjects. The Lancet, Oct. 8, 1955: 745 Not GLP / Published	No	Published
8.12.2_10 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Lawther, P.J. et al.	1975	Pulmonary function and sulphur dioxide, some preliminary findings. Eviron. Res. 10: 355-367 Not GLP / Published	No	Published
8.12.2_11 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Linn, W.S. et al.	1982	Respiratory responses of young adult asthmatics exposed to sulphur dioxide in a controlled-environment chamber. Am. Rev. Respir. Dis. 125: 151 Not GLP / Published	No	Published
8.12.2_12 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Linn, W.S. et al.	1983	Respiratory effects of sulphur dioxide in heavily exercising asthmatics. A dose-response study. Am. Rev. Respir. Dis. 127: 278-283 Not GLP / Published	No	Published
8.12.2_13 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Linn, W.S. et al.	1985	Controlled exposures of volunteers with chronic obstructive pulmonary disease to sulphur dioxide. Environ. Res. 37: 445-451 Not GLP / Published	No	Published
8.12.2_14 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Linn, W.S. et al.	1987	Replicated dose-response study of sulphur dioxide effects in normal, atopic and asthmatic volunteers. Am. Rev. Respir. Dis. 136: 1127-1134 Not GLP / Published	No	Published
8.12.2_15 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Magnussen, H. et al.	1990	Relationship between the airway response to inhaled sulphur dioxide, isocapnic hyperventilation and histamine in asthmatic subjects. Int. Arch. Occup. Environ. Health 62: 485-491 Not GLP / Published	No	Published

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8.12.2_16 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Sandström, T. et al.	1989	Cell response in bronchoalveolar lavage fluid after sulphur dioxide exposure. Scand. J. Work Environ. Health 15: 142-146 Not GLP / Published	No	Published
8.12.2_17 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Schachter, E.N. et al.	1984	Airway effects of low concentrations of sulphur dioxide: dose response characteristics. Arch. Environ. Health 39: 34-42 Not GLP / Published	No	Published
8.12.2_18 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Sheppard, D. et al.	1981	Exercise increases sulphur dioxide-induced bronchoconstriction in asthmatic subjects. Am. Rev. Respir. Dis. 123: 486-491 Not GLP / Published	No	Published
8.12.2_19 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	van Sim, M. and Pattle, R.E.	1957	Effect of possible smog irritants on human subjects. J.A.M.A., Vol. 165, No. 15: 1908 Not GLP / Published	No	Published
8.12.2_20 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Snell, R.E. and Luchsinger, P.C.	1969	Effects of sulphur dioxide on expiratory flow rates and total respiratory resistance in normal human subjects. Arch. Environ. Health 18: 693-698 Not GLP / Published	No	Published
8.12.2_21 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Stacy, R.W. et al.	1981	Effects of 0.75 ppm sulphur dioxide on pulmonary function parameters of normal human subjects. Arch. Environ. Health 36: 172-178 Not GLP / Published	No	Published
8.12.2_22 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Andersen, I. et al.	1974	Human response to controlled levels of sulphur dioxide. Arch. Environ. Health 28: 31-39 Not GLP / Published	No	Published

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8.12.2_23 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Andersen, I. et al.	1981	Human response to controlled levels of combinations of sulphur dioxide and inert dust. Scand J. Work Environ. Health 7: 1-7 Not GLP / Published	No	Published
8.12.2_24 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Charan, N.B. et al.	1979	Pulmonary injuries associated with acute sulphur dioxide inhalation. American Review of Respiratory Disease, Vol. 119: 555 Not GLP / Published	No	Published
8.12.2_25a Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Speizer, F.E. and Frank, N.R.	1966	Uptake and release of SO2 by the human nose. Arch. Environ. Health 12: 725-728 Not GLP / Published	No	Published
8.12.2_25b Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Frank, N.R. and Speizer, F.E.	1964	Uptake and release of SO2 by the human nose. Physiologist 7: 132 Not GLP / Published	No	Published
8.12.2_26 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Gunnison, A.F. and Palmes, E.D.	1974	S-sulphonate in human plasma following inhalation of sulphur dioxide. Am. Ind. Hyg. Assoc. J. 35: 288-291 Not GLP / Published	No	Published
8.12.2_27 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Galea, M.	1964	Case report - Fatal Sulphur Dioxide Inhalation. Canad. Med. Ass. J. , Vol. 91, p. 345-347 Not GLP / Published	No	Published
8.12.2_28 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Woodford, D.M. et al.	1979	Obstructive lung disease from acute sulphur dioxide exposure. Respiration 38: 238-245 Not GLP / Published	No	Published

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8.12.2_29 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Sandström, T. et al.	1989	Is the short term limit value for sulphur dioxide exposure safe? Effects of controlled chamber exposure investigated with bronchoalveolar lavage. Brit. J. Ind. Med. 46: 200-203 Not GLP / Published	No	Published
8.12.2_30a Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Frank, N.R.	1964	Studies on the effects of acute exposure to sulphur dioxide in human subjects. Proc. Roy. Soc. Med. 57: 1029-1033 Not GLP / Published	No	Published
8.12.2_30b Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Frank, N.R. et al.	1962	Effects of acute controlled exposure to SO2 on respiratory mechanics in healthy male adults. J. Appl. Physiol. 17: 252-258 Not GLP / Published	No	Published
8.12.2_31 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Frank, N.R. et al.	1964	A comparison of the acute effects of SO2 administered alone or in combination with NaCl particles on the respiratory mechanisms of healthy adults. Int. J. Air. Wat. Poll. 8: 125-133 Not GLP / Published	No	Published
8.12.2_32 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Kirkpatrick, M.B. et al.	1982	Effect of oronasal breathing route on sulphur dioxide-induced bronchoconstriction in exercising asthmatic subjects. Am. Rev. Respir. Dis. 125: 627-631 Not GLP / Published	No	Published
8.12.2_33 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Islam, M.S. and Ulmer, W.T.	1979	Untersuchungen zur Schwellenkonzentration von Schwefeldioxyd bei besonders Gefährdeten (Border-line concentrations of SO2 for patients with oversensitivity of the bronchial system). Wiss. Umwelt 1: 41-47 Not GLP / Published	No	Published
8.12.2_34 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Sheppard, D. et al.	1980	Lower threshold and greater bronchomotor responsiveness of asthmatic subjects to sulphur dioxide. Am. Rev. Respir. Dis. 122: 873-878 Not GLP / Published	No	Published

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8.12.2_35 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Bethel, R.A. et al.	1983	Effect of exercise rate and route of inhalation on sulphur-dioxide-induced bronchoconstriction in asthmatic subjects. Am. Rev. Respir. Dis. 128: 592-596 Not GLP / Published	No	Published
8.12.2_36 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Bedi, J.F. and Horvath, S.M.	1989	Inhalation route effects on exposure to 2.0 ppm sulphur dioxide in normal subjects. J. Air. Pollut. Control Assoc. 39: 1448-1452 Not GLP / Published	No	Published
8.12.2_37 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Kulle, T.J. et al.	1986	Pulmonary effects of sulphur dioxide and respirable carbon aerosol. SO Environm. Res. 41: 239-250 Not GLP / Published	No	Published
8.12.2_38 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Witek, T.J. and Schachter, E.N.	1985	Airway responses to sulphur dioxide and methacholine in asthmatics. J. Occup. Med. 27: 265-268 Not GLP / Published	No	Published
8.12.2_39 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Sheppard, D. et al.	1984	Magnitude of the interaction between the bronchomotor effects of sulphur dioxide and those of dry (cold) air. Am. Rev. Respir. Dis. 130: 52-55 Not GLP / Published	No	Published
8.12.2_40 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Linn, W.S. et al.	1985	Effects of heat and humidity on the responses of exercising asthmatics to sulphur dioxide exposure. Am. Rev. Respir. Dis. 131: 221-225 Not GLP / Published	No	Published
8.12.2_41 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Stacy, R.W. et al.	1983	A survey of effects of gaseous and aerosol pollutants on pulmonary function of normal males. Arch. Environ. Health 38: 104-115 Not GLP / Published	No	Published

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8.12.2_42 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Carson, J.L. et al.	1987	The appearance of compound cilia in the nasal mucosa of normal human subjects following acute, in vivo exposure to sulphur dioxide. Environ. Res. 42: 155-165 Not GLP / Published	No	Published
8.12.2_43 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Heath, K.S. et al.	1994	Effects of sulphur dioxide exposure on African-American and Caucasian asthmatics. Environ. Res. 66: 1-11 Not GLP / Published	No	Published
8.12.2_44 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Bedi, J.F. et al.	1984	Pulmonary function effects of 1.0 and 2.0 ppm sulphur dioxide exposure in active young male non-smokers. J. Air. Pollut. Control. Assoc. 34: 1117-1121 Not GLP / Published	No	Published
8.12.2_45 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Bedi, J.F. et al.	1982	Human exposure to sulphur dioxide and ozone in a high temperature-humidity environment. Am. Ind. Hyg. Assoc. J. 43: 26-30 Not GLP / Published	No	Published
8.12.2_46 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Bethel, R.A. et al.	1983	Sulphur dioxide-induced bronchoconstriction in freely breathing, exercising, asthmatic subjects. Am. Rev. Respir. Dis. 128: 987-990 Not GLP / Published	No	Published
8.12.2_47a Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Bethel, R.A. et al.	1983	Potentiation of sulphur dioxide-induced bronchoconstriction by airway cooling. Am. Rev. Respir. Dis. 127: A 161 Not GLP / Published	No	Published
8.12.2_47b Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Bethel, R.A. et al.	1984	Interaction of sulphur dioxide and dry cold air in causing bronchoconstriction in asthmatic subjects. J. Appl. Physiol. 57: 419-423 Not GLP / Published	No	Published

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8.12.2_48 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Bethel, R.A. et al.	1985	Effect of 0.25 ppm sulphur dioxide on airway resistance in freely breathing, heavily exercising, asthmatic subjects. Am. Rev. Respir. Dis. 131: 659-661 Not GLP / Published	No	Published
8.12.2_49 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Burton, G.G. et al.	1969	Response of healthy men to inhaled low concentrations of gas-aerosol mixtures. Arch. Environ. Health 18: 681-692 Not GLP / Published	No	Published
8.12.2_50 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Delohery, J. et al.	1984	The relationship of inhaled sulphur dioxide reactivity to ingested metabisulphite sensitivity in patients with asthma. Am. Rev. Respir. Dis. 130: 1027-1030 Not GLP / Published	No	Published
8.12.2_51 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Folinsbee, L.J. et al.	1985	Pulmonary response to threshold levels of sulphur dioxide (1.0 ppm) and ozone (0.3 ppm). J. Appl. Physiol. 58: 1783-1787 Not GLP / Published	No	Published
8.12.2_52 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Hazucha, M. and Bates, D.V.	1975	Combined effect of ozone and sulphur dioxide on human pulmonary function. Nature 257: 50-51 Not GLP / Published	No	Published
8.12.2_53 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Islam, M.S. et al.	1992	Bronchomotoric effect of low concentration of sulphur dioxide in young healthy volunteers. Fresenius Envir. Bull. 1: 541-546 Not GLP / Published	No	Published
8.12.2_54 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Jaeger, M.J. et al.	1979	Effect of sulphur dioxide on the respiratory function of normal and asthmatic subjects. Lung 156: 119-127 Not GLP / Published	No	Published

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8.12.2_55 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Kehrl, H. et al.	1983	Pulmonary responses of young male adult asthmatics to SO2 with moderate exercise. Am. Rev. Respir. Dis. 127: 160 Not GLP / Published	No	Published
8.12.2_56 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Kehrl, H.R. et al.	1987	Differing response of asthmatics to sulphur dioxide exposure with continuous and intermittent exercise. Am. Rev. Respir. Dis. 135: 350-355 Not GLP / Published	No	Published
8.12.2_57 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Koenig, J.Q.	1989	Effects of inhalation of acidic compounds on pulmonary function in allergic adolescent subjects. Environ. Health Perspect. 79: 173-178 Not GLP / Published	No	Published
8.12.2_58 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Koenig, J.Q. et al.	1990	Prior exposure to ozone potentiates subsequent response to sulphur dioxide in adolescent asthmatic subjects. Am. Rev. Respir. Dis. 141: 377-380 Not GLP / Published	No	Published
8.12.2_59a Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Koenig, J.Q. et al.	1982	Effects of inhaled sulphur dioxide (SO2) on pulmonary function in healthy adolescents: exposure to SO2 alone or SO2 + sodium chloride droplet aerosol during rest and exercise. Arch. Environ. Health 37: 5-9 Not GLP / Published	No	Published
8.12.2_59b Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Koenig, J.Q. and Pierson, W.E.	1985	Pulmonary effects of inhaled sulphur dioxide in atopic adolescent subjects: A review. Frank R et al. (Ed.), Inhalation Toxicology of Air Pollutants: Clinical Research Considerations, ASTM STP 872, American Society for Testing and Materials, Philadelphia, p. 85-91 Not GLP / Published	No	Published
8.12.2_60 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Koenig, J.Q. et al.	1980	Acute effects of inhaled SO2 plus NaCl droplet aerosol on pulmonary function in asthmatic adolescents. Environ. Res. 22: 145-153 Not GLP / Published	No	Published

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8.12.2_61 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Koenig, J.Q. et al.	1981	Effects of SO2 plus NaCl aerosol combined with moderate exercise on pulmonary function in asthmatic adolescents. Environ. Res. 25: 340-348 Not GLP / Published	No	Published
8.12.2_62 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Koenig, J.Q. et al.	1982	Bronchoconstrictor responses to sulphur dioxide or sulphur dioxide plus sodium chloride droplets in allergic, nonasthmatic adolescents. J. Allergy. Clin. Immunol. 69: 339-344 Not GLP / Published	No	Published
8.12.2_63 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Kulle, T.J. et al.	1984	Sulphur dioxide and ammonium sulphate effects on pulmonary function and bronchial reactivity in human subjects. Am. Ind. Hyg. Assoc. J. 45: 156-161 Not GLP / Published	No	Published
8.12.2_64 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Linn, W.S. et al.	1988	Effect of metaprotenerol sulphate on mild asthmatics' response to sulphur dioxide exposure and exercise. Arch. Environ. Health. 43: 399-406 Not GLP / Published	No	Published
8.12.2_65 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Linn, W.S. et al.	1982	Respiratory responses of young adult asthmatics exposed to sulphur dioxide in a controlled-environment chamber. Am. Rev. Respir. Dis. 125: 151 Not GLP / Published	No	Published
8.12.2_66 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Linn, W.S. et al.	1984	Comparative effects of sulphur dioxide exposures at 5 °C and 22 °C in exercising asthmatics. Am. Rev. Respir. Dis. 129: 234-239 Not GLP / Published	No	Published
8.12.2_67 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Magnussen, H. et al.	1987	Relationship between the airway response to inhaled sulphur dioxide and histamine in asthmatics. Am. Rev. Respir. Dis. 135, Suppl 2: A442 Not GLP / Published	No	Published

Section No / Reference No/ ESR in IUCLID	Substance	Author(s)	Year	Title Source, Report No. GLP / (Un)Published	Data Protection Claimed (Yes/No)	Owner
8.12.2_68 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Myers, D.J. et al.	1986	The inhibition of sulphur dioxide-induced bronchoconstriction in asthmatic subjects by cromolyn is dose dependent. Am. Rev. Respir. Dis. 133: 1150-1153 Not GLP / Published	No	Published
8.12.2_69 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Wolff, R.K. et al.	1975	Sulphur dioxide and tracheobronchial clearance in man. Arch. Environ. Health 30: 521-527 Not GLP / Published	No	Published
8.12.2_70 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Trenga, C.A.	1999	Sulphur dioxide sensitivity and plasma antioxidants in adult subjects with asthma. Occup. Environ. Med. 56: 544-547 Not GLP / Published	No	Published
8.12.2_71 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Speizer, F.E. and Frank, N.R.	1966	A comparison of changes in pulmonary flow resistance in healthy volunteers acutely exposed to SO2 by mouth and nose. Brit. J. Ind. Med. 23: 75-79 Not GLP / Published	No	Published
8.12.2_72 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Nadel, J.A. et al.	1965	Mechanism of bronchoconstriction during inhalation of sulphur dioxide. J. Appl. Physiol. 20: 164-167 Not GLP / Published	No	Published
8.12.2_73 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Newhouse, M.T. et al.	1978	Effect of TLV levels of SO2 and H2SO4 on bronchial clearance in exercising man. Arch. Environ. Health 33: 24-32 Not GLP / Published	No	Published
8.12.2_74 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Sheppard, D. et al.	1983	Tolerance to sulphur dioxide-induced bronchoconstriction in subjects with asthma. Environ. Res. 30: 412-419 Not GLP / Published	No	Published

Section No / Reference No/ ESR in IUCLID	Substance	Author(s)	Year	Title Source, Report No. GLP / (Un)Published	Data Protection Claimed (Yes/No)	Owner
8.12.2_75 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Nowak, D. et al.	1997	Airway responsiveness to sulphur dioxide in an adult population sample. Am. J. Respir. Crit. Care Med. 156: 1151-1156 Not GLP / Published	No	Published
8.12.2_76 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Rondinelli, R.C.A. et al.	1987	The effects of sulphur dioxide on pulmonary function in healthy nonsmoking male subjects aged 55 years and older. Am. Ind. Hyg. Assoc. J. 48: 299-303 Not GLP / Published	No	Published
8.12.2_77 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Myers, D.J. et al.	1986	Interaction of cromolyn and a muscarinic antagonist in inhibiting bronchial reactivity to sulphur dioxide and to eucapnic hyperpnea alone. Am. Rev. Respir. Dis. 133: 1154-1158 Not GLP / Published	No	Published
8.12.2_78 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Roger, L.J. et al.	1985	Bronchoconstriction in asthmatics exposed to sulphur dioxide during repeated exercise. J. Appl. Physiol. 59: 784-791 Not GLP / Published	No	Published
8.12.2_79 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Boushey, H.	1982	Asthma, sulphur dioxide and the clean air act. West. J. Med. 136: 129-135 Not GLP / Published	No	Published
8.12.2_80 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Koenig, J.Q. et al.	1985	The effects of sulphur oxides on nasal and lung function in adolescents with extrinsic asthma. J. Allergy. Clin. Immunol. 76: 813-818 Not GLP / Published	No	Published
8.12.2_81 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Linn, W.S. et al.	1984	Combined effect of sulphur dioxide and cold in exercising asthmatics. Arch. Environ. Health 39: 339-346 Not GLP / Published	No	Published

Section No / Reference No/ ESR in IUCLID	Substance	Author(s)	Year	Title Source, Report No. GLP / (Un)Published	Data Protection Claimed (Yes/No)	Owner
8.12.2_82 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Härkönen, H. et al.	1983	Long-term effects of exposure to sulphur dioxide. Lung function four years after a pyrite dust explosion. Am. Rev. Respir. Dis. 128: 890-893 Not GLP / Published	No	Published
8.12.2_83 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Rabinovitch, S. et al.	1989	Clinical and laboratory features of acute sulphur dioxide inhalation poisoning: two-year follow-up. Am. Rev. Respir. Dis. 139: 556-558 Not GLP / Published	No	Published
8.12.2_84 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Sandström, T. et al.	1989	Cell response in bronchoalveolar lavage fluid after exposure to sulphur dioxide: a time-response study. Am. Rev. Respir. Dis. 140: 1828-1831 Not GLP / Published	No	Published
8.12.2_85 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Lazarus, S.C. et al.	1997	The Leukotriene Receptor Antagonist Zafirlukast Inhibits Sulphur Dioxide-induced Bronchoconstriction in Patients with Asthma. Am. J. Respir. Crit. Care. Med. 156: 1725-1730 Not GLP / Published	No	Published
8.12.2_86 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Islam, M.S. et al.	1994	Non-specific airway responsiveness to hyperventilation of low doses of sulphur dioxide and cold air of non-smoking healthy volunteers of different ages. Zbl. Hyg. 195: 556-566 Not GLP / Published	No	Published
8.12.2_87 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Melville, G.N.	1970	Changes in specific airway conductance in healthy volunteers following nasal and oral inhalation of SO2. West. Indian Med. J. 19: 231-235 Not GLP / Published	No	Published
8.12.2_88 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Tunnicliffe, W.S. et al.	2003	The effect of sulphurous air pollutant exposures on symptoms, lung function, exhaled nitric oxide, and nasal epithelial lining fluid antioxidant concentrations in normal and asthmatic adults. Occup. Environ. Med. 60: e15 Not GLP / Published	No	Published

Section No / Reference No/ ESR in IUCLID	Substance	Author(s)	Year	Title Source, Report No. GLP / (Un)Published	Data Protection Claimed (Yes/No)	Owner
8.12.2_89 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Devalia, J.L. et al.	1994	Effect of nitrogen dioxide and sulphur dioxide on airway response of mild asthmatic patients to allergen inhalation. Lancet 344: 1668-1671 Not GLP / Published	No	Published
8.12.2_90 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Field, P.I. et al.	1996	Evidence for opioid modulation and generation of prostaglandins in sulphur dioxide (SO)2-induced bronchoconstriction. Thorax 51: 159-163 Not GLP / Published	No	Published
8.12.2_91 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Prügger, F.	1974	Ein Fall von sublethaler, akuter Schwefeldioxidvergiftung und deren Folgeerscheinungen auf die Lungenfunktion. (Case report of acute sulphur dioxide poisoning and its effects on lung function.) Pneumologie 150: 97-98 Not GLP / Published	No	Published
8.12.2_92 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Piirilä, P. et al.	1996	A thirteen-year follow-up of respiratory effects of acute exposure to sulphur dioxide. Scand. J. Work Environ. Health 22: 191-196 Not GLP / Published	No	Published
8.12.2_93 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Koksal, N. et al.	2003	Apricot sulphurization: an occupation that induces an asthma-like syndrome in agricultural environments. Am. J. Ind. Med. 43: 447-453 Not GLP / Published	No	Published
8.12.2_94 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Jäppinen, P.; Tola, S.	1986	Smoking among Finnish pulp and paper workers - Evaluation of its confounding effect on lung cancer and coronary heart disease. Scand. J. Work. Environ. Health 12: 619-626 Not GLP / Published	No	Published
8.12.2_95 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Mikaelsson B. et al.	1982	The Prevalence of bronchial asthma and chronic bronchitis in an industrialized community in Northern Sweden. Scand. J. Soc. Med. 10: 11-16 Not GLP / Published	No	Published

Section No / Reference No/ ESR in IUCLID	Substance	Author(s)	Year	Title Source, Report No. GLP / (Un)Published	Data Protection Claimed (Yes/No)	Owner
8.12.2_96 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Ferris, B.G. et al.	1979	Mortality and morbidity in a pulp and paper mill in the United States: a ten-year follow-up. Brit. J. Ind. Med. 36: 127-134 Not GLP / Published	No	Published
8.12.2_97 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Stjernberg N. et al.	1985	Prevalence of bronchial asthma and chronic bronchitis in a community in northern Sweden; relation to environmental and occupational exposure to sulphur dioxide. Eur. J. Respir. Dis. 67: 41-49 Not GLP / Published	No	Published
8.12.2_98 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Savic, M. et al.	1987	Discomforts and laboratory findings in workers exposed to sulphur dioxide. Int. Arch. Occup. Environ. Health 59: 513-518 Not GLP / Published	No	Published
8.12.2_99 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Andersson, E. et al.	1998	Mortality from asthma and cancer among sulphite mill workers. Scand. J. Work Environ. Health 24: 12-17 Not GLP / Published	No	Published
8.12.2_100 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Gokirmak, M. et al.	2003	The role of oxidative stress in bronchoconstriction due to occupational sulphur dioxide exposure. Clin. Chim. Acta. 331, 119-126 Not GLP / Published	No	Published
8.12.2_101 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Andersson, E. et al.	2006	Incidence of asthma among workers exposed to sulphur dioxide and other irritant gases. Eur. Respir. J. 27, 720-725 Not GLP / Published	No	Published
8.12.2_102 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Band, P.R. et al.	1997	Cohort mortality study of pulp and paper mill workers in British Columbia, Canada. J. Exp. Anal. Environ Epidem. 3: 371-382 Not GLP / Published	No	Published

Section No / Reference No/ ESR in IUCLID	Substance	Author(s)	Year	Title Source, Report No. GLP / (Un)Published	Data Protection Claimed (Yes/No)	Owner
8.12.2_103 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Band, P.R. et al.	2001	Cohort cancer incidence among pulp and paper mill workers in British Columbia. Scand. J. Work Environ. Health 27: 113-119. Am. J. Epidemiol. 146: 186-194 Not GLP / Published	No	Published
8.12.2_104 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Langseth, H.; Andersen, A.	2000	Cancer incidence among male pulp and paper workers in Norway. Scand. J. Work Environ. Health 26: 99-105 Not GLP / Published	No	Published
8.12.2_105 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Lee, W.J. et al.	2002	Mortality from lung cancer in workers exposed to sulphur dioxide in the pulp and paper industry. Environ. Health Perspect. 110: 991-995 Not GLP / Published	No	Published
8.12.2_106 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Rix, B.A. et al.	1997	Cancer incidence of sulphite pulp workers in Denmark. Scand. J. Work Environ. Health 23: 458-461 Not GLP / Published	No	Published
8.12.2_107 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Matanoski, G.M. et al.	1998	Industry-wide study of mortality of pulp and paper mill workers Am J Ind Med 33: 354-365 Not GLP / Published	No	Published
8.12.2_108 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Shih, V.E. et al.	1977	Sulphite oxidase deficiency. Biochemical and clinical investigations of a hereditary metabolic disorder in sulphur metabolism. Am. Ind. Hyg. Assoc. J. 35: 288-291 Not GLP / Published	No	Published
8.12.2_109 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Bechthold, W.E: et al.	1993	Biological markers of exposure to SO2: S-sulfonates in nasal lavage. New Engl. J. Med. 297: 1022-1028 Not GLP / Published	No	Published

Section No / Reference No/ ESR in IUCLID	Substance	Author(s)	Year	Title Source, Report No. GLP / (Un)Published	Data Protection Claimed (Yes/No)	Owner
8.12.2_110 a Direct observation, e.g. clinical cases, poisoning incidents	Sodium metabisulfite	Vena, G., Foti, C. and Angelini, G.	1994	Sulphite contact allergy. Contact Dermatitis 31:172–175 Not GLP / Published	No	Published
8.12.2_110 b Direct observation, e.g. clinical cases, poisoning incidents	Sodium metabisulfite	Nair, B. and Elmore, A.R.	2003	Final Report on the Safety Assessment of Sodium Sulphite, Potassium Sulphite, Ammonium Sulphite, Sodium Bisulphite, Ammonium Bisulphite, Sodium Metabisulphite and Potassium Metabisulphite. International Journal of Toxicology 2003, Vol. 22: 63-88. Not GLP / Published	No	Published
8.12.2_111 Direct observation, e.g. clinical cases, poisoning incidents	Sodium metabisulfite	Gall, H., Boehncke, W.-H., Gietzen, K.	1996	Intolerance to sodium metabisulphite in beer. Allergy Net Not GLP / Published	No	Published
8.12.2_112 Direct observation, e.g. clinical cases, poisoning incidents	Sodium metabisulfite	Rowe, R.C., Sheykey, P.J., Quinn, M.E.	2009	Handbook of Pharmaceutical Excipients, 6. Edition, published by the Pharmaceutical Press and the American Pharmacists Association, ISBN 978 0 85369 792 3 (UK), ISBN 978 1 58212 135 2 (USA) Not GLP / Published	No	Published
8.12.2_113 Direct observation, e.g. clinical cases, poisoning incidents	Sodium metabisulfite	Sasseville, D., El-Helou, T.	2009	Occupational allergic contact dermatitis from sodium metabisulphite. Contact Dermatitis 2009: 61: 244–245 Not GLP / Published	No	Published
8.12.2_114 Direct observation, e.g. clinical cases, poisoning incidents	Sodium metabisulfite	Petersen, C and Mené, T.	1992	Consecutive patch testing with sodium sulphite in eczema patients. Contact Dermatitis 27:344–345 Not GLP / Published	No	Published
8.12.2_115 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Johns, AD.O. and Linn, W.S.	2011	A review of controlled human SO2 exposure studies contributing to the US EPA integrated science assessment for sulphur oxides. Inhalation toxicology, 2011; 23 (1-4): 33-43. Not GLP / Published	No	Published

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8.12.2_116 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Goodman, J.E., Dodge, D.G. and Bailey, L.A.	2010	A framework for assessing causality and adverse effects in humans with a case study of sulphur dioxide. Regulatory Toxicology and Pharmacology 58: pp. 308-332. Not GLP / Published	No	Published
8.12.2_116 Direct observation, e.g. clinical cases, poisoning incidents (Attachment of tables)	Sulfur dioxide	Goodman, J.E., Dodge, D.G. and Bailey, L.A.	2010	Attachment of tables as contained in reference above (section 8.12.2_116). Not GLP / Published	No	Published
8.12.2_117 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Gong H Jr, Lachenbruch PA, Harber P, Linn WS.	1995	Comparative short-term health responses to sulphur dioxideexposure and other common stresses in a panel of asthmatics. Toxicol Ind Health. 1995 Sep-Oct;11(5):467-87. Not GLP / Published	No	Published
8.12.2_118 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Goodman JE, Dodge DG, Bailey LA.	2010	A framework for assessing causality and adverse effects in humans with a case study of sulphur dioxide. Regul Toxicol Pharmacol. 2014 Feb;68(1):8-15. Not GLP / Published	No	Published
8.12.2_119 Direct observation, e.g. clinical cases, poisoning incidents	Sulfur dioxide	Johns DO, Linn WS.	2011	A review of controlled human SO_2 exposure studies contributing to the US EPA integrated science assessment for sulphur oxides. Inhal Toxicol. 2011 Jan;23(1):33-43 Not GLP / Published	No	Published
8.12.3_01 Health records, both from industry and any other sources	Sulfur dioxide	Kehoe, R.A. et al.	1932	On the effects of prolonged exposure to sulphur dioxide. J. Ind. Hyg. 14: 159-173 Not GLP / Published	No	Published
8.12.3_02 Health records,	Sulfur dioxide	Anderson, A.	1950	Possible long term effects of exposure to sulphur dioxide. Brit. J.Med. 7: 82-86 Not GLP / Published	No	Published

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both from industry and any other sources						
8.12.3_03 Health records, both from industry and any other sources	Sulfur dioxide	Berges, G, et al.	1975	Einfluß der Luftfeuchtigkeit auf die Lungenfunktion bei Schwefeldioxydexposition. Arbeitsmed. Sozialmed. Präventivmed. 10: 17-19 Not GLP / Published	No	Published
8.12.3_04 Health records, both from industry and any other sources	Sulfur dioxide	Snashall, P.D. and Baldwin, C.	1982	Mechanisms of sulphur dioxide induced bronchoconstriction in normal and asthmatic man. Thorax 37: 118-123 Not GLP / Published	No	Published
8.12.3_05 Health records, both from industry and any other sources	Sulfur dioxide	Sandström, T. et al.	1988	Challenge test for sulphur dioxide - symptom and lung function measurements. Scand. J.Work Environ. Health 14 (1): 77-79 Not GLP / Published	No	Published
8.12.3_06 Health records, both from industry and any other sources	Sulfur dioxide	Andersen, I et al.	1978	Human responses to SO2 at controlled conditions. VDI-Berichte 314. 139-141 Not GLP / Published	No	Published
8.12.3_07 Health records, both from industry and any other sources	Sulfur dioxide	Stjernberg, N. et al.	1984	Long-term effects of chronic exposure to sulphuric dioxide. Bull. Int. Union Tuberc. 59: 43-45 Not GLP / Published	No	Published
8.12.3_08 Health records, both from industry and any other sources	Sulfur dioxide	Jäppinen, P. et al.	1987	Cancer indicence of workers in the Finnish pulp and paper industry. Scand. J. Environ. Health 13: 197-202 Not GLP / Published	No	Published

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8.12.3_09 Health records, both from industry and any other sources	Sulfur dioxide	Fabri, L. et al.	1977	Alterazoni respiratorie da esposizione cronica a bassa concentrazioni di SO2. - Respiratory impairment due to chronic exposure to low levels of sulphur dioxide. Med. Lav. 68: 38-50 Not GLP / Published	No	Published
8.12.3_10 Health records, both from industry and any other sources	Sulfur dioxide	Peters, J.M. et al.	1984	Pulmonary effects of exposure in silicon carbide manufacturing. Br. J. Ind. Med. 41: 109-115 Not GLP / Published	No	Published
8.12.3_11 Health records, both from industry and any other sources	Sulfur dioxide	Skalpe, I.O.	1964	Long-term effects of sulphur dioxide exposure in pulp mills. Brit. J. Ind. Med. 21: 69-73 Not GLP / Published	No	Published
8.12.3_12 Health records, both from industry and any other sources	Sulfur dioxide	Ferris, B.G. et al.	1967	Prevalence of chronic respiratory disease in a pulp mill and a paper mill in the United States. Brit. J. Ind. Med. 24: 26-37 Not GLP / Published	No	Published
8.12.3_13 Health records, both from industry and any other sources	Sulfur dioxide	Stjernberg, N. et al.	1986	Chronic bronchitis in a community in northern Sweden; relation to environmental and occupational exposure to sulphur dioxide. Eur. J. Respir. Dis. 69 (146): 153-159 Not GLP / Published	No	Published
8.12.3_14 Health records, both from industry and any other sources	Sulfur dioxide	Osterman, J.W. et al.	1989	Respiratory symptoms associated with low level sulphur dioxide exposure in silicon carbide production workers. British J. Ind. Med. 46: 629-635 Not GLP / Published	No	Published
8.12.3_15 Health records, both from industry and any other sources	Sulfur dioxide	Osterman J.W. et al.	1989	Work related decrement in pulmonary function in silicon carbide production workers. British J. Ind. Med. 46: 708-716 Not GLP / Published	No	Published

Section No / Reference No/ ESR in IUCLID	Substance	Author(s)	Year	Title Source, Report No. GLP / (Un)Published	Data Protection Claimed (Yes/No)	Owner
8.12.3_16 Health records, both from industry and any other sources	Sulfur dioxide	Kremer, A.M. et al.	1995	Airway hyperresponsiveness in workers exposed to low levels of irritants. Eur. Respir. J. 8: 53-61 Not GLP / Published	No	Published
8.12.3_17 Health records, both from industry and any other sources	Sulfur dioxide	Kremer, A.M. et al.	1994	Airway hyperresponsiveness, prevalence of chronic respiratory symptoms, and lung function in workers exposed to irritants. Occup. Environ. Med. 51: 3-13 Not GLP / Published	No	Published
8.12.3_18 Health records, both from industry and any other sources	Sulfur dioxide	Smith, T.J. et al.	1984	Respiratory exposures associated with silicon carbide production: estimation of cumulative exposures for an epidemiological study. Brit. J. Ind. Med. 41: 100-108 Not GLP / Published	No	Published
8.12.3_19 Health records, both from industry and any other sources	Not applicable	Not applicable	n.a.	Study has been deleted. To guarantee the continuous numbering of the study entries, this ESR only serves as placeholder.	No	Published
8.12.3_20 Health records, both from industry and any other sources	Sulfur dioxide	Linn, W.S. et al.	1984	Asthmatics' responses to 6-hr sulphur dioxide exposures on two successive days. Arch. Environ. Health 39: 313-319 Not GLP / Published	No	Published
8.12.3_21 Health records, both from industry and any other sources	Sulfur dioxide	Andersen, I. et al.	1977	Induced rhinovirus infection under controlled exposure to sulphur dioxide. Arch. Environ. Health 32: 120-126 Not GLP / Published	No	Published
8.12.3_22 Health records, both from industry and any other sources	Sulfur dioxide	Frank, N.R. and Speizer, F.E.	1964	Uptake and release of SO2 by the human nose. Physiologist 7: 132 Not GLP / Published	No	Published

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8.12.3_23 Health records, both from industry and any other sources	Sodium metabisulfite	Kaaman, A.-C., Boman, A., Wrangsjö, K., Matura, M.	2010	Contact allergy to sodium metabisulphite: an occupational problem. Contact Dermatitis 2010: 63: 110–112 Not GLP / Published	No	Published
8.12.3_24 Health records, both from industry and any other sources	Sodium metabisulfite	Sasseville, D., El-Helou, T.	2009	Occupational allergic contact dermatitis from sodium metabisulphite. Contact Dermatitis 2009: 61: 244–245 Not GLP / Published	No	Published
8.12.3_25 Health records, both from industry and any other sourcesl	Sodium metabisulfite	Merget, R. and Korn, M.	2005	CASE STUDY - Metabisulphite-induced occupational asthma in a radiographer. Eur Respir J 2005; 25: 386–388 Not GLP / Published	No	Published
8.12.3_26 Health records, both from industry and any other sources	Sodium metabisulfite	Pougnet, R., Loddé, B., Lucas, D., Jégaden, D., Bell, S. Dewitte, J.-D.	2010	CASE STUDY - A case of occupational asthma from metabisulphite in a fisherman. Int Marit Health, 2010; 61, 3: 180–184 Not GLP / Published	No	Published
8.12.4_01 Epidemiological data	Sulfur dioxide	Raulf-Heimsoth, M.	2010	Assessment of low dose effects of acute sulphur dioxide exposure on the airways using non-invasive methods. Arch. Toxicol. 84: 121 – 127 Not GLP / Published	No	Published
8.12.4_02 Epidemiological data	Sulfur dioxide	van Thriel, C et al.	2010	Sensory and pulmonary effects of acute exposure to sulphur dioxide (SO2). Toxicology Letters 196: 42 – 50 Not GLP / Published	No	Published
8.12.4_03a Epidemiological data	Sodium metabisulfite	Vena, G., Foti, C. and Angelini, G.	1994	Sulphite contact allergy. Contact Dermatitis 31:172–175 Not GLP / Published	No	Published
8.12.4_03b Epidemiological data	Sodium metabisulfite	Nair, B. and Elmore, A.R.	2003	Final Report on the Safety Assessment of Sodium Sulphite, Potassium Sulphite, Ammonium Sulphite, Sodium Bisulphite, Ammonium Bisulphite, Sodium Metabisulphite and Potassium Metabisulfit. International Journal of Toxicology 2003, Vol. 22: 63-88 Not GLP / Published	No	Published

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8.12.4_04 Epidemiological data	Sodium metabisulfite	Kaaman, A.-C., Boman, A., Wrangsjö, K., Matura, M.	2010	Contact allergy to sodium metabisulphite: an occupational problem. Contact Dermatitis 2010: 63: 110–112 Not GLP / Published	No	Published
8.12.5_02 Diagnosis of poisoning	Sodium metabisulfite	Chemservice S.A.	2014	Review of public available information - Diagnosis of Sodium metabisulphite poisoning including specific signs of poisoning and clinical tests. Chemservice S.A., Grevenmacher, Luxembourg, Report No CSL-ML-284, Not GLP / Unpublished	Yes	Micro-Pak B.V.
8.12.6_02 Contact dermatitis	Sodium metabisulfite	Sokol, W.N. and Hydick, I.B.	1990	Nasal congestion, urticaria, and angioedema, caused by IgE-mediated reaction to sodium metabisulphite. J. Allerg Clin Immunol. 65, 233-238 Not GLP / Published	No	Published
8.12.6_03 Contact dermatitis	Sodium metabisulfite	Wüthrich, B., Kagi, M.K. and Hafner, J.	1993	Disulphite-induced acute intermittent urticaria with vasculitis, Dermatology. 187, 290-292 Not GLP / Published	No	Published
8.12.6_04 Contact dermatitis	Sodium metabisulfite	Vena, G., Foti, C. and Angelini, G.	1994	Sulphite contact allergy. Contact Dermatitis 31:172–175 Not GLP / Published	No	Published
8.12.6_05 Contact dermatitis	Sodium metabisulfite	Sainte-Laudy, J., Vallon, C. and Guérin, J.-C.	1994	Mise en évidence des IgE spécifiques du groupe des sulphites chez les intolérants à ces conservateurs Allergie et immunologie 26 (4); pp. 132-134, 137-138 Not GLP / Published	No	Published
8.12.6_06 Contact dermatitis	Sodium metabisulfite	Jacobs, M.C. and Rycroft, R.J.G.	1992	Contact dermatitis and asthma from sodium metabisulphite in a photographic technician. Contact Dermatitis. 33, 65-66 Not GLP / Published	No	Published
8.12.6_07 Contact dermatitis	Sodium metabisulphitesulfite	Levanti, C., Ricciardi, L., Isola, I., Cilia, M., Guarneri, F., Purello D'Ambrosio, F.	1996	Burning Mouth Syndrome: Hypersensitivity to Sodium Metabisulphite. Acta dermato-venereologica. 1996; 76 (2): 158-159. Not GLP / Published	No	Published
8.12.6_08 Contact dermatitis	Sodium metabisulfite	Lee, A., Nixon, R.	2001	Contact dermatitis from sodium metabisulphite in a baker. Contact Dermatitis: 2001: 44: 127 Not GLP / Published	No	Published
8.12.6_09 Contact dermatitis	Sodium metabisulfite	Nair, B. and Elmore, A.R.	2003	Final Report on the Safety Assessment of Sodium Sulphite, Potassium Sulphite, Ammonium Sulphite, Sodium Bisulphite, Ammonium Bisulphite, Sodium Metabisulphite and Potassium Metabisulfit. International Journal of Toxicology 2003,	No	Published

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				Vol. 22: 63-88. Not GLP / Published		
8.12.6_10 Contact dermatitis	Sodium metabisulfite	Riemersma, W.A., Schuttelaar, M.L.A., Coenraads, P.J.	2004	Type IV hypersensitivity to sodium metabisulphite in local anaesthetic. Contact Dermatitis 2004: 51: 148–158. Not GLP / Published	No	Published
8.12.6_11 Contact dermatitis	Sodium metabisulfite	Huang, P-Y., Ch, C-Y.	2007	Allergic contact dermatitis due to sodium metabisulphite in a bleaching cream. Contact Dermatitis, 2007: 56: 123-124. Not GLP / Published	No	Published
8.12.6_12 Contact dermatitis	Sodium metabisulfite	Madan, V., Walker, S.L., Beck, M.H.	2007	Sodium metabisulphite allergy is common but is it relevant? Contact Dermatitis: 2007: 57: pp. 173-176. Not GLP / Published	No	Published
8.12.6_13 Contact dermatitis	Sodium metabisulfite	Malik, M.M., Hegarty, M.A., Bourke, J.F.	2007	Sodium metabisulphite -a marker for cosmetic allergy? Contact Dermatitis: 2007: 56: 241-242. Not GLP / Published	No	Published
8.12.6_14 Contact dermatitis	Sodium metabisulfite	Madan, V., Beck, M.H.	2009	Sodium metabisulphite - a contact allergen? Contact Dermatitis: 2009: 61: 58 Not GLP / Published	No	Published
8.12.6_15 Contact dermatitis	Sodium metabisulfite	Rowe, R.C., Sheykey, P.J., Quinn, M.E.	2009	Handbook of Pharmaceutical Excipients, 6. Edition, published by the Pharmaceutical Press and the American Pharmacists Association, ISBN 978 0 85369 792 3 (UK), ISBN 978 1 58212 135 2 (USA) Not GLP / Published	No	Published
8.12.6_16 Contact dermatitis	Sodium metabisulfite	Sasseville, D., El-Helou, T.	2009	Occupational allergic contact dermatitis from sodium metabisulphite. Contact Dermatitis 2009: 61: 244–245 Not GLP / Published	No	Published
8.12.6_17 Contact dermatitis	Sodium metabisulfite	Aalto-Korte, K., Suuronen, K., Alanko, K.	2009	Sodium metabisulphite -a contact allergen? Contact Dermatitis: 2009; 60: 115-117. Not GLP / Published	No	Published
8.12.6_18 Contact dermatitis	Sodium metabisulfite	Kaaman, A.-C., Boman, A., Wrangsjö, K., Matura, M.	2010	Contact allergy to sodium metabisulphite: an occupational problem. Contact Dermatitis 2010: 63: 110–112 Not GLP / Published	No	Published
8.12.6_19 Contact dermatitis	Sodium metabisulfite	Davies, R.F., Johnston, G.A.	2011	New and emerging cosmetic allergens. Clinics in Dermatology (2011) 29, 311-315. Not GLP / Published	No	Published
8.12.6_20 Contact dermatitis	Sodium metabisulfite	Febriana, S.A., Jungbauer, F., Soebono, H.,	2012	Occupational allergic contact dermatitis and patch test results of leather workers at two Indonesian tanneries. Not GLP / Published	No	Published

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		Coenraads, P.J.				
8.12.6_20 Contact dermatitis (Attachment of figure)	Sodium metabisulfite	Febriana, S.A., Jungbauer, F., Soebono, H., Coenraads, P.J.	2012	Attachment of figure as contained in reference above for section 8.12.6_20. Not GLP / Published	No	Published
8.12.6_21 Contact dermatitis	Sodium metabisulfite	Garcia-Gavin, J., Parente, J., Goossens, A.	2012	Allergic contact dermatitis caused by sodium metabisulphite: a challenging allergen. A case series and literature review. Contact Dermatitis: 67, pp. 260-264; © 2012 John Wiley & Sons A/S. Not GLP / Published	No	Published
8.12.6_21 Contact dermatitis (Attachment of tables)	Sodium metabisulfite	Garcia-Gavin, J., Parente, J., Goossens, A.	2012	Attachment of tables as contained in reference above for section 8.12.6_21. Not GLP / Published	No	Published
8.12.6_22 Contact dermatitis	Sodium metabisulfite	Oliphant, T., Mitra, A., Wilkinson, M.	2012	Contact allergy to sodium sulphite and its relationship to sodium metabisulphite. Contact Dermatitis, 66, 128-130// © 2012 John Wiley & Sons A/S. Not GLP / Published	No	Published
8.12.6_23 Contact dermatitis	Sodium metabisulfite	Roberts, D.W., Basketter, D., Kimber, I., White, J., Fadden, J.Mc., White, I.R.	2012	Sodium metabisulphite as a contact allergen - an example of a rare chemical mechanism for protein modification. Contact Dermatitis, 66: 123-127. Not GLP / Published	No	Published
8.12.6_23 Contact dermatitis (Attachment of scheme)	Sodium metabisulfite	Roberts, D.W., Basketter, D., Kimber, I., White, J., Fadden, J.Mc., White, I.R.	2012	Attachment of scheme as contained in reference above for section 8.12.6_23. Not GLP / Published	No	Published
8.12.6_23 Contact dermatitis (Illustration of scheme)	Sodium metabisulfite	Roberts, D.W., Basketter, D., Kimber, I., White, J., Fadden, J.Mc., White, I.R.	2012	Attachment of illustrative scheme as contained in reference above for section 8.12.6_23. Not GLP / Published	No	Published
8.12.6_24 Respiratory	Sodium metabisulfite	Wüthrich, B. and T. Huwyler	1989	Das Disulfit-Asthma. Schweiz. Med. Wschr., 119, 1177-1188. Not GLP / Published	No	Published

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sensitisation						
8.12.6_25 Respiratory sensitisation	Sodium metabisulfite	Wright, W., Zhang, Y.G., Salome, C.M., Woolcock, A.J.	1990	Effect of Inhaled Preservatives on Asthmatic Subjects. I Sodium metabisulphite. The American Review of Respiratory Disease: 1990; 141 (6): 1400-1404. Not GLP / Published	No	Published
8.12.6_26 Respiratory sensitisation	Sodium metabisulfite	Valero, A.L., Bescos, M., Amat, P. and Malet, A.	1993	Bronchial asthma caused by occupational sulphite exposure. Allergol. Immunopathol. (Madr). 21(6), 221-4 Not GLP / Published	No	Published
8.12.6_27 Respiratory sensitisation	Sodium metabisulfite	Field, P.I., McClean, M., Simmul, R., Berend, N.	1994	Comparison of sulphur dioxide and metabisulphite airway reactivity in subjects with asthma. Thorax 1994; 49: 250-256. Not GLP / Published	No	Published
8.12.6_28 Respiratory sensitisation	Sodium metabisulfite	Gastaminza, G., Qujrce, S., Torres, M., Tabar, A., Echechipia, S., Munoz, D., Fernandez de Corres, L.	1995	Pickled onion-induced asthma: a model of sulphite-sensitive asthma? Clinical and Experimental Allergy, 1995, Volume 25, pages 698-703. Not GLP / Published	No	Published
8.12.6_29 Respiratory sensitisation	Sodium metabisulfite	Nannini, L.J., Hofer, D.	1997	Effect of Inhaled Magnesium Sulphate on Sodium Metabisulphite-lnduced Bronchoconstriction in Asthma. Chest / 111 / 4 / APRIL, 1997. Not GLP / Published	No	Published
8.12.6_30 Respiratory sensitisation	Sodium metabisulfite	Nicol, G.M., Nix, A., Chung, K.F., Barnes, P.J.	1989	Characterisation of bronchoconstrictor responses to sodium metabisulphite aerosol in atopic subjects with and without asthma. Thorax 1989;44:1009-1014. Not GLP / Published	No	Published
8.12.6_31 Respiratory sensitisation	Sodium metabisulfite	Pavord, I.D., Wisniewski, A., Tattersfield, A.E.	1994	Refractoriness to inhaled sodium metabisulphite in subjects with mild asthma. Eur Respir J, 1994, 7, 50–54. Not GLP / Published	No	Published
8.12.6_32 Respiratory sensitisation	Sodium metabisulfite	Pavord, I., Lazarowicz, H., Inchley, D., Baldwin, D., Knox, A., Tattersfiel, A.	1994	Cross refractoriness between sodium metabisulphite and exercise induced asthma. Thorax 1994; 49: 245-249. Not GLP / Published	No	Published
8.12.6_33 Respiratory sensitisation	Sodium metabisulfite	Van Schoor, J., Joos, G.F.,	2000	Indirect bronchial hyperresponsiveness in asthma: mechanisms, pharmacology and implications for clinical research. Not GLP / Published	No	Published

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		Pauwels, R.A.				
8.12.6_34 Respiratory sensitisation	Sodium metabisulfite	Merget, R. and Korn, M.	2005	CASE STUDY - Metabisulphite-induced occupational asthma in a radiographer. Eur Respir J 2005; 25: 386–388 Not GLP / Published	No	Published
8.12.6_35 Respiratory sensitisation	Sodium metabisulphite	Steiner, M, Scaife, A., Semple, S., Hulks, G., Ayres, J.G.	2008	Sodium metabisulphite induced airways disease in the fishing and fish-processing industry. Occupational Medicine 2008; 58: 545–550. Not GLP / Published	No	Published
8.12.6_36 Respiratory sensitisation	Sodium metabisulfite	Pougnet, R., Loddé, B., Lucas, D., Jégaden, D., Bell, S. Dewitte, J.-D.	2010	CASE STUDY - A case of occupational asthma from metabisulphite in a fisherman. Int Marit Health, 2010; 61, 3: 180–184 Not GLP / Published	No	Published
8.12.6_37 Respiratory sensitisation	Sodium metabisulfite	Uriarte , S.A., Fernández-Nieto, M., Arochena, L., Sastre. J.	2015	Occupational Asthma in Seafood Manufacturing and Food Allergy to Seafood. Journal of Investigational Allergology & Clinical Immunology: 2015; Vol. 25(1): 59-60. Not GLP / Published	No	Published
8.12.6_38 Accidents and systemic action	Sodium metabisulfite	Atkinson, D.A., Sim, T.C. and J.A. Grant	1993	Sodium metabisulphite and SO2 release: An under-recognized hazard among shrimp fishermen. Annals of Allergy, Vol. 71 (December 1993), 563-566. Not GLP / Published	No	Published
8.12.6_39 Accidents and systemic action	Sodium metabisulfite	Kounis, N.G., Mazarakis, A., Almpanis, G., Gkouias, K., Kounis, G.N., Tsigkas, G.	2014	The more allergens an atopic patient is exposed to, the easier and quicker anaphylactic shock and Kounis syndrome appear: Clinical and therapeutic paradoxes. J Nat Sci Biol Med. 2014 Jul-Dec; 5(2): 240–244. Not GLP / Published	No	Published
8.12.7_03 Accidents	Sulfur dioxide	Delohery, J. et al.	1984	The relationship of inhaled sulphur dioxide reactivity to ingested metabisulphite sensitivity in patients with asthma. Am. Rev. Respir. Dis. 130: 1027-1030 Not GLP / Published	No	Published
8.12.7_04 Accidents	Sodium metabisulfite	Atkinson, D.A., Sim, T.C. and J.A. Grant	1993	Sodium metabisulphite and SO2 release: An under-recognized hazard among shrimp fishermen. Annals of Allergy, Vol. 71 (December 1993), 563-566. Not GLP / Published	No	Published

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8.12.7_05 Accidents	Sodium metabisulfite	Kounis, N.G., Mazarakis, A., Almpanis, G., Gkouias, K., Kounis, G.N., Tsigkas, G.	2014	The more allergens an atopic patient is exposed to, the easier and quicker anaphylactic shock and Kounis syndrome appear: Clinical and therapeutic paradoxes. J Nat Sci Biol Med. 2014 Jul-Dec; 5(2): 240–244. Not GLP / Published	No	Published
8.12.7_06 Accidents	Sodium metabisulfite	Cussans, A., McFadden, J. and L. Ostlere	2015	Systemic sodium metabisulphite allergy. Contact Dermatitis, Contact Points, pp. 1-2. Not GLP / Published	No	Published
8.13.2_1	Sulfur dioxide	Yargiçoğlu P1, Ağar A, Gümüşlü S, Bilmen S, Oğuz Y.	1999	Age-related alterations in antioxidant enzymes, lipid peroxide levels, and somatosensory-evoked potentials: effect of sulphur dioxide. Arch Environ Contam Toxicol 27:554-60 Not GLP / Published	No	Published
8.13.2_2	Sulfur dioxide	Yun Y, Yao G, Yue H, Guo L, Qin G, Li G, Sang N	2013	O(2) inhalation causes synaptic injury in rat hippocampus via its derivatives in vivo Chemosphere 93: 2426-32, Not GLP / Published	No	Published
8.13.2_3	Sulfur dioxide	Qin G, Wang J, Huo Y, Yan H, Jiang C, Zhou J, Wang X, Sang N	2012	Sulphur dioxide inhalation stimulated mitochondrial biogenesis in rat brains. Toxicology 2012 (200): 67-74 Not GLP / Published	No	Published
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12 Annex of studies on health hazards

Data relating to Table 11: Summary table of human data on respiratory sensitisation

Hein et al. 1996 Pneumologie 50/6: 394-8

39 % of patients with a history of sulfite-sensitive asthma showed significant broncho-constriction after ingestion of metabisulfite (PD_{20} $FEV_{1:}$ 34±56 mg; min: 5, max: 200 mg; n=17); specificity: 100 %, sensitivity: ca. 40 %

Onset of SMB reaction minimal 60, maximal 210 min, average 150 min.

Table 2. Age, sex distribution, atopia and smoking status, percentage of vital capacity (%FVC) and 1- second-capacity (%FEV_1) as well as PC_{20} Histamine and PC_{20} MBS for healthy control subjects and patients with bronchial asthma after a history of a sulfite asthma or after a response to the oral metabisulfite test.

	Control proband		Asthma bronchiale	
			Sulfite anamnesis	Oral metabisulfite test

	No atopia	Atopia	Negative	Positive	Negative	Positive
n	4	4	27	44	27	17
Gender (female/male)	3/1	2/2	16/11	30/14	14/13	16/1
Atopia (y/n)	0/4	4/0	19/8	24/20	14/3	10/7
Smoker (y/n)	0/4	4/0	11/1	3/41	1/26	2/15
%FVC	98±7	105±5	88±13	92±20	91±24	93±13
$\%FEV_1$	98±7	103±6	83±18	86±18	90±17	79±19
PC_{20}Hist. (mg/ml)	>8	>8	1±1.1	1.3±1.8	1.7±1.9	0.3±0.2
PC_{20}SBM (mg)	>390	>390	>390		>390	34±56

Delohery et al. 1984 Am Rev Respir Dis; 130:1027-32

% fall after MB: Group 1: 35±14; group 2: 6±6; group 3: 5±3

Pc20 SO_2 (ppmV): group 1: 1.19±0.78 (0.5 – 2.9); group 2: 2.3±1.42; group 3: >5; Pc20 SO_2 does not correlate with MB PEFR fall. Asthmatics whose asthma is provokes by ingestion of acid MB solutions, are not supersensitive to inhaled SO_2 gas

SO_2 sensitivity does not correlate with histamine reactivity.

TABLE 1

SUBJECT DETAILS AND RESULTS

Subgroup Group	No.	Age	& Sex	Baseline PEFR	% Fall PEFR Citrate	% Fall PEFR MB	% Fall PEFR Control Gas	Pc20 SO_2 *ppm*	Pc20 Histamine *mg/ml*
A	1	25	M	500	14	24	15	0.75	0.15
	2	21	M	480	4	4	13	4	0.051
	3	20	M	590	5	5	2	> 5	3.5
B	1	26	M	500	0	60	2	1	0.08
	2	24	M	390	0	1	9	1.6	0.18
	3	22	M	630	1	8	3	> 5	> 8.0
C	1	18	F	430	18	43	3	0.54	0.15
	2	22	F	290	10	7	4	2.8	0.04
	3	22	F	475	9	11	8	> 5	> 8.0
D	1	26	F	430	0	37	10	2.9	0.10
	2	31	F	390	0	18	10	2.4	0.14
	3	27	F	400	5	3	5	> 5	4.8
E	1	29	F	345	15	62	0	2	0.023
	2	33	F	395	15	14	4	1.85	0.18
	3	27	F	580	2	5.2	5	> 5	6.5
F	1	38	F	380	1	29	3	1.2	0.05
	2	40	F	240	8	8	3	0.6	0.07
	3	41	F	450	6	2	1	> 5	> 8.0
G	1	44	F	300	14	23	19	0.64	0.03
	2	40	F	270	15	2	17	0.5	0.05
	3	48	F	390	5	0	8	> 5	7.2
H	1	57	F	180	8	22	9	0.67	0.03
	2	69	F	385	14	7	1	1.75	0.5
	3	56	F	355	3	3	6	> 5	2.5
I	1	14	F	340	9	53	14	0.5	0.029
	2	22	F	190	7	0	0	1.9	0.015
	3	22	F	440	11	11	7	> 5	3.8
J	1	24	F	360	10	33	3	1.7	0.10
	2	19	F	400	7	0	3	> 5	0.7
	3	22	F	460	4	9	2	> 5	> 8.0

Definition of abbreviations: Baseline PEFR = baseline PEFR on day 1 prior to MB challenge; PEFR = peak expiratory flow rate; citrate = ingested challenge study with 30 mg control 0.5% citric acid solution; MB = ingested challenge study with 50 mg metabisulfite in 30 ml 0.5% citric acid; control gas = humidified air control gas for inhaled SO_2 gas studies.

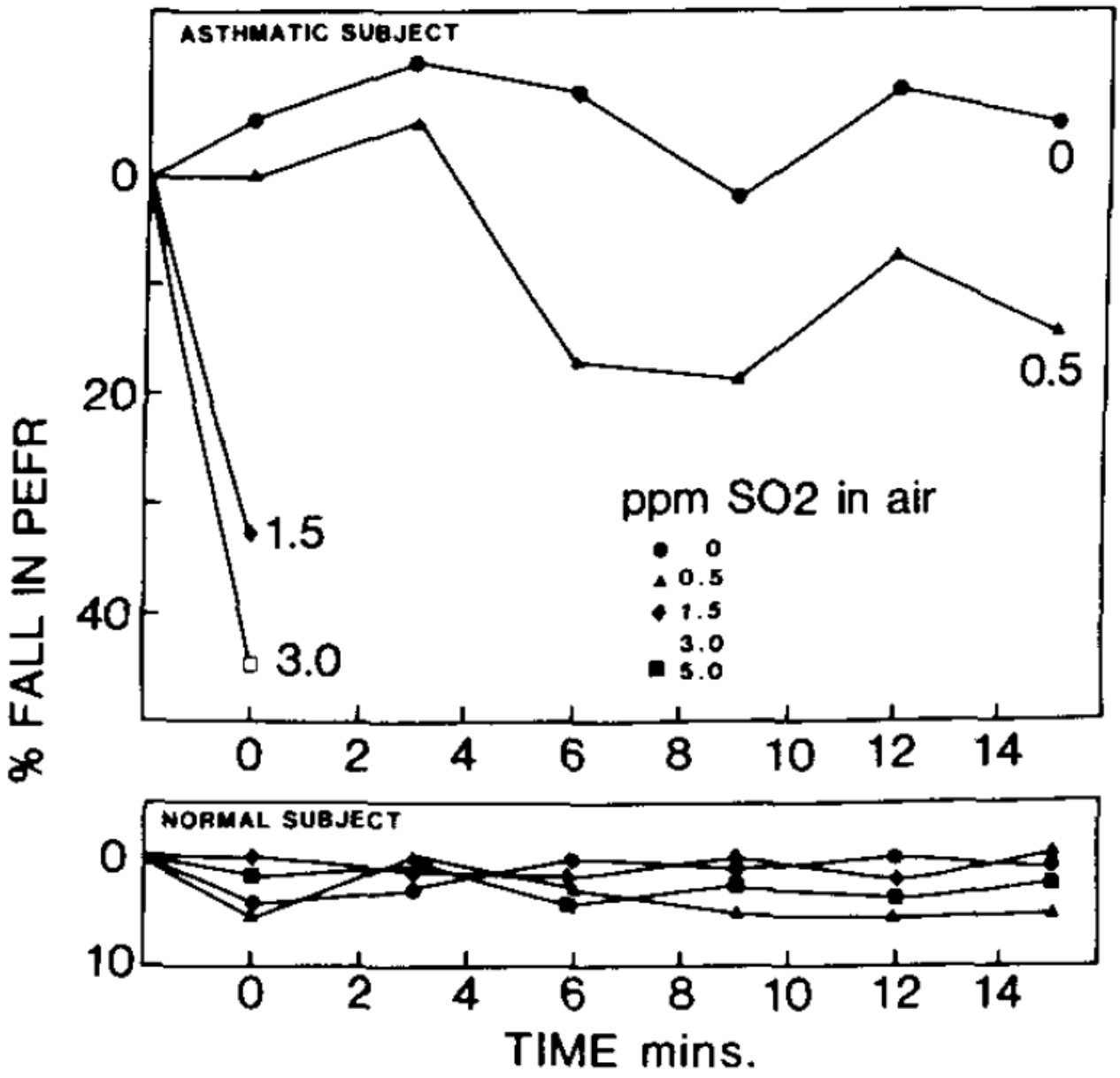

Fig. 4. Typical responses of an asthmatic subject (*upper panel*) and a normal control subject (*lower panel*) to inhalation of the SO_2 gas mixture. The asthmatic subject shows a dose-response relationship with increasing SO_2 gas concentration producing a progressive fall in PEFR.

Schwarz and Chester 1984; J Allergy Clin Immunol. 74:511-3

Bronchospastic response at 1.2 ppm

Aerosol challenge 2/8 negative; 3/8 positive at 0.5 mg/mL, and 5.0 mg/mL, respectively

3/8 positive reactors to 0.5 mg/mL aerosol reacted at 10, 25, 50 mg oral SMB, respectively.
All patients negative in prick tests.

TABLE I. Responses of the study patients to oral metabisulfite challenge

	Dose for positive response*				
	5 mg	10 mg	25 mg	50 mg	Total
Number positive	0	2	1	3	7
Number tested	8	8	6	5	8

*A positive response is defined as having a 50% or higher change in measurement of specific airway resistance.

TABLE II. Aerosol results and characteristics of the asthmatic patients studied*

	Sex		Dose for positive responses				
	Male	Female	0.05 mg/ml	0.5 mg/ml	5.0 mg/ml	Atopic†	Nonatopic
Positive reactors	2	4	0	3	3	5	1
Negative reactors	0	2	0	0	0	1	1

*A positive response is defined as having a 50% or higher change in measurements of specific airway resistance.
†Atopy was defined as the presence of a significant history of allergic disease and immediate wheal-and-flare skin test reactions to standard inhalant allergens.

Data relating to Table 13: Summary table of human data on skin sensitisation

Garcia-Gavin et al. 2012; Contact Dermatitis 67:260-9

124 (4.5 %) positive results (77F/47M), most frequently on the face and the hands, median age: 50; 13 cases (10.5 %) occupational exposure.

Table 1. MOAHLFAs of the overall group of patients tested from 1 January 1990 to 14 October 2010 (n = 12 746), of the patients tested with sodium metabisulfite (SMS) (n = 2763), and of the sodium metabisulfite-positive patients (n = 124)

	SMS-positive patients (n = 124)	SMS-tested patients (n = 2763)	Overall sample (n = 12 746)
Male (%)	37.4	19.6	34.2
Occupational (%)	10.5	15.7	16.8
Atopy (%)	18	20	21.5
Hand (%)	24.2	29.1	38.3
Leg (%)	10.5	7.6	3.5
Face (%)	40.3	51.3	34.5
Age > 40 years (%)	61.3	54.6	47.6

Oliphant et al. 2012 Contact Dermatitis 66/3:128-30

183 patients tested: 5.5 % (n=10) positive to sodium metabisulfite, 3.8 % (n=7) positive to sodium sulfite.

Table 1. Patients with positive patch test reactions to sodium sulfite (SS) and sodium metabisulfite (SMB)

Age (years)	Sex	Site	SMB	Relevance	Comment	SS	Relevance	Comment
25	M	Lips	+/++	?R	?Food preservative	?+/+	?R	?Food preservative
45	M	Generalized	–/++	NR	–	–/?+	NR	–
33	M	Hands	?+/++	PR	Medicament	–/+	XR	–
23	F	Perioral	–/+	?R	?Food preservative	–/–	–	–
42	F	Perioral	+/+	?R	?Food preservative	–/–	–	–
36	M	Lips	–/+	?R	?Food preservative	–/–	–	–
13	F	Face	–/+	NR	–	–/–	–	–
51	F	Hands	–/+	?R	Medicament	–/+	XR	–
92	F	Legs	+/+	PR	Medicament	–/+	XR	–
62	M	Legs	–/+	PR	Medicament	–/+	XR	–
34	M	Scalp	–/–	–	–	+/++	CR	Hair dye

CR, current relevance; F, female; M, male; NR, not relevant; PR, past relevance; ?R, possible relevance; XR, cross-reaction.
First/second readings according to International Contact Dermatitis Research Group criteria.

Madan, V., Walker, S.L., Beck, M.H. 2007, Contact Dermatitis; 57:173-6.

71 (4.1 %) positive reactions, interpreted as allergic. 33/71 with identifiable source (group A), 38 with unknown sources (group B). 47 cases with known sources after reanalysis (3 %).

Sensitization to sodim metabisulfite from parenteral solutions and occupational exposure from food handling may account for some of the otherwise unexplained positive patch test reactions.

Table 4. Relevant occupational/recreational exposure

Occupation	Relevant group (A) (+additional exposure)	Unexplained relevance group (B)
Baker	1 + Timodine	2
Catering	3	1
Chemical processing	1	1
Rubber manufacturing	1 + Trimovate and Timodine	0
Swimming pool	1	0
Brewer (wine)	1 + Trimovate	1
False tan	1	1
Exposure to local anaesthetic solutions (dentists, nurses, etc.)	0	5
Photographic chemical handling	0	0

Data relating to Table 14: Summary table of mutagenicity/genotoxicity tests *in vitro*

Pagano and Zeiger (1987). Mutation Research 179: 159-166

Positive – slight but dose-related increase in # of revertants – increase < 2-fold, with 60 min incubation, >2-fold after 90 or 120 min incubation)

Reproducible weak mutagenic response in *S. typhimurium* strains carrying the *his* D6610 or *his*G46 mutations.

Peak mutagenic response in G46 stains at 0.1 M and in TR3243 at 0.3 M.

Number of induced revertants per dose, the ***his*D6610** site was most responsive, with **TA 97** being the most active.

Mutagenic response highest with 0.1 M sodium phosphate buffer at pH 5.0-6.0.

Base-pair substitution and frameshift mutations

Base-pair substitution (deamination of cytosine):

At higher concentrations (1 M): cytosin bisulfite adducts leading to base substitution

At lower concentrations (approx. 0.01 M) deamination of cytosine via oxidative damage assumed.

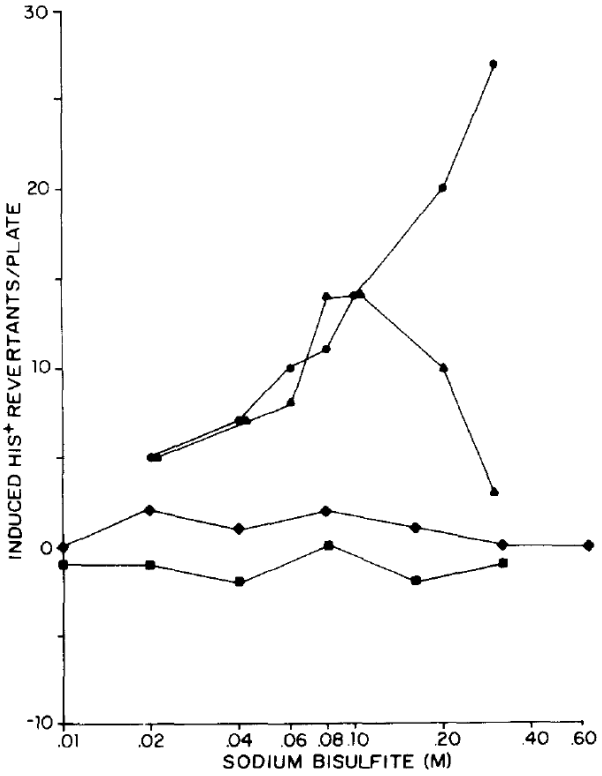

Fig. 1. The mutagenicity of bisulfite in *S. typhimurium* TR3243 (●), G46 (▲), D3052 (◆) and C3076 (■).

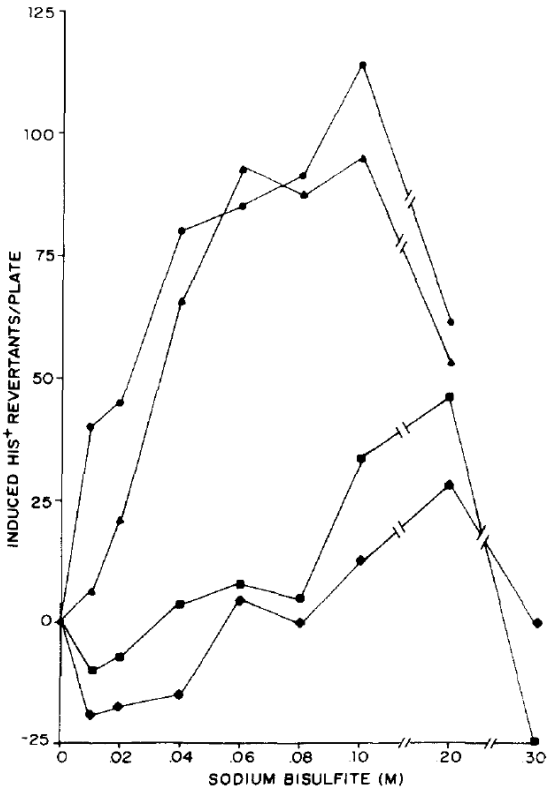

Fig. 2. The effect of pH on the mutagenicity of sodium bisulfite in *S. typhimurium* strain TA97. ●, pH 5.0; ▲, pH 6.0; ■, pH 7.0; ◆, pH 8.0.

TABLE 2

THE MUTAGENICITY OF SODIUM BISULFITE IN DIFFERENT GENETIC BACKGROUNDS OF *Salmonella typhimurium*

(a) *his*D6610 mutation

Dose (M)	TA88	TA110	TA90	TA97
0	40 ± 3 [a]	61 ± 6	34 ± 8	132 ± 14
0.01	46 ± 4 (6)	– [b]	–	161 ± 26 (29)
0.02	61 ± 10 (21)	86 ± 10 (25)	33 ± 7 (−1)	180 ± 12 (48)
0.04	72 ± 24 (32)	101 ± 11 (40)	37 ± 6 (3)	204 ± 11 (72)
0.06	96 ± 11 (56)	108 ± 12 (47)	38 ± 2 (4)	234 ± 16 (102)
0.08	94 ± 12 (54)	123 ± 9 (62)	37 ± 8 (3)	226 ± 9 (94)
0.10	71 ± 16 (32)	110 ± 11 (49)	28 ± 5 (−6)	194 ± 14 (62)
0.20	69 ± 8 (29)	–	8 ± 4 (−26)	135 ± 8 (3)
0.30	53 ± 4 (13)	60 ± 7 (−1)	2 ± 1 (−32)	54 ± 8 (−78)

(b) *his*G46 mutation

Dose (M)	SB2802	TA92	TA1535	TA100
0	3 ± 1	25 ± 2	12 ± 2	141 ± 12
0.01	–	–	–	–
0.02	6 ± 2 (3)	34 ± 5 (9)	9 ± 3 (−3)	117 ± 7 (−24)
0.04	11 ± 4 (8)	36 ± 1 (11)	10 ± 2 (−2)	–
0.06	16 ± 5 (13)	38 ± 6 (13)	9 ± 5 (−3)	117 ± 7 (−24)
0.08	15 ± 2 (12)	41 ± 4 (16)	12 ± 2 (0)	–
0.10	15 ± 3 (12)	50 ± 5 (25)	17 ± 5 (5)	116 ± 8 (−25)
0.20	16 ± 3 (13)	51 ± 4 (26)	15 ± 3 (3)	–
0.30	16 ± 4 (13)	40 ± 2 (15)	4 ± 1 (−8)	0

[a] Mean his^+ revertants per plate ± S.D. (induced revertants); triplicate or quadruplicate plates.
[b] Not done.

Anonymous15 2008; Environ Mol Mutagen 49: 276-281

Positive

Reduction in MI to 56 – 60 – 45 – 42 % (for concentrations: 25 – 50 – 100 – 200 µg/mL) of concurrent negative control, positive control: MI: 43 % of neg. control; OECD 473 for PBLs: MI reduction to 45±5 % of controls

TABLE I. The Structural, Numerical, Total Chromosome Aberrations (CAs), Percentage of Abnormal Cells and Mitotic Index (MI) in Cultured Human Lymphocytes Treated With PMB

Test substance	Treatment: Period (hr)	Treatment: Concentration (μg/ml)	Chromosome aberrations: Structural CA[a] B′ type	Structural CA[a] B″ type	Numerical CA	Structural CA/cell ± SE	Numerical CA/cell ± SE	Total CA/Cell ± SE	Percentage of abnormal cell ± SE	MI ± SE (%)
Control	—	—	9	1	0	0.025 ± 0.006	0.00	0.025 ± 0.006	2.50 ± 0.64	10.2 ± 0.90
BrdU	—	—	9	5	0	0.035 ± 0.008	0.00	0.035 ± 0.008	3.50 ± 0.86	9.00 ± 0.67
EMS	24	125	81	18	3	0.247 ± 0.038 a_2b_1	0.007 ± 0.004	0.255 ± 0.033 a_2b_2	20.00 ± 2.17 a_2b_2	4.42 ± 0.77 a_2b_2
PMB	24	25	28	14	1	0.105 ± 0.013 $a_2b_1c_2$	0.002 ± 0.002	0.107 ± 0.015 $a_1b_1c_2$	10.00 ± 1.41 $a_1b_1c_2$	5.55 ± 0.43 a_2b_2
		50	45	3	1	0.120 ± 0.007 $a_3b_3c_3$	0.002 ± 0.002	0.122 ± 0.009 $a_2b_2c_3$	11.00 ± 0.91 $a_2b_2c_2$	6.13 ± 0.57 a_2b_1
		100	41	9	3	0.125 ± 0.016 $a_2b_1c_2$	0.007 ± 0.004	0.132 ± 0.018 $a_1b_1c_2$	12.25 ± 01.65 $a_2b_1c_2$	4.63 ± 0.42 a_3b_2
		200	60	4	2	0.160 ± 0.026 $a_1b_1c_1$	0.005 ± 0.002	0.165 ± 0.023 $a_2b_1c_1$	14.50 ± 2.10 a_1b_1	4.32 ± 0.62 a_2b_2
EMS	48	125	73	18	2	0.227 ± 0.047 a_1b_1	0.005 ± 0.005	0.232 ± 0.046 a_1b_1	20.25 ± 3.35 a_1b_1	4.54 ± 0.41 a_3b_2
PMB	48	25	39	6	2	0.112 ± 0.008 $a_2b_2c_3$	0.005 ± 0.002	0.117 ± 0.011 $a_2b_2c_2$	11.25 ± 1.03 $a_2b_2c_2$	7.18 ± 0.41 $a_2b_1c_2$
		50	28	11	5	0.097 ± 0.008 $a_2b_2c_3$	0.012 ± 0.009	0.110 ± 0.009 $a_2b_2c_3$	10.25 ± 0.85 $a_2b_2c_3$	6.78 ± 0.47 $a_2b_1c_1$
		100	40	11	2	0.127 ± 0.002 $a_3b_3c_3$	0.005 ± 0.002	0.132 ± 0.002 $a_3b_3c_3$	12.50 ± 0.28 $a_3b_3c_3$	5.58 ± 0.83 a_1b_2
		200	90	9	5	0.247 ± 0.042 a_1b_1	0.012 ± 0.002 a_1b_1	0.260 ± 0.044 a_1b_1	21.00 ± 2.91 a_2b_1	3.23 ± 0.06 $a_3b_2c_3$

a, significant from control; b, significant from BrdU control, c, significant from positive control (EMS); $a_1b_1c_1$, $P < 0.05$; $a_2b_2c_2$, $P < 0.01$; $a_3b_3c_3$, $P < 0.001$.
[a]B′, chromatid-type breaks; B″, chromosome-type breaks.

TABLE III. The Percentage of Micronucleus (MN), the Percentage of Micronucleated Binuclear Cell and Nuclear Division Index (NDI) in Cultured Human Lymphocytes Treated With PMB

Test substance	Treatment: Periods (hr)	Treatment: Concentration (μg/ml)	MN ± SE (%)	Percentage of micronucleated binuclear cell ± SE	NDI ± SE
Control	—	0	0.56 ± 0.02	0.55 ± 0.03	1.51 ± 0.07
EMS	24	125	1.48 ± 0.15 a_2	1.32 ± 0.12 a_2	1.36 ± 0.07
PMB	24	25	1.[illegible] ± 0.20 a_1	1.11 ± 0.11 a_1	1.48 ± 0.09
		50	1.[illegible] ± 0.12 a_2	1.17 ± 0.13 a_1	1.45 ± 0.08
		100	1.50 ± 0.14 a_2	1.18 ± 0.09 a_2	1.33 ± 0.08
		200	1.43 ± 0.17 a_1	1.21 ± 0.11 a_2	1.18 ± 0.05 a_2c_1
EMS	48	125	2.41 ± 0.16 a_2	2.22 ± 0.08 a_3	1.37 ± 0.05
PMB	48	25	1.63 ± 0.08 a_3c_2	1.33 ± 0.11 a_2c_2	1.61 ± 0.10
		50	1.63 ± 0.07 a_3c_2	1.46 ± 0.08 a_2c_2	1.49 ± 0.09
		100	1.60 ± 0.04 a_3c_3	1.46 ± 0.06 a_3c_3	1.25 ± 0.06 a_1
		200	1.71 ± 0.21 a_1c_1	1.68 ± 0.04 a_3c_3	1.06 ± 0.01 a_3c_3

a, significant from control; c, significant from positive control (EMS); a_1c_1, $P < 0.05$; a_2c_2, $P < 0.01$; a_3c_3, $P < 0.001$.

Slightly positive: Concentration dependent significant increase in SCE but not twice as high as controls

TABLE II. The Frequency of the Sister Chromatid Exchanges (SCE) and Replication Index (RI) in Cultured Human Lymphocytes Treated With PMB

	Treatment							
Test substance	Periods (hours)	Concentration (μg/ml)	Min–max SCE	SCE/cell ± SE	M1	M2	M3[a]	RI ± SE
BrdU Control	—	0	1–12	5.830 ± 0.43	43	96	261	2.55 ± 0.06
EMS	24	125	15–70	27.96 ± 2.95 b_2	69	147	184	2.29 ± 0.08
PMB	24	25	3–24	8.240 ± 0.46 b_1c_3	67	99	234	2.42 ± 0.18
		50	3–20	8.710 ± 0.59 b_1c_3	31	103	266	2.59 ± 0.04 c_2
		100	4–27	10.45 ± 0.63 b_2c_3	64	138	198	2.34 ± 0.08
		200	6–30	14.19 ± 0.74 b_2c_3	103	178	119	2.04 ± 0.12 b_1
EMS	48	125	18–46	31.18 ± 0.45 b_3	79	155	166	2.22 ± 0.08b_1
PMB	48	25	3–19	8.790 ± 0.56 b_1c_3	50	93	257	2.52 ± 0.02 c_3
		50	3–28	9.540 ± 0.32 b_3c_3	57	85	258	2.50 ± 0.05 c_1
		100	4–24	11.09 ± 0.27 b_3c_3	52	112	236	2.46 ± 0.06 c_1
		200	10–32	17.65 ± 1.13 b_2c_3	180	206	14	1.59 ± 0.02 b_3c_3

b, significant from BrdU control; c, significant from positive control (EMS); b_1c_1, $P < 0.05$; b_2c_2, $P < 0.01$; b_3c_3, $P < 0.001$.
[a]The number of the cells under first mitosis (M1), second mitosis (M2), and third mitosis (M3).

Data relating to Table 15: Summary table of *in vivo* genotoxicity studies

Anonymous11 (2005) Environmental and Molecular Mutagenesis. 46 (3): 150–155

Positive

Dose-dependent increase OTM from 14 mg/m^3 onwards in blood lymphocytes. Cells derived from brain, lung, liver, spleen, kidney, and intestine in both sexes and in testicles of males. No effects on food consumption and body weight

gain; no deaths, morbidity or distinctive clinical signs.

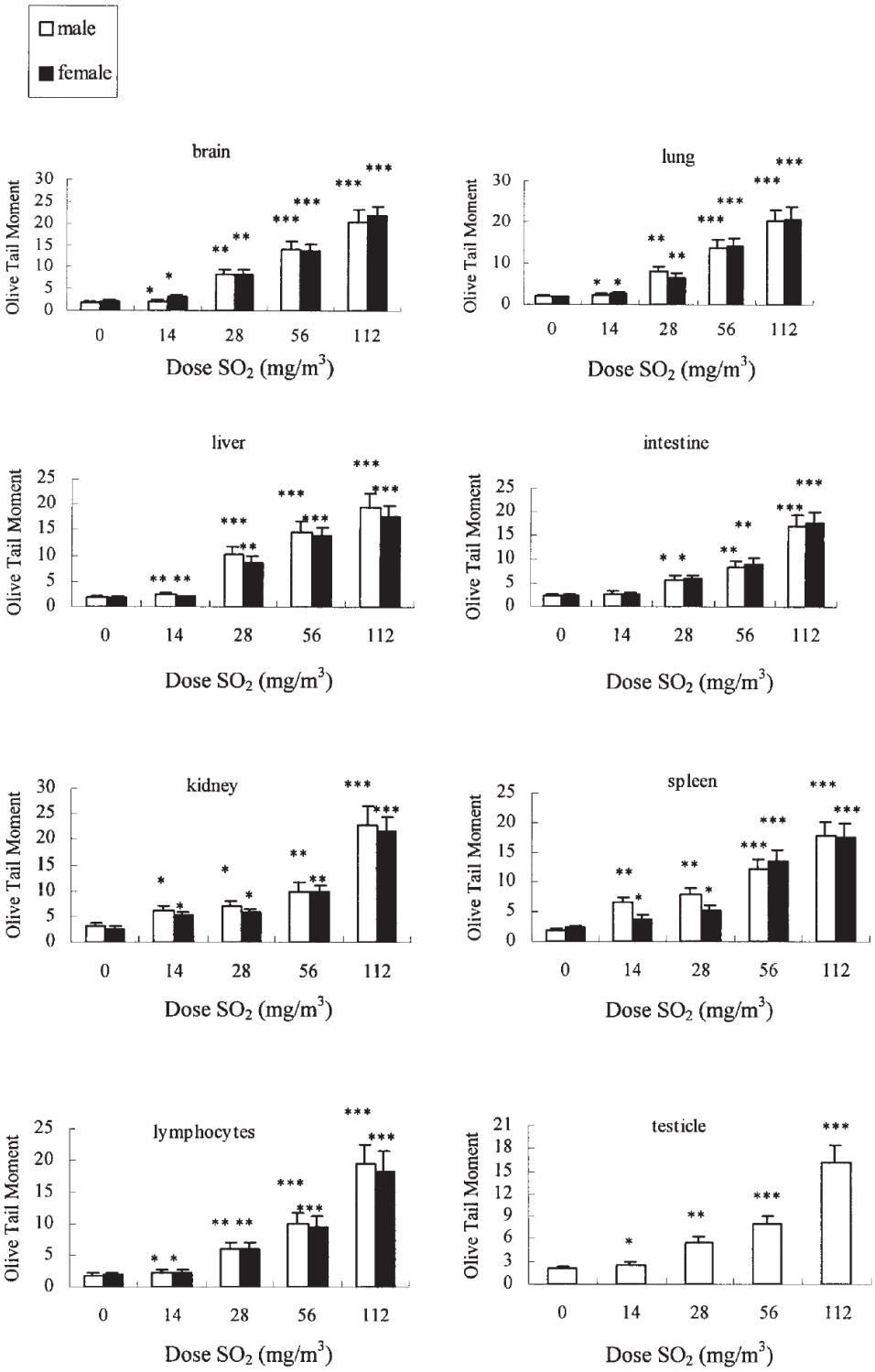

Fig. 1. DNA damage, expressed as OTM, in various organs of mice treated with SO_2 by inhalation. Untreated mice served as negative controls. Data are averages of 50 cells scored from each of six mice per group. Error bars denote SE. Treated mice different from control at $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Anonymous6 (2008) also published as: Anonymous7, (2010) Mutation Res. 697: 38–46; reference

Negative

The number of micronuclei not increased; however not proven that the substance reached the target organ. No signs of overall toxicity.

PCE:NCE ratio unchanged.

Table 3
Ratios of PCE:NCE and frequencies of micronucleated PCE (MNPCE) per 2000 PCE in the bone marrow of NMRI mice exposed to SO_2 or of their respective controls.

Treatment	Sex	PCE:NCE[a]	MNPCE/2000 PCE		% MNPCE[b]
			Number[a]	Range	
Clean air[c]	m	0.99 ± 0.062	2.8 ± 1.30	1–4	0.14
	f	0.90 ± 0.095	2.0 ± 0.71	1–3	0.10
CP, 60 mg/kg b.w.[d]	m	0.89 ± 0.070	70.6 ± 20.57**	43–101	3.53**
	f	0.92 ± 0.119	49.4 ± 8.41**	41–60	2.47**
SO_2, 1 ppm[c]	m	0.96 ± 0.049	2.3 ± 0.82	1–3	0.11
	f	0.99 ± 0.350	2.8 ± 1.94	1–6	0.14
SO_2, 3 ppm[c]	m	0.96 ± 0.024	2.5 ± 1.87	1–6	0.13
	f	0.90 ± 0.226	2.0 ± 1.26	1–4	0.10
SO_2, 10 ppm[c]	m	0.98 ± 0.022	2.7 ± 0.52	2–3	0.13
	f	0.98 ± 0.055	2.0 ± 1.26	1–4	0.10
SO_2, 30 ppm[c]	m	0.94 ± 0.690	3.2 ± 1.47	1–5	0.16
	f	0.95 ± 0.019	2.5 ± 1.22	1–4	0.12

CP, cyclophosphamide monohydrate; SO_2, sulfur dioxide; b.w., body weight; m, males; f, females.
[a] Group mean ± standard deviation.
[b] Derived from group mean.
[c] Whole-body inhalation, for 4 h/day on 7 consecutive days.
[d] Oral application.
** Significantly different from negative control animals (clean air), $P \leq 0.01$, *U*-test according to Mann–Whitney.

Meng et al., (2002) Inhalation Toxicity, 14: 303-309

Positive

Dose dependent increase in micronuclei in PCE, no sex differences. Increase statistically significant at 14 mg/m^3 SO_2 and higher.

TABLE 1. Induction effect of SO_2 on micronuclei in the PCE of mouse bone marrow

		Female		Male		Total	
SO_2 (mg/m^3) (X ± SD)	Number of PCE	MN (%)	PCE with MN (%)	MN (%)	PCE with MN (%)	MN (%)	PCE with MN (%)
Control	1000 × 8	0.11 ± 0.10	0.11 ± 0.10	0.13 ± 0.09	0.13 ± 0.09	0.12 ± 0.09	0.12 ± 0.09
14.0 ± 0.38	1000 × 8	0.40 ± 0.08[a]	0.40 ± 0.08[a]	0.39 ± 0.10[a]	0.39 ± 0.10[a]	0.39 ± 0.09[a]	0.39 ± 0.09[a]
28.0 ± 0.34	1000 × 8	0.83 ± 0.05[a]	0.80 ± 0.06[a]	0.83 ± 0.21[a]	0.78 ± 0.13[a]	0.83 ± 0.15[a]	0.79 ± 0.10[a]
56.0 ± 0.32	1000 × 8	1.59 ± 0.10[b]	1.45 ± 0.12[b]	1.38 ± 0.13[b]	1.26 ± 0.11[b]	1.48 ± 0.16[b]	1.36 ± 0.15[b]
84.0 ± 0.27	1000 × 8	1.93 ± 0.13[b]	1.73 ± 0.10[b]	1.73 ± 0.09[b]	1.60 ± 0.12[b]	1.83 ± 0.15[b]	1.69 ± 0.14[b]

[a]Significantly different from control without SO_2 by *t*-test at $p < .01$.
[b]Significantly different from control without SO_2 by *t*-test at $p < .001$.

TABLE 2. SO_2 and number of micronuclei per PCE of mouse bone marrow

		Female			Male		
SO_2 (mg/m^3) (X ± SD)	Number of PCE	PCE with monoMN (%)	PCE with biMN (%)	PCE with triMN (%)	PCE with monoMN (%)	PCE with biMN (%)	PCE with triMN (%)
Control	1000 × 8	0.11 ± 0.10	0	0	0.13 ± 0.09	0	0
14.0 ± 0.38	1000 × 8	0.40 ± 0.08[a]	0	0	0.39 ± 0.10[a]	0	0
28.0 ± 0.34	1000 × 8	0.77 ± 0.10[a]	0.03 ± 0.05	0	0.74 ± 0.11[a]	0.03 ± 0.05	0.01 ± 0.03
56.0 ± 0.32	1000 × 8	1.33 ± 0.15[b]	0.11 ± 0.06[a]	0.01 ± 0.03	1.15 ± 0.12[b]	0.11 ± 0.06[a]	0
84.0 ± 0.27	1000 × 8	1.63 ± 0.15[b]	0.15 ± 0.09[a]	0	1.48 ± 0.19[b]	0.13 ± 0.09[a]	0

Note. PCE with monoMN: PCE with one MN per cell; PCE with biMN: PCE with two MN per cell; PCE with triMN: PCE with three MN per cell.
[a]Significantly different from control without SO_2 by *t*-test at $p < .01$.
[b]Significantly different from control without SO_2 by *t*-test at $p < .001$.

Anonymous10 (2003) Inhalation Toxicity, 15: 1053-58

Positive

Significant increase in micronuclei (mono-, bi, and polymicronuclei) in PCE at 28 mg/m^3 compared to controls.

TABLE 3. Defense of seabuckthorn seed oil for MNs in the PCE of mice induced by SO_2

Group	Monomicronuclei (‰)	Bimicronuclei (‰)	Polymicronuclei (‰)	Rate of micronuclei (‰)	Rate of micronuclear cells (‰)
Group A	1.6 ± 1.14	0	0	1.6 ± 1.14	1.6 ± 1.14
Group B	12.33 ± 1.75***	1.83 ± 0.75***	0.5 ± 0.55*	17.5 ± 1.87***	14.67 ± 1.51***
Group C	1.50 ± 1.64$^{\#\#\#}$	0.17 ± 0.41$^{\#\#\#}$	0$^{\#}$	1.83 ± 2.14$^{\#\#\#}$	1.67 ± 1.86$^{\#\#\#}$
Group D	8.67 ± 1.75$^{***\#\#}$	0.83 ± 1.17	0.67 ± 0.82	12.33 ± 5.57$^{***\#\#}$	10.17 ± 3.19$^{***\#\#}$
Group E	8.17 ± 2.93$^{***\#\#}$	0.67 ± 0.52$^{*\#\#}$	0$^{\#}$	9.50 ± 3.27$^{***\#\#\#}$	8.83 ± 3.06$^{***\#\#}$
Group F	7.87 ± 2.14$^{***\#\#}$	0.67 ± 0.82$^{\#}$	0$^{\#}$	8.83 ± 2.59$^{***\#\#\#}$	8.50 ± 2.23$^{***\#\#\#}$
Group G	5.50 ± 1.05$^{***\#\#\#}$	0.67 ± 0.52$^{*\#\#}$	0$^{\#}$	6.83 ± 1.47$^{***\#\#\#}$	6.17 ± 1.17$^{***\#\#\#}$

Note. Group A is normal group without injection and toxicant and group B has only SO_2 inhalation. Using *t*-test and comparing with group A, significance is shown by $^{*}p < .05$; **, $p < .01$; $^{***}p < .001$. Comparing with group B, $^{\#}p < .05$; $^{\#\#}p < .01$; $^{\#\#\#}p < .001$.

Meng, Z. & Zhang, B. (2002). Mutagenesis 17: 215-217.

Positive

Dose and duration dependent increase in aberrant cells, dose dependent decrease of mitotic index in both sexes

Chromosome and chromatide breaks at 56 mg/m^3 SO_2; at lower concentrations chromatide breaks only sign. at ≥ 14 mg/m^3.

Table I. Effects of SO_2 inhalation on mitoses and CA formation in bone marrow cells of mice

Treatment	Gap[a]	CAs per 100 cells (mean ± SD)[b]		Aberrant cells (%) (mean ± SD)[c]	Mitotic index (%) (mean ± SD)[c]
		Chromatid break	Chromosome break		
Control	5	1.61 ± 0.15	0.20 ± 0.02	1.81 ± 0.45	3.85 ± 0.78
SO_2 (mg/m^3)					
7.00 ± 0.27	8	2.14 ± 0.28	0.20 ± 0.05	2.30 ± 0.56	3.05 ± 0.46
14.0 ± 0.38	10	3.12 ± 0.30[d]	0.25 ± 0.06	3.28 ± 0.66[d]	2.61 ± 0.52[d]
28.0 ± 0.34	13	3.96 ± 0.40[e]	0.32 ± 0.03[d]	4.26 ± 0.44[f]	2.48 ± 0.43[d]
56.0 ± 0.32	14	4.68 ± 0.36[f]	0.55 ± 0.05[e]	4.86 ± 0.45[f]	2.32 ± 0.44[d]

[a] Total chromatid and chromosome gaps at each dose were recorded but not included as CAs/cell.
[b]Number of chromatid or chromosome breaks per 100 metaphase cells scored per dose group.
[c]Results at each dose were compared to those of the control using Student's t-test. Results are from eight male and eight female animals (100 cells/animal).
[d,e,f]Significantly different from control without SO_2 inhalation by χ^2 at [d]$P < 0.05$, [e]$P < 0.01$ and [f]$P < 0.001$.

Table II. Relationship between the relative duration of SO_2 exposure and CA formation in bone marrow cells of mice

Treatment	CAs per 100 cells (mean ± SD)[a]		Aberrant cells (%) (mean ± SD)[b]
	Chromatid break	Chromosome break	
Control	1.60 ± 0.14	0.20 ± 0.02	1.80 ± 0.16
SO_2 (14.0 ± 0.24 mg/m^3)			
1 day	1.80 ± 0.12	0.20 ± 0.04	2.00 ± 0.10
3 days	2.00 ± 0.16	0.20 ± 0.02	2.20 ± 0.14
5 days	2.24 ± 0.24	0.20 ± 0.05	2.30 ± 0.36
7 days	3.42 ± 0.28[c]	0.28 ± 0.04	3.58 ± 0.46[c]

[a]Number of chromatid or chromosome breaks per 100 metaphase cells scored per dose group, significantly different from control without SO_2 inhalation by χ^2. Results are from 10 male animals (100 cells/animal).
[b]Results for each SO_2 group were compared to those of the control using Student's t-test.
[c]$P < 0.05$.

Anonymous15 2008; Environ Mol Mutagen 49: 276-281

Positive: Dose related increase of aberrant cells

TABLE IV. The Structural, Numerical, Total Chromosome Aberrations (CAs), Percentage of Abnormal Cell and Mitotic Index (MI) in Rat Bone Marrow Cells Treated With PMB

Test substance	Treatment		Chromosome aberrations			Structural CA/ cell ± SE	Numerical CA/cell ± SE	Total CA/cell ± SE	Percentage of abnormal cell ± SE	MI ± SE
			Structural CA[a]							
	Periods (hr)	Concentration (mg/kg)	B′ type	B″ type	Numerical CA					
Control	—	0	21	1	0	0.055 ± 0.006	0	0.055 ± 0.006	5.50 ± 0.64	3.60 ± 0.103
Urethane	12	400	34	6	3	0.100 ± 0.010	0.007 ± 0.004	0.107 ± 0.014	10.00 ± 1.41	3.04 ± 0.181
PMB	12	150	35	2	3	0.092 ± 0.014	0.007 ± 0.002	0.100 ± 0.012 a_1	8.50 ± 0.50 a_2	2.48 ± 0.262 a_1
		300	52	2	5	0.135 ± 0.005 a_3c_2	0.012 ± 0.009	0.147 ± 0.011a_2c_1	13.00 ± 1.00 a_2	2.41 ± 0.146 a_2c_1
		600	51	5	6	0.140 ± 0.016 a_1	0.015 ± 0.005	0.155 ± 0.015 a_2c_1	14.50 ± 1.32 a_2c_1	1.85 ± 0.060 a_3c_3
Urethane	24	400	61	9	7	0.175 ± 0.025	0.017 ± 0.004	0.192 ± 0.028	16.75 ± 1.18	1.93 ± 0.066
PMB	24	150	39	4	6	0.107 ± 0.011 a_1c_2	0.015 ± 0.006	0.122 ± 0.006 a_2c_2	11.25 ± 0.62 a_2c_2	4.20 ± 0.416 c_1
		300	49	5	2	0.135 ± 0.014 a_1	0.005 ± 0.002 c_1	0.140 ± 0.014 a_2c_1	13.25 ± 1.10 a_2c_1	2.90 ± 0.285 c_1
		600	79	8	2	0.217 ± 0.030 a_1	0.005 ± 0.002 c_1	0.222 ± 0.029 a_1	18.50 ± 0.86 a_3	2.44 ± 0.276 a_1

a, significant from control; c, significant from positive control (urethane); a_1c_1, $P < 0.05$; a_2c_2, $P < 0.01$; a_3c_3, $P < 0.001$.
[a]B′, chromatid-type breaks; B″, chromosome-type breaks.

Anonymous14 (2011) Mutat. Res. 2011 Feb 28;720(1-2):58-61

Positive

Increased frequency of micronuclei in bone marrow and peripheral blood cells at 2g/kg (limit dose); significant reduction of PCE:NCE ratio at **2 g/kg**

Table 2
Detection of micronuclei mean (±S.D.) in peripheral blood reticulocytes (MNRET) and in polychromatic erythrocytes (MnPCEs) of bone marrow cells of mice exposed for 24 h to sodium metabisulfite For each group, $n = 10$ (five males and five females). 2000 cells/animal.

Group	Gender	Blood		Bone marrow		Ratio (PCE:NCE)[a]
		Per gender	Per group	Per gender	Per group	Per group
Negative control[b]	Male Female	2.4 ± 0.54 3.0 ± 0.70	2.7 ± 0.67	2.8 ± 0.45 3.2 ± 0.45	3.0 ± 0.47	1.67 ± 0.67
0.5 g/kg	Male Female	2.6 ± 0.54 2.8 ± 0.44	2.7 ± 0.48	3.2 ± 1.10 2.4 ± 0.55	2.8 ± 0.92	1.37 ± 0.35
1 g/kg	Male Female	4.6 ± 1.14 5.0 ± 1.22	4.8 ± 1.14	4.2 ± 1.30 5.0 ± 1.23	4.6 ± 1.27	1.77 ± 0.62
2 g/kg	Male Female	7.0 ± 1.22 7.0 ± 1.41	7.0 ± 1.25**	7.0 ± 1.58 6.8 ± 1.48	6.9 ± 1.45**	0.74 ± 0.18**
Positive control[c]	Male Female	11.8 ± 0.84 13.2 ± 1.10	12.5 ± 1.18***	11.4 ± 1.52 13.2 ± 1.10	12.3 ± 15.7***	0.75 ± 0.19**

[a] PCE, polychromatic erythrocytes; NCE, normochromatic erythrocytes.
[b] Water.
[c] Cyclophosphamide, 25 mg/kg.
** Significant difference from negative control in the same tissue at $P < 0.01$.
*** $P < 0.001$; tested by ANOVA-Kruskal–Wallis test.

Data relating to Table 16: Summary table of human data relevant for germ cell mutagenicity

Nordenson et al (1980) Hereditas 93: 161-164. (published)

SO_2 group: All types of aberrations were significantly increased in comparison to the control group with $p<0.01$ or $p<0.001$.

Smoking was the only possible confounder recorded.

Due to lack of evaluation/ matching for possible confounders and low number of participants, no final conclusion can be drawn from the study.

Table 3. Chromosomal aberrations in workers at a sulphite pulp factory and in controls

	Work place			Controls (Umeå)
	Boiling (SO_2)	Bleeching (chlorine)	Paper Mill (dust)	
Number of individuals	7	6	6	15
Number of cells	1156	621	662	1500
Gaps				
No.	44	8	18	31
Per cell	0.038	0.013	0.027	0.021
Chromatid aberrations				
No.	24	4	4	9
Per cell	0.021	0.006	0.006	0.006
Chromosome aberrations				
No.	19	7	4	1
Per cell	0.016	0.011	0.006	0.001
All aberrations				
No.	87	19	26	41
Per cell	0.075	0.031	0.039	0.027

Sorsa, M. *et al.* (1982). Hereditas 97: 159-161.

Frequencies of CA and SCE were similar in all groups. However, due to lack of evaluation/ matching for possible confounders and low number of participants, no final conclusion can be drawn from the study. In addition, exposure towards SO_2 was very low.

Table 1. Chromosome aberrations and SCEs of SO_2-exposed male workers and controls

Subject and occupation	Age (yr)	Employment time (yr)	Smoking habits	Cells with chromosomal aberrations: No. cells analyzed	Including gaps (%)	Excluding gaps (%)	SCEs: No. cells analyzed	Mean ± SD
Exposed group								
High-exposure								
1, smelter	62	39	–	100	2	1	30	8.1±3.9
2, molder	50	26	+	100	6	4	30	9.0±3.4
3, molder	32	6	–	100	2	2	30	8.6±3.0
4, castbreaker	52	5	–	100	1	1	30	10.2±3.4
5, castbreaker	62	9	+	100	4	2	30	8.9±3.9
Low-exposure								
6, foreman	51	30	–	45	4	4	26	7.8±3.3
7, castmaker	41	25	–	100	1	1	30	8.8±3.4
8, truckdriver	33	16	+++	100	2	1	30	10.4±4.7
n = 8	$\bar{x}$ = 47.9	$\bar{x}$ = 19.5		n = 745	$\bar{x}$ = 2.8±1.8	$\bar{x}$ = 2.0±1.3	n = 236	$\bar{x}$ = 8.9±0.9
Control group								
9	43		–	100	4	3	30	9.1±3.5
10	52		+++	100	2	1	30	10.3±4.6
11	44		+++	100	3	2	30	10.1±4.7
12	59		–	100	4	4	30	9.0±3.4
13	59		+	100	4	3	30	6.6±3.0
14	31		(+)	100	1	0	30	9.5±3.5
15	46		–	100	2	1	30	7.0±3.5
16	37		+++	100	4	4	30	12.2±5.0
n = 8	$\bar{x}$ = 46.4			800	$\bar{x}$ = 3.0±1.2	$\bar{x}$ = 2.25±1.5	n = 240	$\bar{x}$ = 9.2±1.8

–, nonsmoker +, <10 cigarettes/day +++, ≥20 cigarettes/day (+), recent smoker

Table 2. Chromosome aberrations and SCEs among smoking and nonsmoking subjects

No.	Smoking/exposure category		Cells with chromosomal aberrations (%): Incl. gaps	Excl. gaps	SCEs: Mean ± SD
All smokers					
16	+++	control	4	4	12.2±5.0
11	+++	control	3	2	10.1±4.7
10	+++	control	2	1	10.3±4.6
8	+++	exposed	2	1	10.4±4.7
14	(+)	control	1	0	9.5±3.5
2	+	exposed	6	4	9.0±3.4
5	+	exposed	4	2	8.9±3.9
13	+	control	4	3	6.6±3.0
n = 8			$\bar{x}$ = 3.3±1.6	$\bar{x}$ = 2.1±1.5	$\bar{x}$ = 9.6±1.6
All nonsmokers					
4	–	exposed	1	1	10.2±3.4
9	–	control	4	3	9.1±3.5
12	–	control	4	4	9.0±3.4
7	–	exposed	1	1	8.8±3.4
3	–	exposed	2	2	8.6±3.0
1	–	exposed	2	1	8.1±3.9
6	–	exposed	4	4	7.8±3.3
15	–	control	2	1	7.0±3.5
n = 8			$\bar{x}$ = 2.5±1.3	$\bar{x}$ = 2.1±1.4	$\bar{x}$ = 8.6±1.0

+++, ≥20 cigarettes/day +, ≤10 cigarettes/day (+), recent smoker

Meng and Zhang (1989). Mutation Research 241:15-20 (published)

Exposed *vs*. controls (p<0.001):

Lymphocytes with MN:

w/o:

0 % *vs* . 31 %

>0.1 %:

72.5 % *vs* . 16.7 %

>0.2 %:

17.5 % *vs.* 0 %

Higher frequency of MN in smokers in both groups, but always higher in exposed workers whether smoking or not.

Exposed *vs*. controls (p<0.01):

CA chromosome type:

165 *vs* . 25 aberrant cells

(2.1 ± 0.23 % vs. 0.3 ± 0.1 %)

CA chromatid type:

77 vs. 24 aberrant cells
(1.0 ± 0.2 % vs. 0.3 ± 0.1 %)

CA total number of cells:

242 vs. 49
(3.0 ± 0.3 % vs. 0.6 ± 0.1 %)

SCE per cell:

6.7 ± 0.2 vs. 2.7 ± 0.1

No difference of CA and SCE between smokers and non-smokers.

TABLE 8

SMOKING AND SCE IN LYMPHOCYTES

	Number of persons	Number of cells observed	SCEs/cell ($\bar{x} \pm$ SE)
Non-smokers			
Control group	21	1 330	2.65 ± 0.13
Worker group	20	1 225	6.88 ± 0.41
Smokers			
Control group	21	1 330	2.80 ± 0.25
Worker group	20	1 225	6.67 ± 0.25