Committee for Risk Assessment

RAC

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

Adopted

26 November 2021

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CLH-O-0000007055-78-01/F

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

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

Chemical name: **Sulfur dioxide**

EC Number: **231-195-2**

CAS Number: **7446-09-5**

The proposal was submitted by **Germany** and received by RAC on **11 August 2020.**

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

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **14 September 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **13 November 2020**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Christina Tsitsimpikou**

Co-Rapporteur, appointed by RAC: **Nikolaos Spetseris**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **26 November 2021** by **consensus**.

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

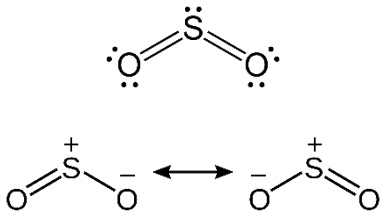
	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	016-011-00-9	sulfur dioxide	231-195-2	7446-09-5	Press. Gas Acute Tox. 3* Skin Corr. 1B	 H331 H314	GHS04 GHS05 GHS06 Dgr	 H331 H314		*	U, 5
Dossier submitters proposal	016-011-00-9	sulfur dioxide	231-195-2	7446-09-5	**Retain:** Press. Gas Skin. Corr. 1B **Add:** Muta. 2 STOT SE 3 Skin Sens. 1 **Modify:** Acute Tox. 3	**Retain:** H314 **Add:** H341 H335 H317 **Modify:** H331	**Retain:** GHS04 GHS05 GHS06 Dgr **Add:** GHS08	**Retain:** H314 **Add:** H341 H335 H317 **Modify:** H331		**Add:** inhalation: ATE = 1041 ppmV (gases)	**Retain** U, 5
RAC opinion	016-011-00-9	sulfur dioxide	231-195-2	7446-09-5	**Retain:** Press. Gas Skin. Corr. 1B **Add:** STOT SE 1 **Modify:** Acute Tox. 3	**Retain:** H314 **Add:** H370 (respiratory system, inhalation) **Modify:** H331	**Retain:** GHS04 GHS05 GHS06 Dgr **Add:** GHS08	**Retain:** H314 **Add:** H370 (respiratory system, inhalation) **Modify:** H331		**Add:** inhalation: ATE = 1000 ppmV (gases)	**Retain** U, 5
Resulting Annex VI entry if agreed by COM	016-011-00-9	sulfur dioxide	231-195-2	7446-09-5	Press. Gas Acute Tox. 3 Skin. Corr. 1B STOT SE 1	H331 H314 H370 (respiratory system, inhalation)	GHS04 GHS05 GHS06 GHS08 Dgr	H331 H314 H370 (respiratory system, inhalation)		inhalation: ATE = 1000 ppmV (gases)	U, 5

GROUNDS FOR ADOPTION OF THE OPINION

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.

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. Thus, RAC supports the DS' proposal to **classify sulfur dioxide as Press. Gas (H331).**

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HUMAN HEALTH HAZARD EVALUATION

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 changes of the lower respiratory tract; Non-guideline; Non-GLP; Reliability of 2.	Rats (Sprague Dawley) /M, 12 animals /dose	**SO_2** (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).	Mean survival time: Susceptibility to lethal toxic action of SO_2 highest in mice, intermediate in guinea pigs, least in rats	Anonymous23
	Mice (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).		
	Guinea pigs ♂; 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)**.

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 groups of 10 rats (plus control group)	41-751 ppm/ 2 hours	*General effects*: sneezing, coughing and lachrymation, intermittent burst 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

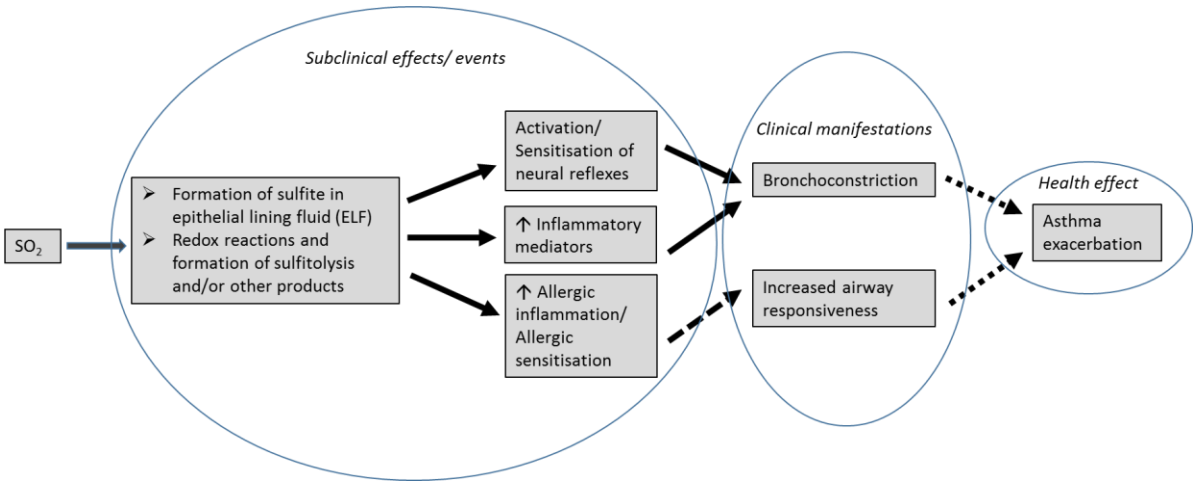
Andersen *et al.* 1974	15 healthy male volunteers; age: 20 – 28; 4 smokers, 11 non-smokers	0, 2.6, 13.2, 65.8/ 360 min	**2.6 mg/m³** cross-sectional nasal airway significantly ↓ (more pronounced after 1-3 hours than after 4-6 hours exposure), FEF_{25-75} significantly ↓ **13.2 mg/m³** mucus flow rate 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**.

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.

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**.

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 for mutagenicity due to inconclusive data is warranted.**

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. Neoplastic 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)

Kind of study (e.g. case reports)	Examination methods, number of individuals examined	Results	References
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 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 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).	Langseth and Andersen, 2000
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.

Kind of study (e.g. case reports)	Examination methods, number of individuals examined	Results	References
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 determined using the nearest air monitoring station to the patient	– 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

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.**

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APPENDIX Background information on Toxicokinetics

ANNEXES:

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

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

APPENDIX

Background information on Toxicokinetics

Inhalation is the predominant route of exposure for SO_2 since it is a gaseous substance. The chemistry of the inhaled SO_2, its reaction products and metabolites as well as whether these substances reach and/or persist at specific sites within the respiratory tract or systemically after exposure, are important aspects in the evaluation of the toxicity of SO_2.

Chemistry

The physicochemical properties of SO_2 most relevant to its toxicological profile include its solubility in biological fluids (e.g. the epithelial lining fluid) and its chemical transformations and reactions that occur within the human body. SO_2 is highly soluble in water with a very low effective Henry's law constant for SO_2 in water. Once SO_2 contacts the fluids lining the airways, it dissolves into the aqueous compartment and rapidly hydrates to form sulfurous acid (H_2SO_3), which forms hydrogen (H^+) ions, bisulfite (HSO_3^-) anions, and sulfite (SO_3^{2-}) anions.

$$SO_2 + H_2O \rightleftharpoons H_2SO_3 \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} HSO_3^- \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} SO_3^{2-}$$

The prevalence of the different sulfur species is primarily pH dependent and in the human respiratory tract (pH of 7.4 and 37 °C), dissolved SO_2 exists exclusively as a mixture of bisulfite and sulfite, with the latter being predominant (US EPA 2017). Subsequent reactions of bisulfite and sulfite such as sulfitolysis, enzymatic detoxification, and auto-oxidation play an important role in the chemical and biological properties of SO_2 and are described further in the ODD.

Read-Across

SO_2 is very soluble in water and upon inhalation into the lungs forms sulfurous acid. Since all physiological processes within the human body proceed in aqueous solutions, chemical equilibria exist among the quadrivalent-sulfur substances: SO_2, sulfites, hydrogensulfites and metabisulfites. These equilibria primarily depend on pH and secondarily on ionic strength and temperature. The chemical equilibria in aqueous solutions are summarised in the following equations:

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

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

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

The nature of the cation (i.e., sodium, potassium, ammonium) is not expected to contribute substantially to differences in toxicity and solubility (all compounds are very water soluble) and consequently the chemical and biological properties of the sulfite anion are considered as the relevant determinants. The species that dominates among these rapidly interconvertible hydration products depends primarily upon pH and therefore, SO_2 is transported through aqueous systems at neutral pH almost totally in its hydrated form. Because of this rapid hydration, the interactions of SO_2 with biological molecules in an aqueous medium will probably be those of sulfite and bisulfite.

Acidification will release SO_2 vapours; in alkaline solutions, sulfites, bisulfites, and metabisulfites are produced. At concentrations > 1M, bisulfite anions will dimerize with the elimination of water to form metabisulfite $(S_2O_5)^{2-}$; at low concentrations metabisulfite will hydrolyse to form bisulfite $(HSO_3)^-$.

Based on the described equilibrium correlations, unrestricted read-across between SO_2 and the groups of sulfites, hydrogensulfites and metabisulfites is proposed by the DS and supported by RAC for the inhalation route and systemic exposure. The proposed read-across was also supported by one Industry or trade association and one MSCA.

Sulfur Dioxide

Absorption

Inhalation is the predominant route of exposure for SO_2 as a gas. It is rapidly absorbed in the moist epithelium of the upper respiratory tract both in humans and in laboratory animals under resting conditions. During nasal breathing, the majority of available data suggests that 95% or greater SO_2 absorption occurs in the nasal passages. Approximately 15% is subsequently desorbed and eliminated with exhaled air. Although some SO_2 degradation products and metabolites rapidly move from the respiratory tract into the blood and are distributed throughout the body, experiments using radiolabeled ^{35}S indicate that the majority of sulfur in SO_2-derived degradation products and metabolites in the body at any given time following exposure are found in the respiratory tract and may be detected there for up to a week following inhalation.

However, there is a shift in the absorption pattern from the upper airways to the tracheobronchial airways in conjunction with a shift from nasal to oronasal breathing and is associated with increased ventilatory rates. Due to their greater amount of oral breathing, children (particularly boys and perhaps the obese) and individuals with allergies or upper airway infections may be expected to have greater SO_2 penetration into the lower respiratory tract than healthy adults.

Distribution

Inhaled SO_2 is readily dissolved in the epithelial lining fluid where it exists as a mixture of bisulfite and sulfite anions with the latter predominating. The SO_2 metabolites and/or degradation products can diffuse across cell membranes, reach the circulation and are readily distributed throughout the body. Although the majority of SO_2-derived metabolites and/or degradation products remain in the respiratory tract following exposure, extrapulmonary SO_2-derived metabolites and/or degradation products are found in the liver, with lesser amounts found in the heart, spleen, kidney, brain, and other tissues. The amount of SO_2-derived species in blood and other tissues increases with the concentration of SO_2 in inhaled air, while the distribution within the body is generally unaffected. A substantial portion of SO_2-derived products appear to be retained within the upper airways, particularly during nasal breathing, with only slow absorption into the blood.

Metabolism

The inhaled SO_2 readily dissolves in biological fluids and forms sulfite anions. The SO_2-derived metabolites and/or degradation products can undergo subsequent reactions such as sulfitolysis, enzymatic detoxification and auto-oxidation with the generation of free radicals. Sulfites can diffuse across cell membranes, and bisulfite can react with disulfide bonds (R−S−S−R) to form thiols (R−SH) and S-sulfonates ($R-S-SO_3^-$) by a process termed sulfitolysis. Sulfite is a strong nucleophile and reacts with disulfite bonds in cellular molecules such as cysteine, albumin, and glutathione.

Sulfitolysis 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-sulfonate ($RS\text{-}SO_3^-$) compounds in an organ or tissue is an indication for recent exposure to sulfite.

The primary route of sulfite metabolism is by sulfite oxidase (SOX) catalysed enzymatic oxidation of sulfite to sulfate (SO_3^{2-} to SO_4^{2-}) with ferricytochrome C being the physiological electron acceptor. Sulfite oxidase is located in the intermembrane space of mitochondria. High activity of this enzyme has been found in the liver, kidney, and heart, with the highest enzyme expression being in the liver, whereas activity is low in brain, spleen, lungs, and testis. The high sulfite oxidase activity in the liver plays a major role in detoxification of circulating sulfite. A deficiency in sulfite oxidase activity may lead to toxicity even in the absence of exogenous sulfite or bisulfite exposures. For example, humans and mice with homozygous genetic defects in the sulfite oxidase protein or in the enzymes required to synthesize the essential molybdenum cofactor ultimately develop lethal neurologic disease attributable to accumulation of endogenous sulfite postnatally. Sulfite oxidase activity is highly variable among species. Liver sulfite oxidase activity in the rat is 10−20 times that in humans. Rapid metabolism of circulating sulfite to sulfate may explain the lack of sulfite/S-sulfonates found in blood of rats exposed by inhalation to 30 ppm SO_2, whereas these products were found in other species conditions. Deficiency of SOX leads to accumulation of SO_3^{2-}, a strong nucleophile, which is capable of reacting with a wide variety of cell components. Organs with low activity of sulfite oxidase are suggested to be target organs.

Glutathione (GSH) is proposed to play a role in SO_2 detoxification through the sulfitolysis of glutathione disulfide (GSSG) to S-sulfoglutathione ($GSSO_3^{2-}$). Repeated inhalation exposure to 5 ppm of SO_2 did lead to depletion of the GSH reserves in the cell tissues of lung, liver, heart, and kidney of rats (Anonymous44). In addition, several 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) following repeated exposure to SO_2 in various species (guinea pig, rabbit, mouse, rat) (Anonymous11, 43, 47, 48, 49, 51, 53). These results are in agreement with the wide distribution of metabolites of SO_2 within the body.

Elimination

When the partial pressure of SO_2 on mucosal surfaces exceeds that of the gas phase, such as during expiration or following exposure, partial desorption of SO_2 initially occurs through

desorption from the fluids lining the respiratory tract. SO_2 that does not desorb is transformed to bisulfite/sulfite. The majority of the circulating sulfites are excreted in the urine as sulfates (> 80%) primarily from the SOX catalyzed oxidation of sulfites (US EPA 2017).

Sulfites

When ingested, sulfites are absorbed almost 100% and react with water to form bisulfite, sulfite and SO_2. The prevailing species found in the stomach are bisulfite and SO_2, and the balance between these is determined by the acidity of the different stomach phases. SO_2 gas is highly soluble in aqueous media but some may be inhaled and absorbed in the lungs as either SO_2 and/or sulfite during and after oral ingestion. Once absorbed, sulfite is excreted in the urine along with endogenously formed sulfate by the reactions mentioned above.

The half-life of sulfites in humans is estimated to be 15 min, but this can vary particularly in very old people and patients with Down's syndrome who can have a lower activity of sulfite oxidase.

Dermal absorption studies for sulfites are not available. However, using the default values according to the EFSA guidance on dermal absorption, sulfites, metabisulfites, bisulfites and sulfates can be assumed to have a dermal absorption below 25/75% (at concentrations > 5% and < 5% respectively (CLH report, EFSA Guidance on Dermal Absorption, 2012).

The oral and dermal routes are not relevant exposure routes for gaseous SO_2.