Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Sulfur dioxide

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

CLH-O-0000007055-78-01/F

Adopted

26 November 2021

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Substance name: Sulfur dioxide
EC number: 231-195-2
CAS number: 7446-09-5
Dossier submitter: Germany

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
12.11.2020	Germany	Sulphuric Acid REACH Consortium (SAC)	Industry or trade association	1
Comment received				
The statement is submitted on behalf of: Lead Registrant for Sulfur Dioxide (LR) Contact: <confidential> Grillo-Werke AG, Weseler Str. 1, 47169 Duisburg, Germany Sulphuric Acid REACH Consortium (SAC) Contact: <confidential> TSG Consulting Concordia House, St James Business Park, Grimbald Crag Court Knaresborough, North Yorkshire HG5 8QB, United Kingdom European Sulphuric Acid Association (ESA) Contact: <confidential> Sector Group of Cefic, Rue Belliard 40, 1040 Brussels, Belgium EU Transparency Register n° 64879142323-90 Sulfur Dioxide based Chemicals REACH Consortium (SDIOC) Contact: <confidential> EBRC Consulting GmbH, Raffaelstr.4, 30177 Hannover, Germany				

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Final CLH-Comment SO2 EBRC 11NOV2020_Redacted.pdf
Dossier Submitter's Response
Specific responses to individual comments are given in the respective sections below.
RAC's response
Comments are addressed individually in each hazard endpoint.

Date	Country	Organisation	Type of Organisation	Comment number
10.11.2020	Netherlands		MemberState	2
Comment received				
NL-CA agrees with the justification on read-across for sulfur dioxide on metabisulfite, sulfite and bisulfite. Hydrolyzation of sulfur dioxide in aqueous medium is rapid and distribution of sulfite, sulfur dioxide and hydrogen sulfite, depending on pH, has been extensively described in literature. Therefore, read-across for sulfur dioxide is justified.				
Dossier Submitter's Response				
Thank you for the support. No response required.				
RAC's response				
RAC appreciates the support for the read-across from sulfites for systemic routes of exposure. For dermal exposure, relevant for the skin sensitisation endpoint, read-across is discussed separately in the said endpoint.				

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2020	Netherlands	Micro-Pak Europe BV	Company-Downstream user	3
Comment received				
Micro-Pak Europe BV is an applicant for the biocidal active substance approval of "sulfur dioxide released from sodium metabisulfite". Micro-Pak does not consider the classification of sulfur dioxide as Muta. 2 and Skin Sens. 1 as proposed by the dossier submitter BAuA as warranted based on the reasons described below. The arguments will be provided in the respective fields, and in addition also as an attachment. Sulfur dioxide is ubiquitously occurring in the natural environment. Moreover, sulfites are also constantly generated as part of biological processes. Thus, mammalians and other organisms are well adapted to these molecules; in mammalians, excess sulfites are converted by the endogenous enzyme sulfite oxidase to sulfates followed by excretion to maintain the internal concentration at a physiological level. ECHA note – An attachment was submitted with the comment above. Refer to public attachment CLH comments Micro-Pak_public.pdf ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment CLH comments Micro-Pak.pdf				
Dossier Submitter's Response				
Specific responses to individual comments are given in the respective sections below. The CLH procedure addresses hazard properties irrespective of the origin of the active substance. Detoxification mechanisms for sulphites are known to the DS. However, detoxification and repair mechanisms exist for virtually all compounds classified for germ cell mutagenicity but do not provide 100% protection.				

RAC's response
Comments are addressed individually in each hazard endpoint.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
10.11.2020	Netherlands		MemberState	4
Comment received				

The dossier submitter proposes a 'no classification' for carcinogenicity for sulfur dioxide, because of the lack of sufficient evidence.

We agree that the animal data do not warrant classification for carcinogenicity. Results of carcinogenicity of metabisulfites and sulfur dioxide in in vivo animal studies are mixed. Multiple in vivo animal studies show negative results for carcinogenicity for sulfur dioxide and metabisulfites, administered via inhalation or oral route, respectively. Some studies were not reliable because of high tumor incidence observed in control groups and limitations with respect to study design. Furthermore, no dose-related tumor incidence was observed or no formation of malignant tumors was demonstrated upon exposure to sulfur dioxide or metabisulfites. In vivo studies supporting a classification for sulfur dioxide-induced carcinogenicity are thus clearly lacking.

With respect to the available human data, the following is noted.
- Various epidemiological studies have shown a correlation between exposure to sulfur oxide and lung cancer, but also various types of other cancers (prostate, stomach, leukemia, rectal). Confounders such as smoking or exposure to other carcinogens (e.g. arsenic, formaldehyde) could not be excluded. Sufficient evidence for carcinogenic potential of sulfur dioxide in humans is thus not available. Therefore, we agree with the dossier submitter that category 1A is not warranted.

- NL-CA points out that although evidence of carcinogenicity of sulfur dioxide is limited, a positive correlation between tumor formation and exposure to sulfur dioxide in workers has been demonstrated by Henneberger et al. (1989), Langseth et al. (2000) and Band et al. (2001). In addition, a dose-related correlation of sulfur dioxide exposure and lung cancer was found in workers (Lee et al. 2002). Confounders (e.g. smoking etc.) could not be excluded with confidence in these studies, as pointed out by the dossier submitter, 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 this dossier. In other publication evidence was found against smoking as confounder in case-control studies focused on lung cancer mortality, in a cohort study in workers in the pulp and paper industries (Henneberger et al. 1998; Int J Occup Environ Health 4(3): 147-154).

- Carcinogenic potential of sulfur dioxide for human is thus suspected, based upon limited evidence of sulfur dioxide-induced carcinogenicity in humans. In the Guidance on the Application of the CLP Criteria, limited evidence is defined in Annex I: 3.6.2.2.3: "Limited evidence of carcinogenicity: a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence."

- Criteria for category 2 are: "(Suspected human carcinogens) The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal

studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2 of CLP Guidance). Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies." This limited evidence could warrant a classification on category 2 for carcinogenicity but is not discussed by the dossier submitter.

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

Dossier Submitter's Response

Thank you for the comment.

The DS agrees that there are epidemiological studies from the open literature available suggesting an association between sulphur dioxide exposure and cancer incidence or mortality.

As reported in the CLH report (Table 20), the following six cohort studies were reported:
Henneberger, P.K. et al. (1989) Brit. J. Ind. Med. 46: 658-664. (published)
Jäppinen, P. (1987). Brit. J. Ind. Med. 44: 580-587. (published)
Robinson, C.F. et al. (1986). Scand. J. Work Environ. Health 12: 552-560. (published)
Band et al. (2001). Scand J Work Environ Health. 27/2:113-119
Langseth and Andersen (2000) Scand J Work Environ Health. 26/2: 99-105
Lee et al. (2002) Environ Health Perspect. 110:991-995

Cohort size was between 883 and 57613 workers from the pulp and paper industry. Here, lignin is solubilised in either an alkaline sulfate based process (kraft milling) or an acidic sulphite based method (sulphite milling). The latter is associated with exposure to sulphites and sulphur dioxide, but also a range of other potentially carcinogenic substances. Band et al. (2001), Langseth & Andersen (2000) and Lee et al. (2002) may be considered as the more powerful studies.

Band et al. (2001) reported an SMR of 4.8 (90% Cl 1.29-12.37) for Hodgkin's disease for workers occupied exclusively in sulphite mills and an increased SMR for a range of cancer types for the total cohort. A follow-up case control study was announced but could not be located.

Langseth & Andersen (2000) specifically investigated lung cancer incidences and reported an increased SIR of 1.5 (95% CI 1.09-1.99) for sulphite mill workers but similar values for other subgroups. Most of the effect was attributed to asbestos exposure and smoking, but other contributors such as occupational exposure to SO_2 could not be excluded.

The study by Lee et al. (2002) included an exposure assessment for SO2. 40704 of the workers in this cohort were classified as exposed to SO_2 and the dose–response relationship between SO_2 exposure and cancer mortality risk was explored. There were 7613 deaths among exposed workers, including 488 from lung cancer. Following adjustment for confounding (sex, age, employment status, calendar year, country) including co-exposures (asbestos, combustion products, welding fumes), an SMR of 1.5 (95% CI, 1.14–1.96) for lung cancer was obtained when SO_2 exposed were compared to unexposed workers. Increased SMRs were also reported for non-Hodgkin lymphoma (2.55, 95% CI 1.06–6.13) and leukaemia (2.49, 95% CI 1.13–5.49). When weighted cumulative SO_2 exposure was determined taking into account frequency, level and duration of SO_2 exposure, a linear

trend was observed across 4 exposure categories for relative risk (apparently not adjusted for co-exposures) of lung cancer (p=0.009) and non-Hodgkin lymphoma (p=0.05). Results suggest an association of occupational exposure to SO_2 in the pulp and paper industry with increased risk of lung cancer and non-Hodgkin lymphoma, while residual confounding effects could not be fully excluded by the authors.

More recent data were not yet available at the time the CLH report was prepared. Analyses published by 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. DOI: 10.1186/s12889-019-7849-z) provide some indication for an association between increased environmental SO_2 exposure and cancer incidence. This study was focused on the correlation of exposure to PM2.5 with increased incidences of cancers. A significant positive correlation (alpha = 0.05), before adjusting for multiple testing, was also found between the exposure to SO_2 and a higher age and sex-adjusted total increased incidences of cancers. However, after a Bonferroni correction for multiple testing (a total of 70 correlations were tested), this association was no longer significant. Thus, as also concluded by the authors of the study, further data would be necessary in order to confirm a positive correlation of increased incidences of cancers and SO2 exposure.

A correlation analysis by Yue et al., 2017 (Relationships Between Lung Cancer Incidences and Air Pollutants. Technol Health Care 2017 25:411-422. DOI: 10.3233/THC-171344) comparing lung cancer incidence and environmental concentrations for various pollutants in Tianjin disctricts concluded "When SO_2 concentrations are high, lung cancer incidences are high." Notably, a confounding effect of socio-economic status and education on the association between environmental SO_2 exposure and lung cancer has also been discussed for China (Guo et al., 2021. Do socioeconomic factors modify the effects of PM1 and SO2 on lung cancer incidence in China? Sci Total Environ. 2021 Feb 20;756:143998. DOI: 10.1016/j.scitotenv.2020.143998. Epub 2020 Nov 28).

Collarile et al., 2017 (Residence in Proximity of a Coal-Oil-Fired Thermal Power Plant and Risk of Lung and Bladder Cancer in North-Eastern Italy. A Population-Based Study: 1995-2009. Int J Environ Res Public Health. 14(8):860.DOI: 10.3390/ijerph14080860) reported a lung cancer risk ratio of 1.7 (95% CI 1.07-2.73) for women of the tertile with highest SO2 exposure from power plant emissions in northern Italy, but the analysis could not be adjusted for established confounders such as smoking and was limited to women over the age of 75 albeit there was a good dose dependency.

RAC's response

RAC, considering the animal studies in Table 17, pages 69-71 of the CLH report, as well as human studies both from the CLH report and from the public consultation (Su *et al*. 2019; Yue et *al.* 2017; Guo *et al*., 2021), notes that the available animal data set for the SO_2 classification is rather limited and the quality of the studies is not high enough (low duration, tested concentration, inadequate control group, inadequate assessment of tumours etc) to provide unequivocal evidence for the carcinogenicity classification of SO_2, especially since essentially inflammatory reaction to SO_2 cannot be excluded. 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, alcoholism), as well as uncertainties about the concentrations of SO_2 exposure.

Overall and in a weight of evidence approach, RAC believes that the existing evidence does not support classification of SO_2 as a carcinogen.

Date	Country	Organisation	Type of Organisation	Comment number
06.11.2020	France		MemberState	5
Comment received				
Experimental studies are not considered of adequate quality to properly conclude on classification for this endpoint (ex. low duration, one tested concentration, inadequate control group, inadequate assessment of tumours etc). Some excess risks of cancers are reported in workers. However, the results are not consistent and the excess risk may be attributable to confounding factors. Thus, FR considers that no classification can be proposed based on inadequate database.				
Dossier Submitter's Response				
Thank you for the support. No response required.				
RAC's response				
RAC appreciates the support.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2020	France	<confidential>	Industry or trade association	6
Comment received				
We argue a lack of clastogenic and aneugenic activity (genotoxicity) based on: A) Among the various tests to assess genotoxicity, the mouse bone marrow micronucleus test along with hematological endpoints, is one of the key model to assess genotoxicity and one of the most recent one (Ziemann 2010) clearly states that SO2 is not genotoxic. The german eCA did not take into consideration these facts and in our opinion wrongly interpret the oxidative stress measurement as genotoxicity. The presence of ROS does not lead to genotoxicity because of the many natural enzymes responsible for ROS detoxification. B) Reliability of the studies wrongly assessed : Ziemann 2010 study demonstrates that the studies used by the German eCA (Meng studies) to suggest the genotoxicity of SO2 and its read-across can not be trusted (deficient strain, overall sensitivity for all organs while it is known that there is a strong variation between organs, irregular SO2 exposition of the animals, critical data not measured, detoxification mechanisms not taken into consideration). C) Lack of in vitro mutagenicity, leaving only the in vivo assays which, as demonstrated above, have been falsely interpreted and assessed. D) Bacterial genotoxicity could not be demonstrated. E) Several studies considered by the eCA do not satisfy ECHA and OECD minimum guidelines (some with positive and some with negative results). One of the main issue is the lack of knowledge on the direct cytotoxic effect which would influence many other activities of the cells.				

F) The enzymatic detoxification potential in humans is more than sufficient to avoid genotoxicity at the level considered in food consumption.
G) The natural level of SO2 exposure (exogenous and metabolic) is similar to the one brought by food consumption.
H) EFSA 2016 conclusion were the absence of genotoxicity
I) EBRC's scientific analysis of SO2 regarding the absence of genotoxicity as part of this CLH consultation.
J) AFEPASA's scientific analysis of SO2 regarding the absence of genotoxicity as part of this CLH consultation.
K) The largest epidemiological study of all times: the consumption of wine for 1000 of years..

Dossier Submitter's Response

Individual response to the specific points is given below:

(A) The limitations of the Ziemann study are described in the CLH dossier (e.g. no evidence that test compound reached the target organ). In fact, ROS generation as also indicated by the results of Ziemann is one of the key mechanisms leading to genotoxicity and ultimately to mutagenic responses. This is of particular importance if detoxification and repair mechanisms are saturated. Biomarkers for oxidative stress were not only measured in the study by Ziemann, but also in the study by Etlik et al., 1997 (Protective effect of antioxidant vitamins on red blood cell lipoperoxidation induced by SO2. inhalation. Journal of Basic & Clinical Physiology & Pharmacology 8, 1-2.doi: DOI: 10.1515/jbcpp.1997.8.1-2.31). In this study, inhalation exposure of rats to SO_2 resulted in a statistically significant increase in MDA blood levels in comparison to the control group.

(B) The study reliability is assessed individually study-by-study and the outcome of one study should not be taken as evidence for lack of reliability of another study. The DS also described deficiencies of the Meng study in the CLH report. However, the dose-dependent increase in micronuclei cannot be ruled out by the negative outcome of the Ziemann study.

(C) This comment is incorrect. It is referred to section 10.8 (germ cell mutagenicity) listing several positive in vitro genotoxicity studies.

(D) The most important mechanism underlying genotoxicity is clastogenicity as decribed in the CLH report.

(E) The applicants failed to submit studies being fully acceptable from the regulatory perspective. Unfortunately, only little of the available data has been acquired and reported in a way complying with current OECD and EU guidelines for the testing of chemicals. Therefore, the DS (the eCA) had to adopt a WoE-based approach based on a large number of studies with a range of individual limitations. Appropriate care needs to be taken in its interpretation. Nevertheless, the data package provides the information required for an assessment of the human health effects of sulphur dioxide. This approach was also taken to avoid further testing in animals.

(F) Hazard identification is independent of risk.

(G) Same response as for (F).

(H) EFSA opinion from 2016 also points out that there are "[…] several uncertainties and limitations in the database." It was therefore concluded that the current group acceptable daily intake 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. EFSA remarked that the recommended studies could require 5 years for completion.

(I) Specific response to individual comments are given in the respective sections below.

(J) Same response as for (I).

(K) The CLH dossier is focussed on sulphur dioxide which is a gas.

RAC's response

Regarding mutagenic properties of SO_2, the following key points could be summarised:

1. The *in vitro* data provide evidence for the possible genotoxic (clastogenic/ aneugenic) properties of sulfur dioxide and its metabolites, stemming mainly from the cytogenicity studies in mammalian cells

2. In the *in vivo* studies a series of shortcomings have been observed both in studies reporting positive and those reporting negative findings

3. The positive *in vivo* results from the Meng group studies weren't reproduced by the Ziemann study, possibly due to the strain specificity to SO_2 exposure. Nevertheless, Ziemann *et al*. themselves do not negate the results of the Meng *et al*. study

4. 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 processes exist is not sufficient to disregard the results of the genotoxicity studies (hormesis could be also considered)

5. 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. Dominant lethal assay with sulphites was reported negative but with inconsistencies (dose selection, no positive control)

6. Worker exposure to SO_2 in three different occupational settings showed a potential association between SO2 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

RAC considering all the above notes that the available data set for the evaluation of the genotoxic properties for SO_2 is rich, but the quality of the studies is not high enough to provide unequivocal evidence for the mutagenicity classification of SO_2

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2020	Netherlands	Micro-Pak Europe BV	Company-Downstream user	7

Comment received

According to the CLH report, the proposal to classify sulfur dioxide as Muta. 2 is based on positive evidence from in vivo studies supported by in vitro findings considering studies on sulfur dioxide as well as inorganic sulfites; furthermore indications for genotoxicity in lymphocytes of exposed workers, strandbreaking activity in testes in an in vivo comet assay and genotoxic effects in occupational studies are listed.

Among the occupational studies evaluated, one study (Sorsa et al., 1982) did not find any increase in the incidence of chromosomal aberrations (CA) and sister chromatid exchanges (SCE) in lymphocytes of exposed workers, whereas other studies report increased frequency of CA, SCE and/or micronuclei (MN) in lymphocytes of exposed workers. The occupational studies summarized by the dossier submitter were also already evaluated by the German MAK Commission (Supplement 1998, published 2015). In brief, upon careful evaluation of these reported findings, the MAK Commission concluded that effects observed in these studies cannot unambiguously be considered as caused by sulfur dioxide as co-exposure to e.g. radioactivity, quartz, chromium or arsenic cannot be excluded in the described occupational settings. Potential relevant co-exposure does not allow the conclusion that these studies unambiguously provide indications for genotoxicity of sulfur dioxide in exposed workers.

Unfortunately, the anonymization of references for in vivo and in vitro studies including publications hampers the independent assessment of these studies. Due to the BPR dossier for application of approval of sulfur dioxide as active substance, the identity of the cited studies is available to us.

In brief, all in vivo studies reporting genotoxic effects in mice upon inhalation exposure to sulfur dioxide were conducted by the same group of researchers at Shanxi University, China. The non-GLP studies were all conducted in Kunming mice, a strain known for genetic diversity between different populations and for which differences in drug reactions among populations have already been reported (Shang et al., 2009). Additional shortcomings diminishing the reliability of these studies are already mentioned in the CLH report. In contrast, the highly reliable GLP study conducted at the Fraunhofer Institute for Toxicology and Experimental Medicine, Germany published by Ziemann et al. (2010) did not find any increased formation of micronuclei in NMRI mice upon inhalation exposure to SO2, although this study was specifically designed to reproduce findings reported by the above-mentioned researchers from Shanxi University and thus used overlapping test concentrations. In conclusion, the available in vivo genotoxicity studies on sulfur dioxide do not provide a clear evidence for a genotoxic potential of sulfur dioxide.

Further in vivo genotoxicity studies in mice, rats or hamster exposed to inorganic sulfites via oral, subcutaneous or intraperitoneal administration, and in vitro studies are included which yielded both positive and negative results. None of the in vivo studies were conducted according to an OECD Test Guideline and in compliance with GLP. Based on the available dataset and also considering the individual limitations and methodological deficiencies of the aforementioned studies, EFSA (2016) as well as the MSCA Hungary (CoRAP report, 2014) concluded that sulfur dioxide and/or inorganic sulfites are not genotoxic. More detailed information including in-depth discussions of the limitations of

the genotoxicity studies referred to in the CLH report can be found in these documents prepared by EFSA or MSCA Hungary.

In line with this, sulfur dioxide or related inorganic sulfites have been evaluated as non-genotoxic/non-mutagenic by a series of other scientific organizations including SCCS (2003), German MAK Commission (2014), and very recently also the US EPA re-evaluated sulfur dioxide as non-genotoxic (2020). Consequently, sulfur dioxide and inorganic sulfites are considered as non-genotoxic regarding their uses in cosmetic products and as important food additives in the EU as well as as pesticide in the US.

In the absence of any new study providing clear evidence for a genotoxic potential of sulfur dioxide, and considering the intended harmonized assessment of a substance under various regulations, we do not consider the classification of sulfur dioxide as Muta. 2 according to CLP regulation as justified.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment CLH comments Micro-Pak_public.pdf
ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment CLH comments Micro-Pak.pdf

Dossier Submitter's Response

Unfortunately, only little of the available data has been acquired and reported in a way complying with current OECD and EU guidelines for the testing of chemicals. All strengths and weaknesses of the individual studies (including Sorsa et al., 1982) have been taken into account by the DS in a weight of evidence approach to conclude on the hazard genotoxicity.

Therefore, appropriate care needs to be taken in its interpretation. Nevertheless, the data package provides the information required for an assessment of the human health effects of sulphur dioxide. This approach was also taken to avoid further testing in animals. As stated in the CLH report, the proposal for Muta. 2 is based on positive evidence from experiments in mammals supported by some in vitro findings. Some indications for genotoxicity in lymphocytes of exposed workers were also available. The studies which failed to show genotoxic responses are not considered sufficient reliable to discredit positive genotoxicity studies in vitro and in vivo.

Of note, the Kunming mouse is an established strain in China (Cui, L.-B. et al. (2013) The Kunming mouse: as a model for age-related decline in female fertility in human. Zygote, 21, 2013. DOI: 10.1017/S0967199412000123). According to the study authors, the Kunming mouse "[…] has been a native breed, making up 70 per cent of all mice used in reaerch in China."

It was noted by Micro-Pak Europe BV that the US EPA re-evaluated sulphur dioxide and concluded that it is non-genotoxic. An in-depth comparison of the database and reliability assessment between the CLH report and the re-evaluation by US EPA would be needed before concluding on the relevance and impact of the US EPA evaluation.

RAC's response

See response to comment #6.

Date	Country	Organisation	Type of Organisation	Comment number
12.11.2020	Germany	Sulphuric Acid REACH Consortium (SAC)	Industry or trade association	8

Comment received

The analysis of the available data as employed in the CLH report does not appear to transparently weigh the relevance, reliability and adequacy of the data sources. The dossier submitter states on page 65 that "conflicting results are in line with the observation that results are highly dependent on test conditions", which is not in-line with the legal requirements, whereby tests shall be conducted according to accepted test guidelines (cf. CLP regulation Art. 8(3), REACH regulation Art. 13(3), BPR Annex II 2(5)). Test guidelines were implemented in order to obtain reliable, reproducible data under standardised conditions. Modifications of the test conditions that compromise the validity of the study need to be taken into account and render such studies less relevant for regulatory purposes such as classification and labelling. Further, the selection of studies to be compared against the CLP criteria remains unclear and unexplained, therefore the decision on the classification lacks transparency.

The Registrant has undertaken a thorough evaluation of all available data and has compared the data against the classification criteria as laid down in the Guidance on the Application of the CLP criteria (ECHA, 2017) in a weight-of-evidence analysis. A detailed analysis is presented in Table 1 below and a detailed study-by-study specific quality evaluation is given in Annex III. The outcome of this weight-of-evidence analysis can be summarised as follows:

- No evidence for in vitro mutagenicity in bacteria
- Equivocal evidence for in vitro clastogenicity/aneugenicity in a large number of references considered unreliable
- No evidence for in vitro mutagenicity in mammalian cells
- No evidence for in vivo clastogenicity, positive findings originate largely from unreliable studies via unphysiological routes of exposure
- Positive findings were largely obtained from studies published by one and the same working group of Meng and Zhang (Shanxi University), whose study design and reporting shows recurring deficiencies (such as using a mouse strain with questionable suitability for genetic toxicity testing)

In contrast to the CLH report, the LR and SAC is of the opinion that the available body of evidence on genetic toxicity supports the conclusion that sulfur dioxide does not elicit any mutagenic activity.
Without considering the reporting quality of the publications, both positive and negative findings are reported in in vitro and in vivo test systems. However, following rigorous relevance and reliability screening, it can be concluded that sulfur dioxide/sulfites do not show any clastogenic potential. The references discussed under in vitro clastogenicity are rated as not reliable due to experimental and reporting deficiencies and do not show a consistent pattern on the induction of chromosome and genome mutations. A high-quality in vivo study with sodium sulfite via subcutaneous injection in mice did not show an increase of micronuclei formation up to the maximum tolerated dose. This finding is supported by a negative dominant lethal test in rats after single and repeated oral administration (feed) in rats. A number of in vivo clastogenicity studies were assessed as being of limited reliability, since these exhibit reporting and/or other experimental

deficiencies and lack biological plausibility. Overall, there is no consistent evidence documenting genetic toxicity with relevance to humans for sulfites.
This conclusion is for example also confirmed by the EFSA panel after review of more than 60 studies with sulfur dioxide, sodium sulfite, sodium bisulfite, sodium metabisulfite and potassium metabisulfite (EFSA, 2016), with an overall conclusion as follows: "Overall, based on these data the Panel concluded that the use of sulfur dioxide and sulfites (sodium sulfite, sodium bisulfite, sodium metabisulfite, potassium metabisulfite, potassium bisulfite, calcium sulfite and calcium bisulfite) as food additives does not raise a concern with respect to genotoxicity."
Finally, we wish to note that the substance disodium disulfite (synonym sodium metabisulfite) was subject 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. In their concluding report dated 30 October 2015, the following conclusion concerning the endpoint genetic toxicity was drawn by the eMS: "the evaluating Member State is of the opinion that there is very vague and inconsistent evidence of induction of genetic toxicity with relevance to humans for sulphites, and considers, based upon the available information, that the concern for mutagenicity is no longer substantiated. Thus, also classification for mutagenicity seems not warranted".

Overall conclusions
The data base cited by the DS is incomplete and the selection of the studies to be compared against the CLP criteria remains unclear and unexplained. The CLH proposal does not transparently weigh the relevance, reliability and adequacy of the selected data sources. Positive findings were largely obtained from studies published by one and the same working group of Meng and Zhang (Shanxi University), whose study design and reporting shows substantial deficiencies.
In contrast to the opinion of the DS, the LR and SAC are of the opinion that there is no evidence for in vitro mutagenicity, on bacteria, at best equivocal evidence for in vitro clastogenicity (with a large number of unreliable references) or in vitro mutagenicity, no evidence of in vivo clastogenicity (with positive findings only from studies with unphysiological routes of exposure). The cited studies in humans suffer from a lack of consideration of coexposures with other chemical agents, which however cannot be ruled out under the described industrial operations.
Finally, the genotoxicity data base has already been recently reviewed by several other reputable scientific organisations (including EFSA), all concluding on an absence of concern for genotoxicity.
For more details on the weight-of-evidence analysis for in vitro and in vivo genetic toxicity data please refer to Table 1 in the attached document.

General and detailed scientific comments
General comments on data selection and reliability and quality assessment
Overall, the quality assessment of the underlying hazard data did not follow the criteria laid down in ECHA guidance. On page 22 of the CLH report it is stated: "As human health effect assessment bases almost completely on published information, reliability can rarely be scored better than "reliable with restrictions" which is equivalent to Klimisch score 2. As a consequence, key studies are generally defined on the basis of studies with reliability scores of 2 if the results of these are supported by other studies." To this, we must note that the reliability of a study is not per-se reduced due to the fact that the work is published but instead requires an evaluation of the inherent quality of a test report or publication, relating to a standardised methodology.
Furthermore, the selection of references on sensitisation and genotoxicity referred to in the CLH report appears arbitrary, since the criteria for study selection are not stated. On

page 22 of the CLH report it states: "Not all references available were considered relevant for hazard assessment. Due to the vast amount of studies submitted and additionally retrieved from scientific literature search, the DS refrained from listing of all studies that were not used for hazard assessment (e.g. due to poor reliability)."
The omission of relevant information without proper justification is clearly not in compliance with the legal requirements (CLP regulation Art. 37(1) in conjunction with Annex VI, Part 2 and regulation 1907/2006 Annex I, Section 1-3) and raises concerns that the hazard assessment presented in the CLH report is based on a biased position.
In the sake of brevity, this document includes a listing (in Appendices II and III) of the limited data selected by the DS in comparison to the more comprehensive data bases, for example, of the EFSA (2016) opinion and the REACH registration dossier on sulfur dioxide, demonstrating the incomplete and selective choice of references in the Dossier submitters CLH proposal.

Read-across concept for sulfur dioxide, sulfites, hydrogensulfites and metabisulfites
Sulfur dioxide is very soluble in water and forms – as an anhydride – sulfurous acid. Since all physiological processes within e.g. the human body are bound to proceed in aqueous solutions, a comprehensive read-across concept has been developed for sulfur dioxide, sulfites, hydrogensulfites and metabisulfites, based on the pH-dependent equilibrium in aqueous solutions which is summarised in the following equations:
SO2+ H2O <->`H2SO3´ H2SO3<->H+ + HSO3-<->2H++SO32- 2HSO3-<->H2O +S2O52-
Since the nature of the cation (i.e., sodium, potassium, ammonium…) is not assumed to contribute substantially to differences in toxicity and solubility (all compounds are very water soluble), with only the chemical and biological properties of the anion considered as relevant determinants. Based on the described equilibrium correlations, unrestricted read-across between the groups of sulfites, hydrogensulfites and metabisulfites is considered justified.
A detailed read-across assessment framework (RAAF) document is attached as Appendix I in the attachment.

Endogenous role of SO2/sulfites and toxicokinetic considerations
Human organ tissues are continuously exposed to endogenous levels of sulfite (SO32-), generated from sulfur-containing amino acids via the cysteine metabolism pathway. These endogenous sulfite anions are transformed to sulfate via the enzyme sulfite oxidase. Sulfite oxidase is present in all mammalian tissues at varying concentrations, except in rare cases of individuals suffering from sulfite oxidase deficiency, a rare autosomal recessive disease. This can lead to severe neurological abnormalities, seizures, mental retardation, and dislocation of the ocular lenses and often leads to death in infancy. Such sulfite oxidase deficiency can arise either from a mutation in (i) the sulfite oxidase gene (isolated sulfite oxidase deficiency), or (ii) that of genes involved in the synthesis of molybdenum cofactors, usually leading to combined deficiencies of molybdoenzyme activities (Johnson & Wadman, 1995).
The mean concentrations (± SD) of "normal" background total serum sulfite in female (n = 41) and male (n = 35) human subjects are 4.63 ± 2.3 and 5.16 ± 2.68 µmol/L, respectively (not statistically significant: P = 0.368). The combined mean concentration of total sulfite in both sexes is 4.87 ± 2.49 µmol/L (Ji et al, 1995).
It has been estimated that humans excrete about 25 mmol (2400 mg) in their urine each day, the majority (up to 24 mmol) of which is generated from endogenous sulfite (Institute of Food Technologists Expert Panel on Food Safety and Nutrition, 1975).
Upon systemic uptake, sulfites are distributed widely between tissues because of their high solubility/bioavailability and are cleared almost exclusively by oxidation to sulfate

with subsequent renal excretion. Sulfite administered intravenously is cleared rapidly in the rhesus monkey. It has a biological half-life of 10 minutes for doses in the range of 0.3 to 0.6 mmole/kg. Based on data from rats and monkeys, Gunnison and Jacobsen (1983) extrapolated that the half-life of sulfite in man is ca. 15 minutes. Thus, for example, approximately 0.25 mg of a lag dose of potassium metabisulfite would remain in body fluids 30 minutes after ingestion which is in agreement with the findings by Gunnison (1981) that chronically ingested sulfite does not accumulate in the tissues and reaches an elevated steady-state level but is rapidly eliminated after absorption.
The capacity of sulfite oxidase (SOX) is usually very high in mammalian species. SOX activity has been measured in the liver, kidney and heart, the highest enzyme expression being in the liver, but the brain, spleen, lungs and testis have been found to have low SOX activity (Gunnison, 1981; Institute of Food Technologists Expert Panel on Food Safety and Nutrition, 1975): based on projections from in vitro assays of sulfite oxidase, Cohen et al. (1973) calculated that the enzyme could theoretically oxidise sulfite at a rate of 750 mmol/kg/day(48g of SO2/kg/day). Using perfused dog livers, Wilkins et al. (1968) demonstrated that sulfite could be oxidised at a rate of 0.8 mmol/kg/hr, which equates to a daily rate of 19 mmol/kg (1200mg of SO2/kg/day). Oshino and Chance (1975) showed that perfused rat livers were capable of even faster sulfite oxidation, with a rate of 2.4 mmol/kg/hr or 58 mmol/kg/day (3700 mg of SO2 / kg/day). In experiments with intact animals, Yokoyama et al. (1971) and Bhagat and Lockett (1960) observed that dogs and rats, respectively, could metabolise inhaled SO2, and ingested bisulfite to sulfate readily, with the majority of the dose appearing in the urine as sulfate within a short time after administration. Gibson and Strong (1973) observed that the majority of an oral dose of sulfite, equivalent to 50 mg SO2/kg, was excreted in the urine as sulfate within 24 hr. They could not detect urinary sulfite, indicating extremely efficient oxidative metabolism. Gibson et al. (1973) demonstrated that 10 and 50 mg/ SO2/kg bw administered as mixture of HSO3/Na235SO3 noted 70-95% of the 35SO32- was absorbed in the intestine and excreted within 24hrs via urine. Rats given oral doses of sodium metabisulfite as a 0.2% solution eliminated 55% of the sulfur as sulfate in the urine within the first four hours (Bhaghat et al. 1960). The physiologically essential rapid oxidation and elimination in sulfite-oxidase competent of the general population renders sulfite substances as being well tolerated. In contrast, the extremely low prevalence of sulfite-sensitive individuals due to their sulfite-oxidase deficiency does not serve as classification argument. Long-term animal studies (e.g. Til et al., 1972) support this assumption.

For a detailed comment on the CLH-proposal, please see the attachment.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Final CLH-Comment SO2 EBRC 11NOV2020_Redacted.pdf

Dossier Submitter's Response

Unfortunately, only little of the available data has been acquired and reported in a way complying with current OECD and EU guidelines for the testing of chemicals. In the EFSA opinion from 2016 it is also 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 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. In order to reach a conclusion based on the available information, all strengths and weaknesses of the individual studies have been taken into account by the DS in a weight of evidence approach to conclude on genotoxicity. Therefore, appropriate care needs to be taken in the interpretation of the

available studies. This decision was also taken to avoid further testing in animals. Please note that studies with a reliability index of 2 are still acceptable from the regulatory perspective.

The weight of evidence analysis by SAC is not comprehensible. Whilst the mode of action of genotoxicity for sulphur dioxide is mainly focussed on clastogenicity, positive results were also observed in bacterial mutagenicity assays with metabisulphites and bisulphites for which a read-across was applied (and supported by NL, comment 2 and SAC).

The DS agrees that many results of the in vitro and in vivo genotoxicity studies are equivocal. However, the DS maintains its opinion that the positive results obtained in the in vivo studies cannot be superseded by studies in which no genotoxic effect was apparent – in particular if the study design is flawed and the acceptability of the study is questioned.

Inclusion of exposure considerations such as endogenous production of sulphur dioxide as well as possible detoxification mechanisms is not foreseen for hazard identification which is the basis for classification purposes. There are several compounds classified for germ cell mutagenicity irrespective of detoxification mechanisms. Such considerations are rather related to risk assessment.

RAC's response

See response to comment #6. Regarding read across for sistemic routes of exposure, thank you for the support.

Date	Country	Organisation	Type of Organisation	Comment number
12.11.2020	Spain	AFEPASA (Azufrera y Fertilizantes Pallarés, S.A.U.)	Company-Manufacturer	9

Comment received

My comments are in the attached public document

ECHA note – An attachment was submitted with the comment above. Refer to public attachment AFEPASA Comments to the August 2020 SO2 CLH Report.pdf

Dossier Submitter's Response

Response to individual considerations presented in the attachment by AFEPASA is given below:

(1) All studies used as key studies for the overall evaluation of mutagenicity are marked as "key study" in column 1 of the respective table. It becomes obvious that more than 2 studies (as mentioned by AFEPASA) have been assigned as key studies by the DS. The study by Ziemann (2008) was judged as most convincing by AFEPASA. The deficiencies of this study are extensively described in the CLH dossier. Considering the limitations in reliability, the (dose-dependent) positive findings in other in vivo studies are not outweighed by the evidence from Ziemann.

(2) Justification for down- or upgrading reliability scores of the individual studies by DS is given transparently in the CLH dossier.

(3) Unfortunately, only little of the available data has been acquired and reported in a way complying with current OECD and EU guidelines for the testing of chemicals. Thus, a weight of evidence approach was applied by DS taking into account all submitted studies. Finally, there was a convincing evidence that the test compound

(evaluated within the read-across concept) has clastogenic activity in vitro and also in vivo.

(4) Argumentation via detoxification mechanisms and endogenous formation of sulphur dioxide are important to consider for risk assessment, but not part of the hazard identification being decisive for classification.

It is further pointed out that the EFSA decision from 2016 is considered temporary as the database was far from optimal. Thus, an improvement of the database was recommended by EFSA.

RAC's response

See response to comment #6.

Date	Country	Organisation	Type of Organisation	Comment number
10.11.2020	Netherlands		MemberState	10

Comment received

NL-CA agrees with the dossier submitter's proposal for classification in category 2 for mutagenicity.

Positive evidence for mutagenicity is found in in vitro studies in bacteria (at pH < 7, physiological less relevant) and mammalian cells, and genotoxicity was demonstrated in vivo studies, though noticing the limitations of some of the studies. Moreover, the in vitro and in vivo studies present somewhat inconsistent findings. Nevertheless, we agree with the dossier submitter that negative results of for example the in vivo micronucleus study of Anonymous 6 (2008)/Anonymous 7 (2010) cannot be used to disregard the positive effects observed in other studies. Indications for genotoxicity was also observed in multiple epidemiological studies related to occupational exposure. Furthermore, no confounding effect for smoking was found on sulfur dioxide-induced genotoxicity in workers exposed to sulfur dioxide by Meng et al. (1989).

No evidence on sulfur dioxide-induced mutagenicity was found in germ cells and therefore category 1B is not warranted. However, there is sufficient data available of in vivo mutagenicity and genotoxicity studies to warrant category 2.

Dossier Submitter's Response

Thank you for the support. No response required.

RAC's response

RAC appreciates the support. See also response to comment #6.

Date	Country	Organisation	Type of Organisation	Comment number
06.11.2020	France		MemberState	11

Comment received

In vitro assays on sulphite, bisulphite, metabisulfite:
Some assays did not include positive controls (ex. Ishidate et al., 1984; Engelhardt, 1989). In case of negative results, it questions the sensitivity of the test to identify mutagenic responses. In addition, interpretation of negative results should be made considering associated cytotoxicity. If no cytotoxicity was observed, the tested doses may be not high enough to detect mutagenic effect.

The MLA assay with sodium metabisulfite is considered by the DS as negative (Stone et al. 2010). However, according to the evaluation and interpretation of results described in the OECD guideline 476, the results should be considered as equivocal since one experiment was positive.

In vitro assays on SO2:
In the Anses opinion on a study of the genotoxicity of sulphur dioxide (Anses, 2013), we have concluded that "SO2 was found to be non-mutagenic in vitro in studies (Pool et al., 1988; Zeller et al., 1988; Pool-Zobel et al., 1990) deemed of little relevance or even unacceptable in view of the presence of numerous defects (studies not following OECD guidelines, SO2 tested in association with genotoxic substances, etc.)."

We note that a new study is available since this opinion, which demonstrate an increased micronucleus in vitro.

In vivo assays on SO2:
The following comments are issued from ANSES opinion on a study of the genotoxicity of sulphur dioxide (Anses, 2013)

Comet assay (Anonymous, 2005): a good cellular viability was observed in this study using the trypan blue protocol. However, high toxicity was found with other more sensitive methods (histological method with haematoxylin and eosin staining, or transmission electron microscopy) in the lungs, liver and spleen of mice exposed to SO2. This difference may have led to a probable underestimation of toxicity in the study and it is not possible to totally rule out the fact that the DNA damage could be the result of interference with cytotoxicity such as apoptosis and necrosis, clearly identified after haematoxylin and eosin staining.

Mouse micronucleus assay (Anonymous, 2008): The PCE/NCE ratio did not significantly decrease, suggesting an absence of cytotoxicity or that the target organ was not reached. This result contradicts the high cytotoxicity observed in the chromosomal aberration study. Furthermore, an increase in the level of erythrocyte malondialdehyde, indicative of lipid peroxidation, suggests the presence of significant oxidative stress and therefore of systemic toxicity.

Mouse micronucleus assays (Anonymous, 2002 and 2003): Both studies found a dose-dependent increase in the frequency of micronuclei in the polychromatic erythrocytes. No determination of the PCE/NCE ratio (polychromatic erythrocytes/normochromatic erythrocytes) was done, which could have provided proof of exposure of bone marrow. However, high cytotoxicity cannot be ruled out, given the effects observed at doses above 14 mg/m3 in the chromosomal aberration study, carried out under similar experimental conditions. It may therefore be the case that this genotoxic effect only occurs at cytotoxic doses.

It is unlikely that the differences in the age and strains of the mice used in these micronucleus studies (6 week-old Kunming mice and 8-to-12-week-old NMRI mice) would have a sufficient effect to explain the discordant results. It is nonetheless worth noting that there is currently little information available about the genetic status of the Kunming strain.

Mouse chromosomal aberration test (Anonymous, 2002): A dose-dependent increase in

chromatid-type aberrations at low concentrations (from 7 to 28 mg/m3) and chromosome-type aberrations at higher concentrations (56 mg/m3), were observed in a context of high cytotoxicity (reduced mitotic index) from 14 mg/m3. No multiple exchanges or rearrangements, indicative of clastogenesis, were observed in this study. In vivo assays on sulphite, bisulphite, metabisulfite: Could you please clarify if the negative assays (NTIS, 1972; Anonymous, 1983; Anonymous, 2008) included a positive control and/or if there was any proof of adequate systemic exposure? Human data: Biomonitoring studies in workers mostly show genotoxic effects such as chromosomal aberrations in peripheral lymphocytes (Nordenson et al, 1980; Sorsa et al, 1982; Yadav et al, 1996; Meng et al, 1990a and b). However, considering the number of confounding factors present (multiple exposure, smoking, etc.) and the small size of the population groups surveyed, it is difficult to draw satisfactory conclusions from these studies. In conclusion, clastogenic effects are reported in in vitro and in vivo assays. In vivo assays with negative results are often associated with a lack of positive control or without a clear demonstration of adequate systemic exposure. Regarding gene mutations, in vitro results are conflicting. However, in vivo Comet assays (positive results in Anonymous, 2005 with SO2 and Anonymous, 2011 with sodium metabisulfite) can also bring information regarding gene mutation endpoint and should be considered in the argumentation of the DS. Inhaled SO2 is rapidly metabolised in the respiratory tract into sulphuric acid, which then breaks down into sulphite/bisulphate and hydrogen ions. The systemic toxicity of SO2 could therefore be due to sulphites. Genotoxicity profile of the sulphites considered in the CLH report is consistent with that of SO2. In addition, it seems that, in vivo, SO2 induces the production of reactive oxygen species, which themselves 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, 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. Based on all the mutagenic effects in somatic cells / organs reported both in vitro and in vivo (mainly as clastogenic effect), FR agrees that SO2 must be classified as a mutagenic agent. Considering the hypothesized mode of action and the absence of evidence that the reproductive organs can be reached, FR agrees with the proposal as Muta category 2.
Dossier Submitter's Response
Thank you for the support. No response required.
RAC's response
RAC appreciates the support and the very clear elaboration of the MS opinion. See also response to comment #6.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
12.11.2020	Germany	Sulphuric Acid REACH Consortium (SAC)	Industry or trade association	12
Comment received				
The LR and SAC have no comments and can agree to the proposed classification. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Final CLH-Comment SO2 EBRC 11NOV2020_Redacted.pdf				
Dossier Submitter's Response				
No response required.				
RAC's response				
RAC appreciates the support.				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
12.11.2020	Germany	Sulphuric Acid REACH Consortium (SAC)	Industry or trade association	13
Comment received				
Overall, the LR and SAC disagree with the proposed classification, because it is scientifically unjustified in our opinion, for the following reasons: Under physiologically and environmentally relevant pH conditions, sulfur dioxide in contact with water reacts immediately to form an equimolar equilibrium of sulfite and hydrogensulfite ("bisulfite") anions. For this reason, the dossier submitter (DS) makes extensive use of read-across from several sulfite substances to sulfur dioxide. Also, in the REACH registration dossiers for a wide range of these sulfite substances, extensive read-across between these substances is made, since all these substances upon dissolution in water (as also for sulfur dioxide) will form "sulfite" anions. Among others, these substances cover the sodium, potassium and calcium sulfites and their hydrogensulfite (bisulfite) counterparts, as well as sodium and potassium disulfites (synonym "metabisulfite"). The data referred to in the CLH proposal are however only a fragmentary reflection of data available in the public domain. In contrast, the REACH registration dossiers for sulfur dioxide as well as the read-across sulfite substances contain several dozen case reports or studies involving several patients, the overwhelming majority of which according to their authors do not provide evidence for skin sensitisation. In the selection and interpretation of data, the dossier submitter does not distinguish between a suspected induction of skin sensitisation after topical application (contact allergy) and hypersensitivity. Whereas for the latter several mechanisms are still under discussion, immediate (cutaneous) symptoms in sulfite sensitive patients after ingestion are not widely considered to have the same mechanism usually identified for skin sensitisers: skin sensitisers typically cause a delayed onset of clinical symptoms/skin reactions which are clearly different from an immediate systemic response after ingestion or inhalation. According to the Regulation 1272/2008 (CLP), a skin sensitiser is defined as				

"… a substance that will lead to an allergic response following skin contact". The few cases of immediate systemic reaction including dermal symptoms cited in the CLH proposal do not per se qualify a chemical as a skin sensitiser or contact allergen.
Whether or not sulfites can elicit skin sensitisation has been independently assessed by several renowned scientific organisations, and the outcomes of their evaluations can be summarised as follows:

- WHO in this context has observed that food intolerances sometimes cause symptoms similar to those of food allergies and have assigned the term "pseudoallergic" food intolerance for such cases (WHO, 2012).

- SCF (1997), SCCNFP (2003), CIR (2003), EFSA (2004), MAK (2014), EFSA (2016) have reviewed the available data on skin sensitisation of sulfites; in addition, several OECD SIDS (2001) exist, based upon which all OECD Member States agreed on a mutually agreed dataset ("MAD"), which concluded on an absence of sensitising effects.

All scientific bodies as mentioned above uniformly concluded that sulfites are not to be considered as (relevant) skin sensitisers based on the very low incidence of patch test responses to sulfites in dermatitis patients.

The LR and SAC therefore do not see a qualified basis for the classification proposal, since the DS does not provide any substantial information contrary or beyond that already evaluated by the scientific organisations listed above.
Whereas some human clinical reports suggest that sulfites may elicit allergy-like responses, the incidence is by comparison extremely low considering that the general population is more or less constantly exposed to sulfur dioxide/sulfites through consumption of foodstuffs, cosmetics and/or pharmaceutical products containing these substances either added as antioxidants intentionally or due to their natural background content. Although IgE mediated reactions as a contributing factor have been discussed, these have never been confirmed. Instead, there is a certain prevalence of susceptible individuals with sulfite oxidase deficiency which render these individuals particularly sensitive to ingestion of sulfite substances.
In contrast, the CLH proposal appears to mix any type of response or intolerance after oral, dermal or inhalation exposure regardless of whether immediate or delayed, systemic or skin reaction. The thus presented information in the CLH proposal does therefore not provide sufficient evidence that sulfur dioxide and/or sulfites are potential skin sensitisers. Animal tests have not given any positive response to any of the sulfite substances with no epidemiological study on the general population being available.
The LR and SAC also contend that the substance Disodium disulfite (synonym sodium metabisulfite) was subject 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. In their concluding report dated 30 October 2015, the following conclusion concerning the endpoint sensitisation was drawn by the eMS:
"Based on the evaluated literature data it is unlikely that disodium disulphite 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 disulphite 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. With regard to skin sensitisation the conclusion is also supported by the review of the available study performed by the German MAK Commission in 2014, who also concluded that in view of the widespread use of disodium disulphite, and therefore the numerous possibilities for contact in everyday life and the

occupational field, the number of persons dermally sensitised is, however, very small." Likewise, EFSA (2016) in their most recent and detailed re-evaluation, while noting that there are "reports of sensitivity and/or intolerance reactions in humans exposed to sulfited foods and beverages" among others conclude that "IgE tests were usually negative indicating that the reactions were not immune-mediated, and sensitivity reactions were mostly intolerance reactions".

Summary
Available information from two animal studies on skin sensitisation according to OECD 429 (GLP) of sulfites (sodium sulfite and sodium metabisulfite) do not indicate any sensitisation potential.
Clinical studies suggest that sulfites may elicit allergy-like responses, but the overall incidence is considerably low keeping in mind a more or less continuous exposure of the general population via foodstuffs, cosmetics and/or pharmaceutical products containing sulfur dioxide/sulfites.
In this context, IgE mediated reactions are often discussed (Sokol and Hydick, 1990, Wüthrich and Huwyler (1994) but were never confirmed free of doubt. Belchi-Hernandez et al. (1993) described clinical manifestations (urticaria-angioedema) in a patient that suggested an IgE mediated mechanism, but skin prick tests were all negative and the oral challenge with sodium metabisulfite was not inhibited by prior administration of cromolyn sodium. The latter inhibits chloride channels in activated mast cells and impedes histamine release. Since histamine release was obviously not inhibited another mechanism (not IgE mediated) needs to be considered. The authors suggested e.g. parasympathic stimulation (hypotension associated with bradycardia, flushing and gastrointestinal symptoms).
Instead of assuming a skin sensitising property for sulfite substances, it appears more appropriate to conclude that sulfites as additives in food can cause food intolerances in sensitive individuals.
Combining both sources, the available information and results do not provide sufficient evidence that sulfur dioxide and/or sulfites are potential skin sensitisers. No animal test or non-standard method gave any positive response to any of the sulfite substances and no epidemiological study on the general population is available.
Further, widespread use of sulfur dioxide/sulfites forced several national and international scientific organisations to examine and evaluate the skin sensitisation potential of sulfites quite comprehensively (see above). All organisations uniformly concluded that sulfites are not to be considered as (relevant) skin sensitisers regarding the incidence of patch test responses to sulfites in dermatitis patients considering the widespread use and the resulting frequent worker and consumer exposure.

Finally, in the SUBSTANCE EVALUATION CONCLUSION DOCUMENT as required by REACH Article 48 for Disodium disulphite (EC No 231-673-0, CAS No 7681-57-4), Evaluating Member State(s): Hungary, Dated: 30 October 2015, the following conclusion concerning the endpoint sensitisation was drawn by the eMS:
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occupational field, the number of persons dermally sensitised is, however, very small." Therefore, the classification criteria of sulfur dioxide and sodium metabisulfite as skin sensitisers are not met according to CLP Regulation.

Overall conclusions
Instead of providing a detailed analysis of available data on sensitisation, the DS has cited an arbitrary selection of references on human case reports, thus rendering this assessment essentially incomplete. Most importantly, the DS but has omitted to verify whether the criteria for actual sensitisation are met in the studies the CLH proposal refers to.
The CLH report further fails to consider the scientific opinions of several reputed scientific organisations (including EFSA) which altogether do not recognise a concern for sensitisation.
Thus, the LR and SAC are of the opinion that since the classification criteria for skin sensitisation are not met by the group of read-across "sulfite" substances, a classification of sulfur dioxide as skin sensitiser is likewise not warranted.

General and detailed scientific comments
General comments on data selection and reliability and quality assessment
Overall, the quality assessment of the underlying hazard data did not follow the criteria laid down in ECHA guidance. On page 22 of the CLH report it is stated: "As human health effect assessment bases almost completely on published information, reliability can rarely be scored better than "reliable with restrictions" which is equivalent to Klimisch score 2. As a consequence, key studies are generally defined on the basis of studies with reliability scores of 2 if the results of these are supported by other studies." To this, we must note that the reliability of a study is not per-se reduced due to the fact that the work is published but instead requires an evaluation of the inherent quality of a test report or publication, relating to a standardised methodology.
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In the sake of brevity, this document includes a listing (in Appendices II and III) of the limited data selected by the DS in comparison to the more comprehensive data bases, for example, of the EFSA (2016) opinion and the REACH registration dossier on sulfur dioxide, demonstrating the incomplete and selective choice of references in the Dossier submitters CLH proposal.

Read-across concept for sulfur dioxide, sulfites, hydrogensulfites and metabisulfites
Sulfur dioxide is very soluble in water and forms – as an anhydride – sulfurous acid. Since all physiological processes within e.g. the human body are bound to proceed in aqueous solutions, a comprehensive read-across concept has been developed for sulfur dioxide, sulfites, hydrogensulfites and metabisulfites, based on the pH-dependent equilibrium in aqueous solutions which is summarised in the following equations:
SO2+ H2O <->`H2SO3´ H2SO3<->H+ + HSO3-<->2H++SO32- 2HSO3-<->H2O +S2O52-

Since the nature of the cation (i.e., sodium, potassium, ammonium…) is not assumed to contribute substantially to differences in toxicity and solubility (all compounds are very water soluble), with only the chemical and biological properties of the anion considered as relevant determinants. Based on the described equilibrium correlations, unrestricted read-across between the groups of sulfites, hydrogensulfites and metabisulfites is considered justified.
A detailed read-across assessment framework (RAAF) document is attached as Appendix I in the attachment.

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The mean concentrations (± SD) of "normal" background total serum sulfite in female (n = 41) and male (n = 35) human subjects are 4.63 ± 2.3 and 5.16 ± 2.68 µmol/L, respectively (not statistically significant: P = 0.368). The combined mean concentration of total sulfite in both sexes is 4.87 ± 2.49 µmol/L (Ji et al, 1995).
It has been estimated that humans excrete about 25 mmol (2400 mg) in their urine each day, the majority (up to 24 mmol) of which is generated from endogenous sulfite (Institute of Food Technologists Expert Panel on Food Safety and Nutrition, 1975).
Upon systemic uptake, sulfites are distributed widely between tissues because of their high solubility/bioavailability and are cleared almost exclusively by oxidation to sulfate with subsequent renal excretion. Sulfite administered intravenously is cleared rapidly in the rhesus monkey. It has a biological half-life of 10 minutes for doses in the range of 0.3 to 0.6 mmole/kg. Based on data from rats and monkeys, Gunnison and Jacobsen (1983) extrapolated that the half-life of sulfite in man is ca. 15 minutes. Thus, for example, approximately 0.25 mg of a lag dose of potassium metabisulfite would remain in body fluids 30 minutes after ingestion which is in agreement with the findings by Gunnison (1981) that chronically ingested sulfite does not accumulate in the tissues and reaches an elevated steady-state level but is rapidly eliminated after absorption.
The capacity of sulfite oxidase (SOX) is usually very high in mammalian species. SOX activity has been measured in the liver, kidney and heart, the highest enzyme expression being in the liver, but the brain, spleen, lungs and testis have been found to have low SOX activity (Gunnison, 1981; Institute of Food Technologists Expert Panel on Food Safety and Nutrition, 1975): based on projections from in vitro assays of sulfite oxidase, Cohen et al. (1973) calculated that the enzyme could theoretically oxidise sulfite at a rate of 750 mmol/kg/day(48g of SO2/kg/day). Using perfused dog livers, Wilkins et al. (1968) demonstrated that sulfite could be oxidised at a rate of 0.8 mmol/kg/hr, which equates to a daily rate of 19 mmol/kg (1200mg of SO2/kg/day). Oshino and Chance (1975) showed that perfused rat livers were capable of even faster sulfite oxidation, with a rate of 2.4 mmol/kg/hr or 58 mmol/kg/day (3700 mg of SO2 / kg/day). In experiments with intact animals, Yokoyama et al. (1971) and Bhagat and Lockett (1960) observed that dogs and rats, respectively, could metabolise inhaled SO2, and ingested bisulfite to sulfate readily, with the majority of the dose appearing in the urine as sulfate within a short time after

administration. Gibson and Strong (1973) observed that the majority of an oral dose of sulfite, equivalent to 50 mg SO2/kg, was excreted in the urine as sulfate within 24 hr. They could not detect urinary sulfite, indicating extremely efficient oxidative metabolism. Gibson et al. (1973) demonstrated that 10 and 50 mg/ SO2/kg bw administered as mixture of HSO3/Na235SO3 noted 70-95% of the 35SO32- was absorbed in the intestine and excreted within 24hrs via urine. Rats given oral doses of sodium metabisulfite as a 0.2% solution eliminated 55% of the sulfur as sulfate in the urine within the first four hours (Bhaghat et al. 1960). The physiologically essential rapid oxidation and elimination in sulfite-oxidase competent of the general population renders sulfite substances as being well tolerated. In contrast, the extremely low prevalence of sulfite-sensitive individuals due to their sulfite-oxidase deficiency does not serve as classification argument. Long-term animal studies (e.g. Til et al., 1972) support this assumption.

For a detailed comment on the CLH-proposal, please see the attachment.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Final CLH-Comment SO2 EBRC 11NOV2020_Redacted.pdf

Dossier Submitter's Response

DS clearly disagrees with the IND position in the conclusion that DS has cited an arbitrary selection of references on human case reports. 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. Analyses of human patch tests in different populations of patients were cited and these studies formed the basis of classification according to the criteria of the CLP guidance. Case reports are listed in a separate section of the CLH dossier and indicated as "Case reports (probably IgE-mediated allergic reactions)". The DS agrees that there are more skin sensitisation studies available from the open literature for sulphur dioxide. However, on balance, these would not change the classification proposal and have thus not been included. More importantly, recent data that was not previously available coming from one of the largest dermatology/allergology networks in EU, the IVDK clearly show patch test reactions for sodium metabisulfite in 357 of 12,156 patients tested (Uter et al., 2018, DOI:10.3390/ijerph15061108). This corresponds to a sensitisation rate of 3% and would - according to CLP guidance - warrant classification for skin sensitisation in sub-category 1A as well. In contrast to the statement of IND, 3-6% (see studies below) is not regarded by DS as "very low incidence of patch test responses to sulfites in dermatitis patients". High frequency is defined as 1.0% dermatitis patients (unselected, consecutive) (Guidance on the Application of the CLP Criteria V.5, 2017). High frequency is also the result of human patch test studies listed in the CLH-Report: 5% in Garcia-Gavin et al. (2012), 6% in Oliphant et al. (2012), and 4% in Madan et al. (2007).

To the knowledge of the DS, dermatologists distinguish between immediate urticaria/irritation/pseudo-allergic reactions and the delayed-type reaction ACD. In the EU, clinics for dermatology/allergology conduct standardised patch testing according to EU-accepted guidelines. In addition, sodium metabisulfite is a standard allergen included in testing baseline series number 38 for preservatives of the German Contact-Allergy-Group (DKG). The studies cited by 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) were all much older and performed under previous guidelines and therefore not used in this CLH report.

For assessment of skin sensitisation, the DS does not see a contradiction with the EFSA evaluation (EFSA Journal 2016;14(4):4438) with respect to sulfited foods and beverages assessed in this opinion. In this EFSA opinion, all but one study report on the oral, but not the dermal route of exposure even if skin or airway symptoms were observed. Pseudo-allergic food intolerances and food allergy are mediated by different routes and represent different mechanisms of action (food allergy: IgE by plasma cells, mast cells release vasodilating factors, anaphylaxis) than relevant for skin allergy (less-no IgE and mast cells; killer cells, macrophages cause ekzema). For the majority of skin allergy-causing substances (not inducing rare cases of cross-reactions), IgE tests are negative, but therefore do not necessarily indicate the absence of a skin sensitisation potential. Nevertheless, this point concerning IgE tests is raised by IND in the context with skin allergy, not food allergy, which seems in appropriate. For the only study on skin allergy mentioned in the EFSA opinion, García-Gavín et al. (2012), the information is presented on p68 as: ”reported that 124 (4.5%) of 2,763 patients patch tested positively to sodium metabisulfite. A total of 13 cases (10.5%) were occupational and 10 of them presenting with 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.” This is completely in agreement with the DS`s assessment of the García-Gavín study in the CLH dossier.

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

In humans, studies on the relevant dermal route 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 proposal for classification was based and justified by relevant human data as required by the CLP Guidance.

General note: The argumentation relating to detoxification and endogenous formation of sulphur dioxide is important to consider for risk assessment, but not part of the hazard identification.

RAC’s response

RAC notes that all data on skin sensitisation included in the CLH report (both animal and human) refer to studies with sulphites.

Therefore, RAC, considering the physical state of SO_2 (i.e. gas), decided to discuss the read-across for dermal exposure from sulphites, which is relevant for the evaluation of the skin sensitisation endpoint,separately. There is no direct evidence whether a gas, such as SO_2, can lead to sufficient concentrations of sulfites on the skin to cause sensitisation. There is only indirect evidence from two case reports on sodium metabilsulfite exposure, included and commented accordingly in the CLH report, that describe contact dermatitis located in parts of the body, where direct skin contact to the metabisulfite solutions themselves could not have occurred. Therefore, both the DS and the authors of the studies report that contact dermatitis is suspected to be caused by SO_2 evaporated from these sodium metabisulfite solutions that reached the skin (Jacobs and Rycroft 1995, Vallon *et al.* 1995).

Permeation of SO_2 through skin and the consequences of dermal exposure are still poorly understood. According to a recent 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).

Clothes, on the other hand, are a good media for adsorption of sulfur compounds and interaction with textile surfaces and off-gassing of SO_2 from heavier fabric over time has the potential for secondary exposure (Kim *et al.*, 2008; Chien *et al.*, 2011).

Sulfur dioxide is classified as Skin Corrosive Cat. 1B. Whether SO_2 itself was proven to be corrosive or again read-across from sulfites or H_2SO_4 was applied to classify, is unclear and no evidence could be retrieved. It is noted that for the formation of H_2SO_4 an additional oxidation step is required, while sulfites have not been shown to be corrosive. The relevance of this information on the read-across from sulphites for skin sensitisation is quite high. In case of read-across from H_2SO_4 for skin corrosion, the amount of water on the skin would be the key, since skin is moist, perhaps more so in humans in a hot workplace than in experimental animals. The highest amount of H_2SO_4 that could be formed would correspond to a saturated solution in the skin water environment.

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, the inner layer of the skin (Kacalak-Rzepka *et al.*, Ann Acad Med Stetin, 2008; 54(3): 54–57). Water from the deeper epidermal layers moves upward to hydrate cells in the outermost skin layer, the stratum corneum, eventually being lost to evaporation. Then, an evaporation barrier is needed to maintain body water homeostasis. Variable skin pH values are being reported in literature, all in the acidic range but with a broad range from pH 4.0 to 7.0, with pH values below 5.0 being optimum (Lambers *et al.,* Int J Cosmet Sci, 2006; 28(5): 359-70). Such conditions favour the transformation of gaseous SO_2 to sulfites in the skin.

For skin corrosion, it could be that tests on SO_2 were performed, although no information is available. In any case, a corrosive substance "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.

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.

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, without specifically mentioning, though, that such effects were subject of observing and reporting. It should be noted that workers typically use personal protective equipment that may decrease the risk for skin effects.

Similarly, in a recent review article, where several studies have looked into the relationship between Traffic-related air pollutants (TRAP) exposure, SO_2 included, 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 (eg 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 (Zhang *et al.*, 2014).

Furthermore, association between environmental factors and outpatient clinic visits for eczema are published in the literature. More specifically, data on dermatology clinic outpatient visits for eczema in 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 conclusion, RAC recognises the lack of animal data from SO_2 itself regarding skin sensitisation and the fact that no measurements are available on the extent of SO_2 transformation to sulphites on the skin. Epidemiological data, on the other hand, are abundant and, although rather circumstantial and not according to the specifications set in the Guidance for Application of CLP criteria, version 5.0, 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 sulphur dioxide, **no classification of SO_2 for Skin sensitisation** is warranted.

In that sense, discussion of the sensitisation properties of sulphites is not relevant.

Date	Country	Organisation	Type of Organisation	Comment number
12.11.2020	Spain	AFEPASA (Azufrera y Fertilizantes Pallarés, S.A.U.)	Company-Manufacturer	14

Comment received

My comments are in the attached public document

ECHA note – An attachment was submitted with the comment above. Refer to public attachment AFEPASA Comments to the August 2020 SO2 CLH Report.pdf

Dossier Submitter's Response

The DS disagrees with the conclusion by IND and responds to points I-VII of the AFEPASA Comments to the August 2020 SO_2 CLH Report with respect to 1.3 Skin sensitisation as follows:

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

In the DS`s view, contact allergy (type IV reaction, patch test, relevant for the endpoint skin sensitisation) was distinguished from the mechanistically completely different IgE-mediated type I reaction (prick test), and for the studies the individual route/allergy type was clearly assigned.

(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))

In the dossier, newer human patch test data from three most relevant studies (Garcia-Gavin et al. 2012, Oliphant et al. 2012 and Madan et al. 2007) was evaluated for classification which could not be considered by SCF (1997), SCCNFP (2003), CIR (2003), EFSA, 2004, and OECD SIDS (2001) because of their date of publication. IgE tests and pseudoallergic reactions assessed by EFSA (2016) do not exclude skin allergy and dermal sensitisation.

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

Pseudo-allergic food intolerances and food allergy are mediated by completely different routes and represent different mechanisms of action than relevant for skin allergy (please see reply to comment 13). For the majority of skin allergy-causing substances, IgE tests are negative but therefore do not necessraily prove the absence of an existing skin allergy.

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

This is stated for food allergy, not skin allergy.

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

It is for ethical reasons not allowed to test the general population in epidemiological studies for the reason of possibly inducing an irreversible disease in healthy humans.

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

Several clinical studies on humans listed in the CLH dossier give evidence for classification.

(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".

This is related to the oral route. However, IgE tests assessed by EFSA (2016) do not exclude skin allergy and dermal sensitisation (please see reply to point 13).

RAC's response

See response to comment #13.

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2020	France	<confidential>	Industry or trade association	15

Comment received

Because of the following context and scientific knowledge on SO2 and its read-across, a classification of sulfur dioxide (and its read-across) as skin sensitiser is not warranted:
A) The general lack of scientific differentiation between "contact allergy and hypersensitivity"
B) The existence of numerous reliable reports confirming the lack of ski sensitization (SCF (1997), SCCNFP (2003), CIR (2003), EFSA, 2004, MAK (2014), EFSA (2016), OECD SIDS (2001)), MS Hungary 2015 decision.
C) IgE mediated reactions have been discussed but were never confirmed
D) the very low prevalence of susceptible individuals with sulfite oxidase deficiency
E) the absence of epidemiological study on the general population
F) EFSA 2016 conclusion that "IgE tests were usually negative indicating that (i) the reactions were not immune-mediated, and (ii) sensitivity reactions were mostly intolerance reactions"…
G) EBRC's scientific analysis of SO2 regarding skin sensitisation as part of this CLH consultation.
H) AFEPASA's scientific analysis of SO2 regarding skin sensitisation as part of this CLH consultation.

Dossier Submitter's Response

These comments have also been made in document "AFEPASA's comments relative to the CLH report" p.4-5. Please refer to our reply provided on comment number 14.

RAC's response

See response to comment #13.

Date	Country	Organisation	Type of Organisation	Comment number
13.11.2020	Netherlands	Micro-Pak Europe BV	Company-Downstream user	16

Comment received

With regard to skin sensitization, the dossier submitter summarizes a series of case reports on acute, immediate-type systemic reactions after sulfite exposure via injection of sulfite-containing anesthesia or via ingestion of sulfite-containing food or wine. While allergic contact dermatitis, the clinical manifestation of previous skin sensitization, is

characterized as delayed-type immunologic reaction following dermal exposure, these examples obviously relate to another hazard mechanism. While indeed also oral or parenteral administration of substances can in general lead to sensitization, sulfites (in contrast to allergens) are known to trigger pseudo-allergic food intolerances, i.e. mimicking symptoms of allergy but with no underlying specific immune-mediated responses as e.g. described by the WHO (WHO IPCS, Guidance for Immunotoxicity Risk Assessment for Chemicals, 2012). 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.

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 mentioned. Unfortunately, no further details on these studies are provided in the corresponding tables. However, the cited case report published by Jacobs and Rycroft (1995) describes a female employee of a photographic laboratory who already had eczema on her arms prior to occupational exposure to sulfites. Exposure to sulfites during her work caused asthmatic reactions and worsened the existing eczema. She showed a dermal response to sodium metabisulfite when patch tested and sulfites may have the potential to worsen existing dermal diseases, however this report on an individual case with pre-existing eczema does not provide evidence that sodium metabisulfite or even released gaseous sulfur dioxide can indeed induce skin sensitization in healthy individuals. Taking into account 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 critical for the evaluation of sulfur dioxide.

In addition, human patch test studies reporting responses predominantly to sodium metabisulfite are considered by the dossier submitter. However, considering the very widespread use of sodium metabisulfite and other sulfites in cosmetics, daily life hygiene products and pharmaceuticals in addition to food, wine and beverages as also mentioned in the CLH report based on which a daily exposure of millions of people to sulfites can reasonably be expected, the number of persons responding in these studies can be considered as limited. 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).

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 429 and GLP, 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). Furthermore, sodium sulfite was also negative in a GLP study according to OECD 429 (modified LLNA) in mice (study reported in disseminated ECHA registration dossier). Thus it can be concluded that appropriate predictive animal tests consistently indicate the absence of a skin sensitization potential of inorganic sulfites.

It should be mentioned that none of the human studies provide any indication for the induction of dermal responses after contact with sulfur dioxide. As sulfur dioxide is a gas under standard conditions with a considerable high vapor pressure, skin penetration and

thus dermal bioavailability as prerequisite for the induction of skin sensitization can reasonably be expected to be negligible.

In sum and as mentioned above, all available animal studies on the skin sensitization potential of sulfites do not indicate any sensitization potential of these substances. These tests were conducted according to validated test guidelines, and both studies according to OECD 429 (as the gold standard regulatory toxicology test for skin sensitization) were conducted in compliance with GLP.

In clinical studies conducted with dermatitis patients, sodium metabisulfite can elicit allergic responses, but the frequency is considerably low taking into account the possible occupational exposure as well as the frequent exposure of people using millions of cosmetic and pharmaceutical products per year containing sulfites releasing sulfur dioxide. In addition, the widespread usage of inorganic sulfites in the aforementioned products but also as food preservative makes it difficult to assess the relevance of observed dermal symptoms in many of these studies.

Considering the quality and reliability of the evidence, the available studies do not provide clear evidence that the substance sulfur dioxide is indeed a relevant skin sensitizer. This is supported by all available evaluations conducted by various scientific organizations as mentioned above, with the two most recent reviews of the available data performed in 2014 by the German MAK Commission and the National Institute of Chemical Safety, Hungary due to the listing of sodium metabisulfite in the CoRAP. Furthermore, as no epidemiological study on the general population exists, and no animal test or non-standard method gave any positive response to any of the structurally related sulfite species, the criteria for classification according to CLP are not met.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment CLH comments Micro-Pak_public.pdf
ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment CLH comments Micro-Pak.pdf

Dossier Submitter's Response

The DS would like to point out that data from patch test studies provided the basis for the classification proposal. These findings clearly do not concern pseudo-allergic food intolerances or food allergy. Moreover, as stated in the reply to comment 13, the results of these patch test studies are confirmed by new data in a larger cohort of 12,156 patients (Uter et al., 2018).

Indeed, the case reports refered to in the comment rather concern pseudo-allergic food intolerances or food allergy. Therefore, the DS listed case reports in a separate section of the CLH dossier (version 2, September 2018) and transparently indicated them on p. 50 as "Case reports (probably IgE-mediated allergic reactions)".

The DS regarded the only animal study as less relevant. Data from human patch tests was given priority by DS`s assessment. Reference is made also to the CLP Guidance Chapter 3.4.2.2.6. Decision logic for classification of substances.

For sulphur dioxide, read-across from metabisulfite is accepted and therefore these studies were included in the evaluation. For the overall conclusion from the reports on patch tests, the DS does not agree that frequency is considerably low (IND). Analyses of human patch test studies listed in the CLH Report, in detail 5% in Garcia-Gavin et al. 2012, 6% in

Oliphant et al. 2012, and 4% in Madan et al. 2007 warrant classification for skin sensitisation with sub-category 1A according to CLP guidance.
RAC's response
See response to comment #13.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
12.11.2020	Germany	Sulphuric Acid REACH Consortium (SAC)	Industry or trade association	17

Comment received
The LR and SAC do not disagree with the proposed classification, since according to CLP guidance (ECHA Guidance on the Application of the CLP Criteria, 3.8.2.7) and the decision logic for STOT SE classification, sulfur dioxide clearly falls into category 3 because of its respiratory irritation effects. This is also documented in ECHA Guidance on the Application of the CLP Criteria in section 3.8.5.1.3 (p.456) which explicitly lists sulphur dioxide as an example for a substance fulfilling the criteria for classification in STOT SE category 3 "based on well documented experience in humans on irritating effects to the respiratory system". Therefore, the proposed classification with "Specific Target Organ Toxicity after Single Exposure (STOT SE) Category 3 with the Hazard statement H335 "May cause respiratory irritation" is considered appropriate and adequate and is supported by the LR and SAC. For a detailed comment on the CLH-proposal, please see the relevant attachment. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Final CLH-Comment SO2 EBRC 11NOV2020_Redacted.pdf
Dossier Submitter's Response
No response required.
RAC's response
RAC notes that • following SO_2 exposure in animals, indication is 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 • following SO_2 exposure of healthy humans, 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 sulphites can lead to severe asthma exacerbation and affected lung function parameters.
- reports also account for reactive airway dysfunction syndrome (RADS) due to SO_2 acute exposure with long-lasting pulmonary effects, mainly due to the corrosive/ irritating properties of SO_2

Regarding the mode of SO_2 action on the respiratory system, evidence is gathered both form animal and from human studies that support the presence of at least 3 different mechanisms: activation/ sensitisation of neural reflexes, increase in inflammatory mediators and increase in allergic inflammation/ allergic sensitization, all three to clinical manifestations, such as bronchoconstriction and increased airway responsiveness, with significant health effects.

Therefore, RAC concludes that

- ✓ human data indicate that the respiratory system as a whole is the target organ of SO_2 when subjects are exposed via inhalation
- ✓ animal data support this observation
- ✓ a mode of action is described and substantiated by experimental findings

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

PUBLIC ATTACHMENTS
1. CLH comments Micro-Pak_public.pdf [Please refer to comment No. 3, 7, 16]
2. Final CLH-Comment SO2 EBRC 11NOV2020_Redacted.pdf [Please refer to comment No. 1, 8, 12, 13, 17]
3. AFEPASA Comments to the August 2020 SO2 CLH Report.pdf [Please refer to comment No. 9, 14]

CONFIDENTIAL ATTACHMENTS
1. CLH comments Micro-Pak.pdf [Please refer to comment No. 3, 7, 16]