SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 28 September 2022

doi: 10.2903/j.efsa.2022.7594

Follow-up of the re-evaluation of sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228)

EFSA Panel on Food Additives and Flavourings (FAF),
Maged Younes, Gabriele Aquilina, Laurence Castle, Karl-Heinz Engel, Paul J Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Ursula Gundert-Remy, Rainer Gürtler, Trine Husøy, Melania Manco, Wim Mennes, Peter Moldeus, Sabina Passamonti, Romina Shah, Ine Waalkens-Berendsen, Polly Boon, Karlien Cheyns, Riccardo Crebelli, Rex FitzGerald, Claude Lambré, Manuela Mirat, Beate Ulbrich, Christiane Vleminckx, Agnieszka Mech, Ana Maria Rincon, Alexandra Tard, Zsuzsanna Horvath and Matthew Wright

Abstract

Sulfur dioxide–sulfites (E 220–228) were re-evaluated in 2016, resulting in the setting of a temporary ADI of 0.7 mg SO_2 equivalents/kg bw per day. Following a European Commission call for data, the present follow-up opinion assesses data provided by interested business operators (IBOs) and additional evidence identified in the publicly available literature. No new biological or toxicological data addressing the data gaps described in the re-evaluation were submitted by IBOs. Taking into account data identified from the literature search, the Panel concluded that there was no substantial reduction in the uncertainties previously identified in the re-evaluation. Therefore, the Panel considered that the available toxicity database was inadequate to derive an ADI and withdrew the current temporary group acceptable daily intake (ADI). A margin of exposure (MOE) approach was considered appropriate to assess the risk for these food additives. A lower confidence limit of the benchmark dose of 38 mg SO_2 equivalents/kg bw per day, which is lower than the previous reference point of 70 mg SO_2 equivalents/kg bw per day, was estimated based on prolonged visual evoked potential latency. An assessment factor of 80 was applied for the assessment of the MoE. At the estimated dietary exposures, when using a refined exposure scenario (Data set D), MOEs at the maximum of 95th percentile ranges were below 80 for all population groups except for adolescents. The dietary exposures estimated using the maximum permitted levels would result in MOEs below 80 in all population groups at the maximum of the ranges of the mean, and for most of the population groups at both minimum and maximum of the ranges at the 95th percentile. The Panel concluded that this raises a safety concern for both dietary exposure scenarios. The Panel also performed a risk assessment for toxic elements present in sulfur dioxide–sulfites (E 220–228), based on data submitted by IBOs, and concluded that the maximum limits in the EU specifications for arsenic, lead and mercury should be lowered and a maximum limit for cadmium should be introduced.

Keywords: sulfur dioxide, E 220, CAS 7446-09-5, sodium sulfite, E 221, CAS 7757-83-7, sodium bisulfite, E 222, CAS 7631-90-5, sodium metabisulfite, E 223, CAS 7681-57-4, potassium metabisulfite, E 224, CAS 16731-55-8, calcium sulfite, E 226, CAS 10257-55-3, calcium bisulfite, E 227, CAS 13780-03-5, potassium bisulfite, E 228, CAS 7773-03-7, food additive

Requestor: European Commission

Question number: EFSA-Q-2021-00110

Correspondence: fip@efsa.europa.eu

Panel members: Maged Younes, Gabriele Aquilina, Laurence Castle, Gisela Degen, Karl-Heinz Engel, Paul J Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Ursula Gundert-Remy, Rainer Gürtler, Trine Husøy, Melania Manco, Wim Mennes, Peter Moldeus, Sabina Passamonti, Romina Shah, Ine Waalkens-Berendsen and Matthew Wright.

Declarations of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output: Jose Cortiñas Abrahantes, Irene Da Costa, Christina Kyrkou, Marcello Laganaro, Irene Munoz Guajardo, Salvatore Mutari. The FAF Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

Suggested citation: EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes M, Aquilina G, Castle L, Engel K-H, Fowler PJ, Frutos Fernandez MJ, Fürst P, Gundert-Remy U, Gürtler R, Husøy T, Manco M, Mennes W, Moldeus P, Passamonti S, Shah R, Waalkens-Berendsen I, Boon P, Cheyns K, Crebelli R, FitzGerald R Lambré C, Mirat M, Ulbrich B, Vleminckx C, Mech A, Rincon AM, Tard A, Horvath Z and Wright M, 2022. Follow-up of the re-evaluation of sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228). EFSA Journal 2022;20 (11):7594, 139 pp. https://doi.org/10.2903/j.efsa.2022.7594

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

Summary

Sulfur dioxide–sulfites (E 220–228) were re-evaluated by the EFSA former Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) in 2016. The ANS Panel noted several uncertainties and limitations in the database and concluded that the current group acceptable daily intake (ADI) of 0.7 mg SO_2 equivalents/kg bw per day (derived using a default uncertainty factor) would remain adequate but should be considered temporary while the database was improved.

At the request of the European Commission, the EFSA Panel on Food Additives and Flavourings (FAF Panel) provides in this opinion an updated safety assessment sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228). The Panel was also requested to assess the data provided by interested business operators (IBOs) in support of an amendment of the EU specifications for these food additives in Commission Regulation (EU) No 231/2012. The present opinion deals with the assessment of the data provided by interested business operators (IBOs) as a response to a dedicated European Commission call for data, as well as additional evidence identified in the publicly available literature.

Dietary exposure to sulfur dioxide–sulfites (E 220–228), expressed in SO_2 equivalents, was calculated using five data sets, taking into account different considerations on the available concentration data (maximum permitted levels (MPLs), reported uses and use levels and analytical data).

Data set D considered analytical data for a food category instead of use level data, even if the use levels were higher; use levels were only included in this data set for those food categories for which no analytical data were available. These results were considered to represent the level of SO_2 equivalents in final products, because they take into account losses of sulfur dioxide during processing, storage and the preparation stages. Furthermore, this data set D includes the presence of sulfur dioxide in foods and beverages due to the addition of sulfur dioxide–sulfites (E 220–228); carry-over; and other sources, such as natural occurrence. The Panel considered Data set D to most realistically represent the dietary exposure to sulfur dioxide equivalents. Furthermore, the non-brand-loyal scenario was considered the most appropriate for risk assessment of sulfur dioxide–sulfites (E 220–228), because these food additives are added to a wide range of foods, and they do not impact on taste or flavour.

In the refined non-brand-loyal exposure assessment scenario, mean dietary exposure ranged from < 0.01 mg SO_2 equivalents/kg bw per day in infants to 0.32 mg SO_2 equivalents/kg bw per day in toddlers. The 95th percentile of dietary exposure ranged from 0.05 mg SO_2 equivalents/kg bw per day in infants to 1.17 mg SO_2 equivalents/kg bw per day in adults. Overall, the Panel considered that the uncertainties identified would, in general, result in an overestimation of the dietary exposure to sulfur dioxide–sulfites (E 220–228) from their use as food additives for the refined estimated exposure scenarios considering data set D.

Analytical data on arsenic, lead, cadmium and mercury in commercial samples of E 221 E 222, E 223 and E 224 were provided by three IBOs. The potential exposure to these toxic elements from the use of sulfur dioxide–sulfites (E 220-E 280) was calculated by assuming that they may be present in the food additive up to a certain limit value and then by calculation pro-rata to the estimates of exposure to the food additive itself. Since the exposure to sulfur dioxide–sulfites (E 220–228) is expressed in mg SO_2 equivalents/kg bw per day, to calculate the exposure to impurities from the use of these food additives, the Panel converted the estimates to sulfite and considered two cases: (a) Exposure was expressed as sodium metabisulfite (E 223), that was considered to be the sulfite most typically used; (b) all the exposure expressed as sodium bisulfite (E 222) which is considered to be a worse case for these calculations due to its low yield of SO_2. Data set C, taking into account use levels and analytical data, was considered the most appropriate scenario available for estimating the exposure to toxic elements from the use of these food additives.

The Panel estimated the potential exposure (i) to Pb, Hg and As based on the maximum limits specified in Regulation (EU) No 231/2012 and (ii) to Pb, Hg, Cd and As at the highest reported limit of quantification and by applying a factor of 10. For both scenarios, in particular, the lower end of the range of calculated MOE values for As was considered to be insufficient. For Pb, Hg and Cd based on the outcome of the evaluation for the typical (E 223) and worse case (E 222), the presence of these toxic elements in sulfur dioxide–sulfites (E 220-E 228) either at the current specifications limit values or at the levels selected by the Panel would not give rise to concern.

The Panel noted that the maximum limits in the EU specifications for toxic elements should be established based on actual levels in the commercial food additive. Therefore, the Panel recommended that the maximum limits to be lowered on the basis of the information provided and on the considerations of the Panel. Moreover, the Panel recommends that the European Commission considers introducing a maximum limit for cadmium for these food additives.

An extensive literature search has been performed as requested in the European Commission mandate and genotoxicity and toxicological studies retrieved in the literature search were screened and assessed for their relevance and reliability.

No new data on absorption, distribution, metabolism and excretion (ADME) or reaction products were submitted by IBOs following the European Commission call for additional data. The Panel considered that sulfites undergo high first pass metabolism after oral exposure but that systemic exposure to sulfites may be up to around a quarter of the dose. The available data show distribution of sulfites in brain following intraperitoneal administration of sodium sulfite and both brain and heart after inhalation of sulfur dioxide.

Following the European Commission call for data, no new biological and toxicological data specifically addressing the data gaps described by the ANS Panel in the re-evaluation of sulfur dioxide–sulfites (E 220–228) in 2016 were received from IBOs. In addition, only limited new data were identified from the literature search. Overall, the Panel considered that there was no substantial reduction in the uncertainties previously identified in the re-evaluation. From the literature search, there are no new data on adverse effects following oral and inhalation exposure in the area of general toxicity. However, there were consistent reports that oral sulfite administration produced adverse effects on the central nervous system (CNS) and there were reports in studies of insufficient reliability with respect to their internal validity for adverse effects on the testis at lower doses than for CNS. 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. However, the Panel considered that the available toxicity database was inadequate to derive an ADI. The Panel therefore considered a margin of exposure (MOE) approach appropriate to assess the risk for these food additives at the current exposure levels.

The temporary group ADI established in 2016 was based on gastrointestinal effects in a long-term rat study with an NOAEL of 70 mg SO_2 equivalents/kg bw per day. At that time, it was also noted that numerous in vitro and animal studies reported that sulfites had a neurotoxic potential; however, it was indicated that more data would be needed before a clear conclusion on the possible neurotoxic effects of sulfites could be made, when used as food additives. The new evidence from the literature search support sulfite-induced neurotoxic effects (e.g. prolonged visual evoked potential (VEP) latency) which justifies using data from Ozturk et al. (2011) study.

A lower confidence limit of the benchmark dose (BMDL) of 38 mg SO_2 equivalents/kg bw per day, which is lower than the previous reference point of departure of 70 mg SO_2 equivalents/kg bw per day, was estimated based on prolonged VEP latency reported in the Ozturk et al. (2011) study and used as reference point to calculate the MOE.

In performing the quantitative extrapolation from the rat data to humans, the Panel considered whether the available data would allow modifying the default assessment factor for the MoE approach of 100. The assessment factor for the MoE considers aspects of interspecies toxicokinetics and dynamics as well as intraspecies toxicokinetics and dynamics and also the duration of the study (WHO, 2005).

Data for the toxicodynamics were available (Dyer, 1985; Otto et al., 1988), which, however, did not allow the quantification of respective interspecies differences.

Taking into account the intra-individual human variability in toxicodynamics for the specific endpoint used to derive the reference point, a reduction of the default toxicodynamic factor of 3.2–1.23 was considered, resulting in a total assessment factor of 40. Applying the additional default extrapolation factor of 2 for subchronic to chronic exposure, an overall assessment factor of 80 has been considered for the assessment of the MoE.

The Panel considered that the shortcomings in the toxicity database highlighted by the ANS Panel at the time of the 2016 re-evaluation had not led to the generation of adequate new data that could have addressed these shortcomings. Accordingly, due to the absence of new biological and toxicological data from IBOs and following an assessment of the literature database, the Panel concluded that the available toxicity database was not adequate to derive an ADI, and consequently withdraws the current temporary group ADI for these food additives.

The Panel concluded that the MOE calculated based on the dietary exposure to sulfur dioxide–sulfites (E 220–228) as food additives should be at least 80. At the estimated dietary exposure to sulfur dioxide–sulfites (E 220–228), when using the refined exposure scenario (Data set D), MOEs at the maximum of the 95th percentile ranges were below 80 for all population groups except for adolescents. The dietary exposure estimated using the maximum permitted levels would result in MOEs below 80 in all population groups at the maximum of the ranges of the mean, and for most of the population groups at both minimum and maximum of the ranges at the 95th percentile of exposure. This raises a safety concern for both dietary exposure scenarios.

Table of contents

1. Introduction

The re-evaluation of sulfur dioxide–sulfites (E 220–228) was completed by EFSA in 2016 (EFSA ANS Panel, 2016). The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) noted several uncertainties and limitations in the database and concluded that the group acceptable daily intake (ADI) of 0.7 mg SO_2 equivalents/kg bw per day (derived using a default uncertainty factor) would remain adequate but should be considered temporary while the database was improved. In addition, the ANS Panel issued several recommendations.

The data gaps and uncertainties identified by the ANS Panel required a follow-up by the European Commission by means of a subsequent call for additional data.[1]

The present opinion deals with the assessment of the data provided by interested business operators (IBOs) and additional evidence identified in the publicly available literature.

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background

The use of food additives is regulated under the European Parliament and Council Regulation (EC) No 1333/2008 on food additives.[2] Only food additives that are included in the Union list, in particular in Annex II to that Regulation, may be placed on the market and used in foods under the conditions of use specified therein. Moreover, food additives shall comply with the specifications as referred to in Article 14 of that Regulation and laid down in Commission Regulation (EU) No 231/2012[3].

Sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228) are authorised for use as food additives in the Union. Since sulfur dioxide–sulfites (E 220–228) were permitted in the Union before 20 January 2009, they belong to the group of food additives which are subject to a new risk assessment by the European Food Safety Authority (EFSA), according to Commission Regulation (EU) No 257/20103[4], and in line with the provisions of Regulation (EC) No 1333/2008.

EFSA completed the re-evaluation of sulfur dioxide–sulfites (E 220–228) as food additives and published a scientific opinion on 14 April 2016.[5] In that opinion, EFSA noted several uncertainties and limitations in the database and concluded that the current group ADI of 0.7 mg SO_2 equivalents/kg body weight (bw) per day (derived using a default uncertainty factor of 100) would remain adequate but should be considered temporary while the database was improved. EFSA recommended that the database and the temporary group ADI should be re-evaluated and noted that the recommended studies could require 5 years for completion. EFSA further concluded that exposure estimates to sulfur dioxide–sulfites (E 220–228) were higher than the group ADI of 0.7 mg SO_2 equivalents/kg bw per day for all population groups.

Consequently, the European Commission issued on 10 October 2016 a call for data[6] requesting business operators to submit data addressing the conclusions and recommendations from the EFSA re-evaluation of the safety of sulfur dioxide–sulfites (E 220–228) as food additives. In particular, the call for data requested:

- Data on the lowest achievable limits for the impurities of toxic elements (lead, mercury and arsenic) for sulfur dioxide–sulfites (E 220–228).
- Data on absorption, distribution, metabolism and excretion (ADME) for all the sulfites, including identification of their forms and reaction products, when they are used to treat beverages and solid foods.
- Data on the mode of action of sulfur dioxide–sulfites (E 220–228).

[1] Available online: https://ec.europa.eu/food/system/files/2019-01/fs_food-improvement-agents_reeval_call_20161010_E 220-E 228_data.pdf.
[2] Council Regulation (EC) No 1333/2008: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32008R1333.
[3] Commission Regulation (EU) No 231/2012: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32012R0231
[4] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010.
[5] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2016.4438
[6] https://ec.europa.eu/food/system/files/2019-01/fs_food-improvement-agents_reeval_call_20161010_E 220-E 228_data.pdf

- Data relevant for addressing the estimated exceedance of the ADI of sulfur dioxide–sulfites (E 220–228).

In February 2020 business operators completed the submission of data in reply to that call. With respect to the toxicological data requested in the call, business operators originally committed to generating new ADME data for sulfites and they indicated their willingness to investigate the feasibility of carrying out mode of action studies, depending on the outcome of the ADME studies. However, business operators finally decided not to carry out any new toxicological studies and instead they submitted in reply to the call for data, a report on the re-evaluation of already available ADME information on sulfites used as food additives, as well as a document on a read-across concept for inorganic sulfite substances.

Given that the Commission is now in possession of all the data that business operators decided to submit in reply to the call for data, it is appropriate to ask EFSA re-evaluate the database and the temporary group ADI for the food additives sulfur dioxide–sulfites (E 220–228), and to issue an updated scientific opinion on the safety of sulfur dioxide–sulfites (E 220–228) as food additives.

In addition, the European Commission notes that sulfur dioxide (CAS No 7446-09-5), currently under evaluation by the European Chemicals Agency (ECHA) as an active substance in biocidal products, has also been proposed by the German competent authority for inclusion in the registry of classification and labelling (CLH). The proposed revision to the harmonised classification and labelling, includes the proposal for a classification of the substance for germ cell mutagenicity as "Muta. 2, H341[7]". A public consultation on this proposal took place between September and November 2020, and an opinion of the ECHA Committee for Risk Assessment (RAC) is expected for adoption in February 2022. Therefore, the updated opinion on the food additives sulfur dioxide–sulfites (E 220–228) should also take into account the conclusions from the currently ongoing scientific assessment that is being carried out by the ECHA on sulfur dioxide in the context of the CLH proposal for classification and its ongoing evaluation as an active substance in biocidal products.

1.1.2. Term of reference

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002[8], the European Commission requests the European Food Safety Authority (EFSA) to provide an updated scientific opinion as regards the safety of the food additives sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228).

In particular, EFSA is requested to re-evaluate the database and the temporary group ADI for the food additives sulfur dioxide–sulfites (E 220–228), as well as to refine the exposure assessment for these food additives, taking into account the data submitted by business operators in reply to the call for data issued by the Commission, as well as any new relevant data retrieved from the published literature and the conclusions from the currently ongoing scientific assessment of ECHA on sulfur dioxide.

In accordance with the provisions of Article 30 of Regulation (EC) No 178/20028, EFSA is requested to identify potentially contentious scientific issues with the work of ECHA and to cooperate with ECHA with a view to either resolving the divergence or presenting a joint document to the Commission clarifying the contentious scientific issues and identifying the relevant uncertainties in the data.

2. Data and methodologies

2.1. Data

The Panel based its assessment on:

- Information from publications retrieved in the systematic literature search (see Section 4.5.2);
- Information submitted in response to the public call for data issued by the European Commission and additional information submitted during the assessment process by interested parties in response to follow-up requests from EFSA (Documentation provided to EFSA);

[7] https://echa.europa.eu/it/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e181cb8df5

[8] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, pp. 1–24.

- Genotoxicity studies submitted to EFSA (Documentation provided to EFSA No 7, 8, 9, 10 and 11);
- Additional supporting scientific literature examining possible sulfur dioxide and/or sulfites toxicity mechanisms;
- Food consumption data from the EFSA Comprehensive European Food Consumption Database (Comprehensive Database), which were used to estimate the dietary exposure to sulfur dioxide–sulfites (E 220–228);
- Use levels and analytical data to estimate the dietary exposure to sulfur dioxide–sulfites (E 220–228);
- Information from Mintel's Global New Products Database (GNPD) to identify the use of sulfur dioxide–sulfites (E 220–228) in food and beverage products and food supplements. Mintel's GNPD is an online database that contains the compulsory ingredient information present on the label of numerous products.

2.2. Methodologies

This opinion was formulated following the principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing guidance documents from the EFSA Scientific Committee.

The FAF Panel assessed the safety of sulfur dioxide–sulfites (E 220–228) as food additives in line with the Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012).

A literature search for studies published between January 2014 (overlapping the end of the coverage of the re-evaluation (EFSA ANS Panel, 2016)) and March 2022 was performed following the approach described in Annex A. An additional literature search was conducted for older (pre-2014) inhalation studies not considered in the re-evaluation (EFSA ANS Panel, 2016) that reported data and/or focused on endpoints identified as relevant for the identification of a reference point. The literature search (pre-2014) was subsequently extended to oral studies reporting neurotoxic endpoints since neurotoxicity had been reported in several recent studies (from 2014 on).

Toxicological studies retrieved in the literature search were screened according to the criteria of relevance and reliability described in Annex B. Epidemiological studies examining potential associations between environmental exposure to sulfur dioxide and health effects were not considered in the assessment of sulfur dioxide–sulfites when used as food additives.

In animal studies, when the test substance was administered in the feed or in the drinking water, but doses were not explicitly reported by the authors as mg/kg bw per day based on actual feed or water consumption, the daily intake was calculated by the Panel using the relevant default values. In case of rodents, the values as indicated in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012a) were applied. In the case of other animal species, the default values used by JECFA (2000) were used. In these cases, the dose was expressed as 'equivalent to mg/kg bw per day'. If a concentration in feed or drinking water was reported and the dose in mg/kg bw per day was calculated (by the authors of the study report or the Panel) based on these reported concentrations and on reported consumption data for feed or drinking water, the dose was expressed as 'equal to mg/kg bw per day'.

For converting an inhalation dose (expressed as ppm or mg/m^3 or mg/L by the authors) to an internal dose (mg/kg bw per day), the respiration rate of the test species (45 L/kg bw per h in the rat and 70 L/kg bw per h in the mouse (Brown et al., 1997; ECHA, 2012), 45[9] L/kg bw per h in the rabbit) was used. The duration of daily inhalation exposure in the study and the extent of respiratory absorption were also taken into account. The default assumption was that absorption of sulfur dioxide via the respiratory tract is 100% of the inhaled dose. The Panel did not distinguish between whole body and nose-only inhalation studies. The purity of sulfur dioxide, if not reported, was assumed to be 100%. The following equation based on that proposed by ECHA (2012) was used:

[9] Mean of the respiration rate of New Zealand White (NZW) rabbit https://www.criver.com/sites/default/files/resources/rm_rm_d_NZW_rabbit.pdf.

$$\text{Dose}\left[\frac{\text{mg} * \text{bw}}{\text{kg} * \text{day}}\right] = \text{Dose}(\text{from the individual study})\left[\frac{\text{mg}}{\text{L}}\right] * \text{Purity correction factor of the tested substance}\left[\frac{\text{X}\%}{100}\right] * \text{Hours of exposure per day}(\text{from the individual study})\left[\frac{\text{h}}{\text{day}}\right] * \text{Correction factor days per week of exposure}(\text{in case less than 7 days per week exposure})\left[\frac{\text{X days}}{\text{7 days}}\right] * \text{Inhalation rate}\left[\frac{\text{L} * \text{bw}}{\text{kg} * \text{h}}\right]$$

In the body text of this opinion, inhalation doses are reported as the calculated corresponding internal dose. The original exposure levels as reported by the authors (as ppm or mg/m^3 or mg/L) can be found in Appendix B.

The Panel noted that sulfur dioxide was administered by whole body exposure in all the inhalation studies identified. This would lead to deposition e.g. on the fur, and subsequent oral exposure through animals grooming. In addition, dermal absorption may occur. This could increase exposure to sulfur dioxide–sulfites compared to animals exposed to a similar dose via nose-only inhalation.

The Panel considered that inhalation studies cannot be used to identify a reference point that could be used for deriving an oral health-based guidance value (HBGV) due to the uncertainty around the assumptions used when estimating internal doses resulting from inhalation exposure.

In animal studies, when the test substance was administered as a 'sulfite', an SO_2 equivalent dose was calculated by the Panel using the theoretical sulfur dioxide yield as reported by Ough and Were (2005) (Table 1). In these cases, the dose was expressed as 'corresponding to mg SO_2 equivalents/kg bw per day'.

Reported use levels of sulfites (e.g. E 221, E 222, E 223,…) used in the exposure assessment have also been converted to SO_2 equivalents using the theoretical sulfur dioxide yield as reported in Table 1.

Table 1: Theoretical sulfur dioxide yield (Ough and Were, 2005)

Sulfiting agent	Theoretical yield of SO_2 (%)
Sulfur dioxide (E 220)	100
Sodium sulfite, anhydrous (E 221)	50.8
Sodium sulfite, heptahydrate (E 221)	25.4
Sodium bisulfite (E 222)	61.6
Sodium metabisulfite (E 223)	67.4
Potassium metabisulfite (E 224)	57.6
Potassium bisulfite (E 228)	53.5

During this assessment, as required by the European Commission mandate, discussions have taken place between FAF Working Group (WG) on sulfur dioxide–sulfites and ECHA's Human Health Working Group regarding the safety assessment of sulfur dioxide–sulfites.

3. Background information

Sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228) are authorised as food additives in the EU in accordance with Annex II and Annex III to Regulation (EC) No 1333/2008 and specifications are established in EU Commission Regulation (EU) No 231/2012.

The term 'sulfites' will be used throughout this document whenever these substances are referred to as a group.

Sulfur dioxide–sulfites (E 220–228) were re-evaluated by the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) in 2016. The ANS Panel noted several uncertainties and limitations in the database and concluded that the current group ADI of 0.7 mg SO_2 equivalents/kg bw

per day (derived using a default uncertainty factor) would remain adequate but should be considered temporary while the database was improved. The ANS Panel recommended that the database and the temporary group ADI should be re-evaluated and noted that the recommended studies could require 5 years for completion. The ANS Panel further concluded that exposure estimates to sulfur dioxide and sulfites were higher than the group ADI of 0.7 mg SO_2 equivalents/kg bw per day for all population groups. Furthermore, the ANS Panel recommended that:

- additional studies performed according to recent internationally recognised OECD guidelines would allow more adequate risk assessment of the sulfites that are used as food additives:
  - ADME data for all the sulfites, including identification of their forms and reaction products, when they are used to treat beverages and solid foods. Depending on the outcome of these ADME studies, additional toxicity studies may be required, such as those described in the Guidance for submission of food additives (EFSA ANS Panel, 2012);
- a mode of action analysis should be conducted when the knowledge permits;
- studies on the origin and mechanisms (forms of sulfites involved) of the reactions of individuals who are sensitive or intolerant to sulfites should be conducted;
- the labelling 'contains sulfites' should provide information on the amount of SO_2 equivalent present in solid foods and beverages;
- the maximum limits for the impurities of toxic elements (arsenic, lead and mercury) in the EU specification for sulfur dioxide–sulfites (E 220–228) should be revised in order to ensure that sulfur dioxide–sulfites (E 220–228) as food additives will not be a significant source of exposure to these toxic elements in food.

These data gaps and uncertainties identified by the ANS Panel required a follow-up by the European Commission by means of a subsequent call for additional data.

An RAC opinion proposing harmonised classification and labelling at EU level for sulfur dioxide was adopted in 2021 (ECHA, 2021). RAC noted that 'the available data set for the evaluation of the genotoxic properties for SO_2 is quite extensive, but the quality of the studies is not sufficient to provide unequivocal evidence for the mutagenicity classification of SO_2. Although there are indications for the possible genotoxic properties of SO_2, the evidence is not strong enough to support classification and therefore, no classification for mutagenicity due to inconclusive data is warranted'. In addition, 'in a weight of evidence approach, RAC concluded that based on the existing evidence SO_2 does not warrant classification as a carcinogen'.

The ECHA Biocidal Products Committee (BPC) concluded that sulfur dioxide released from sodium metabisulfite in product type 9[10] and sulfur dioxide generated from sulfur by combustion in product type 4[11] may be approved and be included in the Union list of approved biocide active substances, subject to specific conditions. The detailed grounds for the overall conclusions are described in the respective assessment reports.

4. Assessment

4.1. Identity and specifications E 200–228

A summary of the identity of the food additives, sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228) is presented in Table 2.

[10] To be published at https://echa.europa.eu/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/1419/PT09.
[11] To be published at https://echa.europa.eu/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/1394/PT04.

Table 2: Summary of the identity of the substances

Food additive	Number	Chemical formula	Molecular weight g/mol	CAS	EINECS
Sulfur dioxide	E 220	SO_2	64.06	7446-09-5	231-195-2
Sodium sulfite	E 221	Na_2SO_3 for anhydrous and	126.04	7757-83-7	231-821-4
		$Na_2SO_3 \cdot 7H_2O$ for heptahydrate	252.16	10102-15-5	–[(a)]
Sodium bisulfite	E 222	$NaHSO_3$	104.06	7631-90-5	231-548-0[(b)]
Sodium metabisulfite	E 223	$Na_2S_2O_5$	190.11	7681-57-4	231-673-0
Potassium metabisulfite	E 224	$K_2S_2O_5$	222.33	16731-55-8	240-795-3
Calcium sulfite	E 226	$CaSO_3$	120.14	10257-55-3	233-596-8[(c)]
Calcium bisulfite	E 227	$Ca(HSO_3)_2$	202.22	13780-03-5	237-423-7
Potassium bisulfite	E 228	$KHSO_3$	120.17	7773-03-7	231-870-1

(a): Not registered in the European Commission Inventory.
(b): The EINECS 231-921-4 included in the Commission Regulation (EU) No 231/2012 for E 222 is not registered in the European Commission Inventory.
(c): The EINECS included in the Commission Regulation (EU) No 231/2012 for E 226 is of another substance (calcium dibenzoate 218-235-4).

The theoretical sulfur dioxide yield of the different sulfites is given in Table 3 along with the minimum sulfur dioxide content specified in Commission Regulation (EU) No 231/2012. The Panel noted that the description of sodium bisulfite (E 222) differs in the European Commission specifications and the JECFA (see Appendix A, Table A.3).

Table 3: Theoretical sulfur dioxide yield according to Ough and Were (2005) and minimum specified content according to Commission Regulation (EU) No 231/2012

Sulfiting agent	Theoretical yield of SO_2 (%)	SO_2 minimum specified content (Commission Regulation (EU) No 231/2012)
Sulfur dioxide (E 220)	100	Not less than 99%
Sodium sulfite, anhydrous (E 221)	50.8	Not less than 48%
Sodium sulfite, heptahydrate (E 221)	25.4	Not less than 24%
Sodium bisulfite (E 222)	61.6	Not less than 32% w/w of $NaHSO_3$[(a)]
Sodium metabisulfite (E 223)	67.4	Not less than 64%
Potassium metabisulfite (E 224)	57.6	Not less than 51.8%
Calcium sulfite (E 226)	None given (53.3)[(b)]	Not less than 39%
Calcium bisulfite (E 227)	None given (31.7)[(b)]	6–8% (w/v) (of a solution)
Potassium bisulfite (E 228)	53.5	Content not less than 280 g $KHSO_3$ per litre (or 150 g SO_2 per litre)

(a): At 32% w/w and with a theoretical yield of 61.6% from $NaHSO_3$, the yield of SO_2 from E 222 meeting the EU specifications would be 19.7%.
(b): Calculated by the Panel from the empirical formulae and molecular weights presented in Table 2.

A summary of the maximum limits for toxic elements in sulfites according to the Commission Regulation (EU) No 231/2012 is presented in Table 4 and detailed specifications are presented in Appendix A.

Table 4: Summary of the maximum limits for toxic elements in sulfur dioxide (E 220) and sulfites (E 221-E 224, E 226-E 228) according to Commission Regulation (EU) No 231/2012

	E 220	E 221	E 222	E 223	E 224	E 226	E 227	E 228
Arsenic mg/kg	3	3	3	3	3	3	3	3
Lead mg/kg	5	2	2	2	2	2	2	2
Mercury mg/kg	1	1	1	1	1	1	1	1

4.2. Technical data submitted on toxic elements

The following was requested in the European Commission call for data[12]:

- Data on the lowest achievable limits for the impurities of toxic elements (lead, mercury and arsenic) for sulfur dioxide–sulfites (E 220–228).

EFSA received analytical data on the levels of toxic elements for four food additives: sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223) and potassium metabisulfite (E 224) from three interested business operators (IBOs) (Documentation provided to EFSA No 1,2,3,4 and 5). No data on sulfur dioxide (E 220), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228) were received. An interested business operator stated that he is not aware of any member producing E 226, E 227 or E 228 and that the production of calcium bisulfite (E 227) was stopped by the lead registrant in 2012 (Documentation provided to EFSA No 4).

It has also been clarified by the IBOs that the sulfur dioxide produced for the use as food additive is commercialised only in liquid, pressurised form (Documentation provided to EFSA No 6).

One IBO has submitted information on E 221, E 223 and E 224 provided by two members of the consortium (Documentation provided to EFSA No 3 and 4). It has been noted by the Panel that one of the two members of that consortium has also individually submitted additional data regarding E 221, E 223 and E 224 (analysis performed on different batches and for a different time period) (Documentation provided to the EFSA No 1 and 5).

Although the European Commission call for data requested information only on lead, mercury and arsenic levels, EFSA has also received data on cadmium and additional elements, and therefore, the Panel has evaluated also these data.

Sodium sulfite (E 221)

Information on the content of toxic elements in sodium sulfite (E 221) was submitted by two IBOs (Documentation provided to EFSA No 1,3,4 and 5).

One IBO submitted analytical data for lead (Pb), mercury (Hg), cadmium (Cd) and arsenic (As) in five batches of E 221 (Documentation provided to EFSA No 5). The samples were analysed by means of inductively coupled plasma-mass spectrometry (ICP-MS) after acid digestion, except for mercury content which was analysed by Direct Mercury Analysis with Atomic Absorption Spectroscopy (DAAS). The methods were described, and the limits of quantification (LOQs) reported were the following: Pb 0.03 mg/kg, Hg 0.03 mg/kg, cadmium (Cd) 0.05 mg/kg and As 0.1 mg/kg. For all five analysed batches, the concentrations of the four toxic elements were reported as below the LOQ. No proposal for lowest technologically achievable levels for these toxic elements was provided by the IBO.

Another IBO submitted data on the levels of Pb, Hg, Cd and As for five batches of anhydrous sodium sulfite (E 221) (Documentation provided to EFSA No 4). The methods were described, and the LOQs were provided for three elements: Pb 0.03 mg/kg, Hg 0.03 mg/kg and As 0.1 mg/kg. The levels of Pb, Hg and As were all reported as below the LOQ. The level of Cd was reported in the submitted certificates of analysis as < 0.05 mg/kg in all analysed batches.

Additionally, the IBO reported for all analysed batches of E 221 levels of: Silver (Ag) (< 0.1 mg/kg), Bismuth (Bi) (< 0.1 mg/kg), Chromium (Cr) (< 0.2 mg/kg), Molybdenum (Mo) (< 0.1 mg/kg), Antimony (Sb) (< 0.1 mg/kg) and Tin (Sn) (< 0.1 mg/kg). The levels of copper (Cu) were reported in the range < 0.2–0.5 mg/kg. The nickel (Ni) content was reported for three batches as 0.1 mg/kg, for one batch as < 0.2 mg/kg and for one batch as < 1 mg/kg. For one batch, levels of selenium (Se) and

[12] https://food.ec.europa.eu/system/files/2019-01/fs_food-improvement-agents_reeval_call_20161010_E 220-E 228_data.pdf

iron (Fe) were also analysed and reported as 0.1 mg/kg and 1 mg/kg, respectively (Documentation provided to EFSA No 4).

No proposal for lowest technologically achievable levels for toxic elements was provided by the IBO.

Sodium bisulfite (E 222)

One IBO has provided information on the content of toxic elements in 13 batches of sodium bisulfite (E 222) in solutions labelled as 38–40% sulfur dioxide content. The analyses were performed in the years 2014–2017 (Documentation provided to EFSA No 2). The method of analysis was reported as an internal laboratory code without further information. The IBO reported the contents of Pb < 0.01 mg/kg, Hg < 0.01, mg/kg Cd < 0.01 mg/kg and As < 0.05 mg/kg in all batches. The lowest technologically achievable levels proposed by the IBO were Pb 0.01 mg/kg, Hg 0.01 mg/kg and As 0.05 mg/kg.

Additionally, the IBO reported for all analysed batches levels of Cr (range 0.01–0.03 mg/kg), Sb (12 batches < 0.01 mg/kg, 1 batch < 0.05 mg/kg), zinc (Zn) (range 0.02–0.09 mg/kg), Cu (range 0.018–0.22 mg/kg), Se (range 0.014–0.14 mg/kg) and Fe (range 0.024–0.3 mg/kg).

Sodium metabisulfite (E 223)

Analytical data for the analysis of Pb, Hg, Cd and As in five batches of E 223 were submitted by one IBO (Documentation provided to EFSA No 5). The samples were analysed by ICP-MS after acid digestion, except for Hg which was analysed by DAAS. For all samples, the concentrations were reported as below the LOQs which were Pb 0.03 mg/kg, Hg 0.03 mg/kg, Cd 0.05 mg/kg and As 0.1 mg/kg. The IBO did not provide a proposal for lowest technologically achievable level of toxic elements.

Another IBO submitted data on the levels of Pb, Hg, Cd and As for five batches of sodium metabisulfite (E 223) analysed in September–October 2018 (Documentation provided to EFSA No 4). The samples were analysed by ICP-MS after acid digestion except for Hg which was analysed by DAAS. LOQs were provided for Pb 0.03 mg/kg, Hg 0.03 mg/kg and As 0.1 mg/kg and these three elements were reported as below the LOQs in all analysed batches. The level of Cd was indicated as < 0.05 mg/kg in the submitted certificates of analysis for all analysed batches. Additionally, levels of other elements were retrieved from the certificates of analysis of five batches of E 223: Ag (< 0.1 mg/kg), Bi (< 0.1 mg/kg), Cu (< 0.2 mg/kg), Mo (< 0.1 mg/kg), Ni (< 0.1 mg/kg), Sb (< 0.1 mg/kg). The levels of Sb were in range 0.1–0.3 mg/kg, Cr in range 0.2–1 mg/kg and Sn 0.1–0.2 mg/kg. For one batch, the content of Se, Zn, cobalt (Co) and Fe was also provided as 0.1 mg/kg, 1 mg/kg, 0.5 mg/kg and 1 mg/kg, respectively.

The same IBO also submitted data provided by another consortium member on the content of toxic elements for 37 lots of E 223 analysed during the year 2018 by means of inductively coupled plasma optical emission spectroscopy (ICP-OES) (Documentation provided to EFSA No 4). The method was described but the LOQs and limits of detection (LODs) values were not stated by the IBO. The Pb levels were reported from 0.021 up to 0.077 mg/kg, Hg was reported as below LOQ up to 0.046 mg/kg, Cd was reported as below LOQ up to 0.017 mg/kg and As was reported as below LOQ up to 0.016 mg/kg. This IBO also provided analytical data on selenium (range 0.055–0.169 mg/kg) and iron (range 1–2 mg/kg) for all analysed batches. No proposal for the lowest technologically achievable limits was submitted by the IBO.

Potassium metabisulfite (E 224)

Analytical data for the analysis of Pb, Hg, Cd, As in five batches of E 224 were submitted (Documentation provided to EFSA No 5). The samples were analysed by ICP-MS except for Hg which was analysed by DAAS. For all samples, the concentrations were reported as below the LOQs which were for Pb 0.03 mg/kg, Hg 0.03 mg/kg, Cd 0.05 mg/kg and As 0.1 mg/kg.

Another IBO submitted data on the levels of Pb, Hg, Cd and As for three batches of potassium metabisulfite (E 224) analysed in July–September 2018 (Documentation provided to EFSA No 4). The methods were described and their respective LOQ values were provided; Pb 0.03 mg/kg, Hg 0.03 mg/kg and As 0.1 mg/kg. In all analysed batches, levels of Pb, Hg and As were reported as below the LOQ. The level of Cd was reported in the certificate of analysis as < 0.05 mg/kg.

Additionally, the IBO reported for all analysed batches of E 224 the levels of Ag (< 0.1 mg/kg), Bi (< 0.1 mg/kg), Cr (< 1 mg/kg), Cu (< 0.1 mg/kg), Mo (< 0.1 mg/kg), Sb (< 0.1 mg/kg), Sn (< 0.1 mg/kg). The Ni content was reported for two batches as < 1 mg/kg, and for one batch as

< 0.1 mg/kg. For one batch, levels of Se and Fe were also analysed and reported as below 0.1 mg/kg and 1 mg/kg, respectively (Documentation provided to EFSA No 4).

No proposal for lowest technologically achievable levels for toxic elements was submitted by the IBOs.

4.3. Exposure assessment

4.3.1. Authorised uses and use levels

Maximum levels of sulfur dioxide–sulfites (E 220–228) have been defined in Annex II to Regulation (EC) No 1333/2008 on food additives. These levels are referred to as 'maximum permitted levels (MPLs)' by the Panel.

Sulfur dioxide–sulfites (E 220–228) are authorised in 40 food categories in the EU according to Annex II to Regulation (EC) No 1333/2008 with maximum permitted levels (MPLs) ranging from 10 to 2,000 mg/kg.

Table 5 summarises the food categories that are permitted to contain sulfur dioxide–sulfites (E 220–228) as food additives and the corresponding MPLs.

Table 5: MPLs of sulfur dioxide–sulfites (E 220–228) in food categories according to Annex II to Regulation (EC) No 1333/2008

Food category number	Food category description	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
04.1.1	Entire fresh fruit and vegetables	Only table grapes, fresh lychees (measured on edible parts) and blueberries (*Vaccinium corymbosum*)	10[(a)]
		Only vacuum packed sweetcorn	100[(a)]
04.1.2	Peeled, cut and shredded fruit and vegetables	Only peeled potatoes	50[(a)]
		Only onion, garlic and shallot pulp	300[(a)]
		Only horseradish pulp	800[(a)]
04.1.3	Frozen fruit and vegetables	Only white vegetables including mushrooms and white pulses	50[(a)]
		Only frozen and deep-frozen potatoes	100[(a)]
04.2.1	Dried fruit and vegetables	Only dried coconut	50[(a)]
		Only white vegetables, processed, including pulses	50[(a)]
		Only dried mushrooms	100[(a)]
		Only dried ginger	150[(a)]
		Only dried tomatoes	200[(a)]
		Only white vegetables, dried	400[(a)]
		Only dried fruit and nuts in shell, excluding dried apples, pears, bananas, apricots, peaches, grapes, prunes and figs	500[(a)]
		Only dried apples and pears	600[(a)]
		Only dried bananas	1,000[(a)]
		Only dried apricots, peaches, grapes, prunes and figs	2,000[(a)]
04.2.2	Fruit and vegetables in vinegar, oil or brine	Except olives and golden peppers in brine	100[(a)]
		Only golden peppers in brine	500[(a)]
04.2.3	Canned or bottled fruit and vegetables	Only white vegetables, including pulses and processed mushrooms	50[(a)]
		Only bottled whiteheart cherries; vacuum packed sweetcorn	100[(a)]
		Only bottled, sliced lemon	250[(a)]

Food category number	Food category description	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
04.2.4.1	Fruit and vegetable preparations excluding compote	Only processed white vegetables and mushrooms	50[a]
		Only rehydrated dried fruit and lychees, mostarda di frutta	100[a]
		Only onion, garlic and shallot pulp	300[a]
		Only horseradish pulp	800[a]
		Only Jellying fruit extract, liquid pectin for sale to the final consumer	800[a]
04.2.5.1	Extra jam and extra jelly as defined by Directive 2001/113/EC	Only jams, jellies and *mermeladas* made with sulfited fruit	100[a]
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EC		50[a]
		Only jams, jellies and marmalades made with sulfited fruit	100[a]
04.2.5.3	Other similar fruit or vegetable spreads		50[a]
04.2.6	Processed potato products		100[a]
		Only dehydrated potatoes products	400[a]
05.2	Other confectionery including breath refreshening microsweets	Only glucose syrup-based confectionery (carry-over from the glucose syrup only)	50[a]
		Only candied, crystallised or glacé fruit, vegetables, angelica and citrus peel	100[a]
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	Only toppings (syrups for pancakes, flavoured syrups for milkshakes and ice cream; similar products)	40[a]
		Only glucose syrup-based confectionery (carry over from the glucose syrup only)	50[a]
		Only fruit fillings for pastries	100[a]
06.1	Whole, broken or flaked grain	Only sago and pearl barley	30[a]
06.2.2	Starches	Excluding starches in infant formulae, follow-on formulae and processed cereal-based foods and baby foods	50[a]
07.2	Fine bakery wares	Only dry biscuits	50[a]
08.2	Meat preparations as defined by Regulation (EC) No 853/2004 (M42)	Only breakfast sausages; burger meat with a minimum vegetable and/or cereal content of 4% mixed within the meat	450[a],[b]
		Only salsicha fresca, longaniza fresca and butifarra fresca	450[a],[b]
09.1.2	Unprocessed molluscs and crustaceans	Only fresh, frozen and deep-frozen crustaceans and cephalopods; crustaceans of the Penaeidae, Solenoceridae and Aristaeidae family up to 80 units	150[a],[c]
		Only crustaceans of the Penaeidae, Solenoceridae and Aristaeidae family between 80 and 120 units	200[a],[c]
		Only crustaceans of the Penaeidae, Solenoceridae and Aristaeidae family over 120 units	300[a],[c]

Food category number	Food category description	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
09.2	Processed fish and fishery products, including molluscs and crustaceans	Only cooked crustaceans and cephalopods	50[(a),(c)]
		Only cooked crustaceans of the Penaeidae, Solenoceridae and Aristaeidae family up to 80 units	135[(a),(c)]
		Only cooked crustaceans of the Penaeidae, Solenoceridae and Aristaeidae family between 80 and 120 units	180[(a),(c)]
		Only dried salted fish of the 'Gadidae' species	200[(a)]
		Only cooked crustaceans of the Penaeidae, Solenoceridae and Aristaeidae family over 120 units	270[(a),(c)]
11.1	Sugars and syrups as defined by Directive 2001/111/EC	Only sugars, except glucose syrup	10[(a)]
		Only glucose syrup, whether or not dehydrated	20[(a)]
11.2	Other sugars and syrups		40[(a)]
		Only treacle and molasses	70[(a)]
12.2.1	Herbs and spices	Only cinnamon (Cinnamomum ceylanicum)	150[(a)]
12.2.2	Seasonings and condiments	Only citrus juice-based seasonings	200[(a)]
12.3	Vinegars	Only fermentation vinegar	170[(a)]
12.4	Mustard	Excluding dijon mustard	250[(a)]
		Only dijon mustard	500[(a)]
12.9	Protein products, excluding products covered in category 1.8	Only gelatine	50[(a)]
		Only analogues of meat, fish, crustaceans and cephalopods	200[(a)]
14.1.2	Fruit juices as defined by Directive 2001/112/EC and vegetable juices	Only orange, grapefruit, apple and pineapple juice for bulk dispensing in catering establishments	50[(a)]
		Only grape juice, unfermented, for sacramental use	70[(a)]
		Only lime and lemon juice	350[(a)]
		Only concentrated grape juice for home wine making	2,000[(a)]
14.1.4	Flavoured drinks	Only carry-over from concentrates in non-alcoholic flavoured drinks containing fruit juice	20[(a)]
		Only non-alcoholic flavoured drinks containing at least 235 g/L glucose syrup	50[(a)]
		Only other concentrates based on fruit juice or comminuted fruit; capilé groselha	250[(a)]
		Only concentrates based on fruit juice and containing not less than 2.5% barley (barley water)	350[(a)]
14.2.1	Beer and malt beverages		20[(a)]
		Only beer with a second fermentation in the cask	50[(a)]

Food category number	Food category description	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
14.2.2	Wine and other products defined by Regulation (EC) No 1234/2007, and alcohol-free counterparts	Only alcohol-free	200[(a)]
14.2.3	Cider and perry		200[(a)]
14.2.4	Fruit wine and made wine		200[(a)]
		Only made wine	260[(a)]
14.2.5	Mead		200[(a)]
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Only distilled alcoholic beverages containing whole pear	50[(a)]
14.2.7.1	Aromatised wines		200[(a)]
14.2.7.2	Aromatised wine-based drinks		200[(a)]
14.2.7.3	Aromatised wine-product cocktails		200[(a)]
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol	Only in fermented grape must-based drink	20[(a)]
		Only nalewka na winie owocowym, aromatyzowana nalewka na winie owocowym, nalewka na winie z soku winogronowego, aromatyzowana nalewka na winie z soku winogronowego, napój winny owocowy lub miodowy, aromatyzowany napój winny owocowy lub miodowy, wino owocowe niskoalkoholowe and aromatyzowane wino owocowe niskoalkoholow	200[(a)]
15.1	Potato-, cereal-, flour- or starch-based snacks	Only cereal- and potato-based snack	50[(a)]
15.2	Processed nuts	Only marinated nut	50[(a)]

MPL: maximum permitted level; FCS: Food Categorisation System (food nomenclature) presented in Annex II to Regulation (EC) No 1333/2008.
(a): Maximum levels are expressed as SO_2 and relate to the total quantity, available from all sources; an SO_2 content of not more than 10 mg/kg or 10 mg/L is not considered to be present.
(b): The food additives may be added individually or in combination.
(c): Maximum limits in edible parts.

In addition, sulfur dioxide (E 220), potassium bisulfite (E228) and potassium metabisulfite (E 224) are also authorised for use in wines and liquor wines according to Annex I, Part B, to Regulation (EC) No 2019/934 at a maximum concentration of sulfur dioxide ranging from 200 mg/L to 400 mg/L.

Furthermore, sulfur dioxide–sulfites (E 220–228) may be added to food additive preparations and to food enzymes according to Annex III (Part 2 and Part 3) to Regulation (EC) No 1333/2008. Namely, sulfur dioxide–sulfites (E 220–228) can be added to food colour preparations (except E 163 anthocyanins, E 150b caustic sulfite caramel and E 150d sulfite ammonia caramel) to a maximum level of 100 mg/kg per preparation and 2 mg/kg expressed as sulfur dioxide in the final product. Moreover, E 220 (sulfur dioxide), E 221 (sodium sulfite), E 222 (sodium hydrogen sulfite), E 223 (sodium metabisulfite) and E 224 (potassium metabisulfite) can be added to enzyme preparations in quantities that do not exceed 2 mg/kg in the final food and 2 mg/L in the final beverage.

In this assessment, food categories listed in Annex II to Regulation (EC) No 133/2008 or in Annex I, Part B, to Regulation (EC) No 2019/934 in relation to sulfur dioxide–sulfites (E 220–228) are referred to as food categories for which direct addition of sulfur dioxide–sulfites (E 220–228) is authorised.

4.3.2. Reported use levels or data on analytical levels of sulfur dioxide–sulfites (E 220–228) in food

During the re-evaluation of sulfur dioxide–sulfites (E 220–228), the ANS Panel concluded that the dietary exposure estimates of sulfur dioxide–sulfites at that time were higher than the group ADI of

0.7 mg SO_2 equivalent/kg bw per day for all population groups (EFSA ANS Panel, 2016). A call for data was published by the European Commission to update the dietary exposure estimates and to address the previously estimated exceedance of the ADI of sulfur dioxide.[13]

As a result of the call for data, only two use levels were provided (Documentation provided to EFSA No 15 and 19). Given the limited number of new use levels, the Panel also considered the use level data collected at the time of the 2016 re-evaluation (EFSA ANS Panel, 2016). Additionally, analytical data were extracted (June 2022) from the scientific data warehouse which contains data from European national authorities and similar bodies, research institutions, academia, food business operators and other stakeholders covering the period of 2012–2021. This period included 3 years (2012–2014) that were used in the 2016 re-evaluation by the ANS panel.

4.3.2.1. Summary on reported use levels of sulfur dioxide–sulfites (E 220–228) in foods

In response to the call for data of the European Commission, two use levels for food categories (FCs) '14.1.2 Fruit juices as defined by Directive 2001/112/EC and vegetable juices' and '14.1.4 Flavoured drinks' were submitted by the European Fruit Juice Association (AIJN) (Documentation provided to EFSA No 15) and by Union of European Soft Drinks Associations (UNESDA) (Documentation provided to EFSA No 19).

At the time of the 2016 re-evaluation, information on uses and use levels of sulfur dioxide–sulfites (E 220–228) was made available by FoodDrinkEurope (FDE) (n = 89), the European Starch Industry Association (AAF) (n = 2), the Gelatine Manufacturers of Europe (GME) (n = 8), the British Meat Processors Association (BMPA) (n = 2) and International Organisation of vine and wine (OIV) (n = 5). Two typical and maximum use levels for FC '8.2 Meat preparations' were above the MPL. In addition, for FCs '14.2.2 Wine and other products', '14.2.4 Fruit wine and made wine' and '14.2.7.1 Aromatised wines', five maximum use levels were above the MPL (EFSA ANS Panel, 2016). Furthermore, two uses were provided for food categories for which direct addition of sulfur dioxide–sulfites (E 220–228) is not authorised. These two uses were not further considered in the exposure assessment.

In summary, use levels (n = 106) in foods belonging to 20 of the 40 food categories for which direct addition of sulfur dioxide–sulfites (E 220–228) is authorised, were available for the assessment. Most data were provided for FC '8.2 Meat preparations as defined by Regulation (EC) No 853/2004'.

See Annex C (Table C.1) for an overview of the provided use levels expressed in SO_2 equivalents (See Section 4.3.5 Exposure estimates, Use level data considerations).

4.3.2.2. Summary of analytical data of sulfur dioxide–sulfites (E 220–228) in foods from the Member States

In total, 24,734 analytical results were submitted to EFSA. These data were reported by 22 countries: Austria (n = 526), Belgium (n = 1,152), Croatia (n = 467), Cyprus (n = 268), the Czech Republic (n = 1,589), Denmark (n = 145), Estonia (n = 6), France (n = 93), Germany (n = 4,691), Greece (n = 64), Hungary (n = 155), Ireland (n = 4,744), Italy (n = 2,907), Lithuania (n = 348), Luxembourg (n = 490), Malta (n = 41), Montenegro (n = 374), the Netherlands (n = 80), Portugal (n = 1,696), Slovakia (n = 2,495), Spain (n = 2,390) and the United Kingdom (n = 13).[14] Foods were sampled between 2012 and 2021.

Data from non-accredited laboratories (n = 1,594) or those that provided limited description of analytical methodology (n = 10,789) were not used in the exposure assessment.

Data reported as sodium sulfite (n = 70) or calcium hydrogen sulfite (n = 79) were discarded as the identity of the sulfite entity detected (i.e. SO_2 or the sulfite) was not specified.

Some reported analytical levels (n = 11) from one data provider were very high (three orders of magnitude higher than other analytical levels in the same food category), exceeding the MPL. These data were submitted in 2013, but the data provider could not confirm that these levels were correctly reported. These data were therefore not included in the exposure assessment. Other data reported as non-quantified with a very high LOQ (10,000 mg/kg) were also not included (n = 8).

The final analytical database contained 12,183 results; 10,195 were for food categories authorised to contain sulfur dioxide–sulfites (E 220–228), of which 380 were above the MPL. The remaining 1,988 analytical results could not be linked to any authorised food category (Annex C, Table C.2).

[13] Available online: https://ec.europa.eu/food/system/files/2019-01/fs_food-improvement-agents_reeval_call_20161010_e220-e228_data.pdf

[14] Occurrence data included in the assessment were submitted to EFSA when the UK was a member of the EU (see 22nd-plenary-meeting-faf-panel-minutes.pdf (europa.eu)).

4.3.3. Summarised data extracted from the Mintel's Global New Products Database

The Mintel's GNPD is an online database which monitors new introductions of packaged goods in the market worldwide. It contains information on over 3.6 million food and beverage products of which more than 1,300,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 24 of its 27 member countries plus Norway presented in the Mintel's GNPD.[15]

For the purpose of this assessment, Mintel's GNPD[16] was used for checking the labelling of food and beverage products and food supplements for sulfur dioxide–sulfites (E 220–228) within the EU's food market as the database contains the compulsory ingredient information on the label.

According to Mintel's GNPD, based on a query spanning the period between January 2018 and May 2022, sulfur dioxide–sulfites (E 220–228) were labelled on 3,303 products without specifying the exact E-number. These products were mainly dressings & vinegar and other table sauces (mustard, BBQ, horseradish sauce, etc.), prepared meals, pickled condiments, fish and meat products.

For individual food additives, 2,227 products were labelled to contain sodium metabisulfite (E 223), mostly fish products, sweet and salty biscuits, cookies, potato products, hors d'oeuvres/canapes, processed vegetables and fruit snacks. Moreover, 1,944 products (mainly fruit snacks, snack mixes, cakes, pastries and other sweet goods, as well as other snack, cereal and energy bars) were labelled to contain sulfur dioxide (E 220) and 1,172 products (mainly table sauces, seasonings, dressings, vinegars, cider and salads) were labelled to contain potassium metabisulfite (E 224).

Sodium sulfite (E 221), sodium bisulfite (E 222) and potassium bisulfite (E 228) were labelled in fewer products (29 to 290), while calcium sulfite (E 226) and calcium bisulfite (E 227) were only reported in one product (Table 6).

Table 6: Number of products labelled to contain sulfur dioxide–sulfites (E 220–228) according to Mintel's GNPD (January 2018 to May 2022)

Additive - E number	N of products labelled
Sulfites (E220-E228)[(a)]	3,303
Sulfur dioxide (E 220)	1,944
Sodium sulfite (E 221)	290
Sodium bisulfite (sodium hydrogen sulfite) (E 222)	163
Sodium metabisulfite (E 223)	2,227
Potassium metabisulfite (E 224)	1,172
Calcium sulfite (E 226)	0
Calcium bisulfite (calcium hydrogen sulfite) (E 227)	1
Potassium bisulfite (E 228)	29

(a): The use of sulfur dioxide or a specific sulfite was not indicated on the label.

In addition to the detailed list of Mintel subcategories with foods labelled to contain sulfur dioxide-sulfites (E 220-E228), Annex C (Table C.3) lists the percentage of the food products labelled to contain these additives out of the total number of food products per food subcategory according to Mintel's GNPD food classification. The percentages ranged from less than 0.1% in some food subcategories to 17.8% in Mintel's GNPD food subcategory 'Dressings and Vinegar' for sulfites not specified (E 220–228), 14.5% for potassium metabisulfite (E 223) in 'Cider' and 13.4% for sulfur dioxide (E 220) in 'Snack mixes'.

Note that these percentages do not represent the market share of the products listed per food category. Annex C (Table C.3) also contains the list of corresponding food categories of Annex II to Regulation (EC) No 1333/2008. As a one-to-one linkage between Mintel subcategories and these food categories was not possible, this list should be considered as indicative.

[15] Missing Cyprus, Luxembourg and Malta.
[16] http://www.gnpd.com/sinatra/home/ accessed on 20/05/2022.

4.3.4. Food consumption data used for exposure assessment

4.3.4.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011)). The version of the Comprehensive database taken into account in the exposure assessment was published in July 2021.[17] Data from EU Member States were considered for the estimations.

The food consumption data in the Comprehensive database were collected by different methodologies and thus direct country-to-country comparisons may not be appropriate. Depending on the food category and the level of detail used for the exposure calculations, the exposure estimates may be influenced by subjects' underreporting and/or misreporting consumption amounts. Nevertheless, the EFSA Comprehensive Database includes the currently best available food consumption data across the EU.

Food consumption data from infants, toddlers, children, adolescents, adults and the elderly were used in the dietary exposure assessment of sulfur dioxide–sulfites (E 220–228). For the present assessment, food consumption data were available from 41 different dietary surveys carried out in 22 EU Member States (Table 7). Not all Member States provided consumption information for all population groups, and in some cases, the same Member State provided food consumption data from more than one consumption survey. In most cases, when for one country and age class different dietary surveys were available, only the most recent was used. However, when two national surveys from the same country gave a better coverage of the age ranges, than using only the most recent one, both surveys were kept. For details on each survey, see Annex C (Table C.4).

Table 7: Population groups considered in the dietary exposure assessment of sulfur dioxide–sulfites (E 220–228)

Population	Age range	EU Member States with food consumption surveys covering more than 1 day
Infants	From more than 12 weeks up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia
Toddlers[(a)]	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, Slovenia, Spain
Children[(b)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
The elderly[(b)]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden

(a): The term 'toddlers' in the Comprehensive Database (EFSA, 2011) corresponds to 'young children' in Regulations (EC) No 1333/2008 and (EU) No 609/2013.
(b): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Comprehensive Database (EFSA, 2011).

Since 2018, all consumption records in the Comprehensive Database are codified according to the FoodEx2 classification system (EFSA, 2015). Nomenclature from the FoodEx2 classification system has

[17] https://www.efsa.europa.eu/en/data-report/food-consumption-data

been linked to the food categorisation system of Annex II of Regulation (EC) No 1333/2008, Part D, to perform the exposure assessments of food additives. In practice, the FoodEx2 food codes were matched to the food categories. FoodEx2 also includes facets, which may be used to provide further information about different properties and aspects of foods recorded in the Comprehensive Database. These facets were used in the exposure assessment of sulfur dioxide–sulfites (E 220–228) to optimise the mapping of the concentration data to the foods codified in the Comprehensive Database (e.g. process: drying, packaging: vacuum-packed).

4.3.4.2. Food categories considered for the exposure assessment to sulfur dioxide–sulfites (E 220–228)

A detailed list of the food categories of Annex II of Regulation (EC) No 1333/2008 and Regulation (EC) No 2019/934 and how the restrictions and exceptions were addressed in the exposure assessment can be found in Annex C (Table C.5).

In some cases, restrictions and exceptions were addressed by selecting the specific FoodEx2 codes that were mapped to an authorised food category. In other instances, when the restriction or exception is not referenced in FoodEx2, all food products within a food category were included in the exposure assessment (e.g. for FC '14.1.2 Fruit and vegetable juices, only orange, grapefruit, apple and pineapple juice for bulk dispensing in catering establishments', all consumed orange, grapefruit, apple and pineapple juice were taken into account). However, if this would result in a large overestimation of the exposure, these food products were not considered (e.g. for the same category 14.1.2: 'Only concentrated grape juice used for home wine making' or 'Only grape juice, unfermented, for sacramental use' which were considered by the Panel as specialised uses and therefore were not included).

Similarly, some food categories were not considered in the exposure assessment, as they are not referenced in the FoodEx2 classification system, or no consumption data were available (e.g. '14.2.5 Mead' or '15.2 Processed nuts, only marinated nut').

For specific uses, such as wines, for which more than one MPL is available (e.g. 185 mg/L for quality sparkling wines and 235 mg/L for other sparkling wines), the highest MPL was used.

Overall, of the 40 food categories in which the use of sulfur dioxide–sulfites (E 220–228) is authorised according to Annex II to Regulation (EC) No 1333/2008 and Annex I, Part B, to Regulation (EC) No 2019/934, 36 food categories were considered in the MPL scenario and 34 food categories in the refined scenarios.

4.3.5. Exposure estimates

4.3.5.1. Dietary exposure to sulfur dioxide–sulfites (E 220–228) from its use as a food additive

The Panel estimated the chronic dietary exposure to sulfur dioxide–sulfites (E 220–228) expressed as SO_2 equivalents for the following population groups: infants, toddlers, children, adolescents, adults and the elderly. The methodology to estimate the dietary exposure to sulfur dioxide–sulfites (E 220–228) in the current assessment with different scenarios – MPL exposure assessment scenario and refined exposure assessment scenarios (brand-loyal and non-brand-loyal) – is described in the approach for the exposure assessment of food additives under re-evaluation (EFSA ANS Panel, 2017).

Use level data considerations

Use levels reported as E 220 (n = 17) or E 220–228 (n = 2) were expressed as SO_2 equivalents. Use levels for other E-numbers with a maximum value equal to the MPL (E 221 n = 8, E 222 n = 7, E 223 n = 9, E224 n = 7, E 228 n = 7) were assumed to be already expressed as SO_2 equivalents, as well as when the data provider had indicated that the levels are expressed as E 220. Remaining use levels reported as E 223 (n = 6) or E 224 (n = 5) were converted to SO_2 equivalents considering the theoretical SO_2 content presented in Table 1 (Section 2.2 Methodology).

Use levels reported for E 226 (n = 7) and E 227 (n = 7) were not considered in the exposure assessment since an IBO indicated that they are not aware of any member producing these two additives. Furthermore, the production of E 227 was stopped by the lead registrant in 2012 and this was confirmed by the information in the Mintel GNPD (see also Section 4.3.3). By excluding the data provided on use levels for these two food additives, no food category was eliminated from the exposure assessment.

Two uses for FC 8.2 Meat preparations were excluded from the exposure assessment, because both the typical and maximum use level were above the MPL. In addition, for FCs '14.2.2 Wine and other products', '14.2.4 Fruit wine and made wine' and '14.2.7.1 Aromatised wines', the highest typical values were considered in the assessment, because all five reported maximum use levels for these categories were above the MPL.

Analytical data considerations

The final analytical database contained 12,183 concentrations; 10,234 were codified in food categories authorised to contain sulfur dioxide–sulfites (E 220–228) and among these 380 were above the MPL. The remaining, 1,949 could not be linked to any food in which direct addition of sulfur dioxide–sulfites (E 220–228) is authorised (Table 5); however, their presence could be due to carry-over (see Art. 18 of Regulation (EC) No 1333/2008).

Of these 1,949 concentrations, foods with only one analytical result were not included in the assessment (n = 151). Furthermore, foods with only left censored data (i.e. analytical results below the limit of detection (LOD) or limit of quantification (LOQ)) (n = 446) were not considered in the exposure assessment. As a result, 11,586 concentrations were used in the exposure assessment.

To consider left-censored analytical data in the dietary exposure assessment scenarios described below, the substitution method as recommended in the 'Principles and Methods for the Risk Assessment of Chemicals in Food' (WHO, 2009) and the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010) was used. In the present opinion, analytical data below LOD or LOQ were assigned half of LOD or LOQ, respectively (medium-bound (MB)). Subsequently, per food category the mean or median, as appropriate, MB concentration was calculated.

In order to minimise the impact of possible outliers on the exposure estimates, the 95th percentile concentration was used in the assessment for food categories in which the direct addition of sulfur dioxide–sulfites (E 220–228) is not authorised and in the refined brand-loyal scenario for authorised food categories in which the maximum concentration is above the MPL (see below). When less than 60 analytical levels were available for a food category, the 95th percentile was not calculated (EFSA, 2011) and the maximum exposure value was used instead.

Concentration data sets to calculate the exposure

The dietary exposure to sulfur dioxide–sulfites (E 220–228) was assessed using four sets of concentration data:

1) **Data set MPL**: The MPLs set down in the EU legislation.

   Exposure estimates based on this data set include only the food categories in which sulfur dioxide–sulfites (E 220–228) are authorised and are based on levels of sulfur dioxide in these foods that are equal to the MPL.

2) **Data set A**: Reported use levels and analytical data (results not exceeding the MPL) for food categories for which direct addition of sulfur dioxide–sulfites (E 220–228) is authorised.
   To estimate the exposure based on **Data set A**, the following two refined exposure scenarios were calculated considering the following values:

   - Refined brand-loyal (DSA-BL): Maximum of the reported use levels or analytical data, whichever is higher/available, for the main contributing food category at the individual level, and the mean of the typical reported use levels or analytical data, whichever is higher/available, for the remaining food categories.
   - Refined non-brand-loyal (DSA-NBL): Mean of use level data or analytical data (whichever is higher/available) for all food categories.

These scenarios present the dietary exposure for only the food categories in which sulfur dioxide–sulfites (E 220–228) can be added and provided that foods do not contain sulfur dioxide at levels exceeding the MPL.

3) **Data set B**: Reported use levels and analytical data (results not exceeding the MPL) for food categories for which direct addition of sulfur dioxide-sulfites (E 220–228) is authorised and, in addition, analytical data for food categories in which the presence of sulfur dioxide–sulfites (E 220–228) may be due to carry-over and for food categories for which the direct addition of sulfur dioxide–sulfites (E 220–228) is not authorised and whose presence cannot be explained via carry-over, but e.g. via natural occurrence.

This database should reflect all the dietary sources of sulfur dioxide and not only from those foods in which sulfur dioxide–sulfites (E 220–228) as additives are authorised, provided that foods do not contain sulfur dioxide at levels exceeding the MPL.

To estimate the dietary exposure based on **Data set B,** the same two exposure scenarios were calculated as for Data set A, considering the following:

- Refined brand-loyal (DSB-BL):
  - for food categories where direct addition of sulfur dioxide–sulfites (E 220–228) is authorised: Mean and maximum of use level data or analytical data (whichever is higher/available);
  - for food categories where sulfur dioxide-sulfites (E 220–228) are not authorised: Mean and P95 (for food categories with at least 60 analytical levels) or maximum (for food categories with less than 60 analytical levels) of analytical data. The Panel noted that the maximum analytical level was used for most food categories due to a limited number of analytical data.
- Refined non-brand-loyal (DSB-NBL): Mean of use level data or analytical data (whichever is higher/available) for all food categories.

The exposure estimates using Data set B include all dietary sources of sulfur dioxide based on the use levels and analytical data available, and do not consider analytical data exceeding the MPL.

4) **Data set C:** As Data set B, but also including analytical data exceeding the MPL. To estimate the dietary exposure based on **Data set C**, the same two scenarios were calculated as for Data set A, considering the following:
   - Refined brand-loyal (DSC-BL):
     - Food categories where direct addition of sulfur dioxide–sulfites (E 220–228) is authorised:
       - Analytical data below the MPL, or above the MPL with less than 60 analytical data: Mean and maximum of use level data or analytical data (whichever is higher/available);
       - Analytical data above the MPL and with at least 60 analytical data: Mean and P95 of analytical data, or mean and maximum of use level data (whichever is higher/available);
     - Food categories where sulfur dioxide–sulfites (E 220–228) are not authorised: Mean and P95 (for food categories where number of analytical data is at least 60) or maximum (for food categories where number of analytical data is less than 60) of analytical data.
   - Refined non-brand-loyal (DSC-NBL): Mean of use level data or analytical data (whichever is higher/available) for all food categories.

Based on this data set, dietary exposure was estimated from all dietary sources of sulfur dioxide, including analytical data that exceeded the MPL.

5) **Data set D** considers the same food categories as Data set C, but if analytical levels (including results exceeding the MPL) are available, they were taken into account, even if they are lower than the use levels.

   It is known that sulfur dioxide may be lost during processing, storage and preparation stages. These losses will increase with time and are dependent on pH, temperature, humidity, light and other factors (EFSA ANS, 2016; Documentation provided to EFSA No 14; Documentation provided to EFSA No 16). Since an extensive analytical database was available for the current assessment, the Panel considered an exposure scenario based on analytical results (Data set D), as these results represent the level of SO_2 equivalents in final products. In this case, reported use levels were considered only for those food categories for which no analytical data were available.

The same two scenarios as for Data set A were calculated considering the following values:

- Refined brand-loyal (DSD-BL):
  - Food categories where direct addition of sulfur dioxide–sulfites (E 220–228) is authorised:
    - Analytical data are below the MPL, or above the MPL but number of analytical data is less than 60: Mean and maximum of analytical data;
    - The number of analytical data is at least 60 and data exceed the MPL: Mean and P95 of analytical data.
    - No analytical data are available for a food category: Mean and maximum of reported use levels;
  - For food categories where direct addition of sulfur dioxide–sulfites (E 220–228) is not authorised:
    - Mean and P95 for food categories where number of analytical data is at least 60;
    - Mean and maximum of analytical data for food categories where number of analytical data is less than 60.
- Refined non-brand-loyal (DSD-NBL):
  - Mean of analytical data;
  - If analytical data are not available for a food category where direct addition of sulfur dioxide–sulfites (E 220–228) is authorised, mean of use level data was considered.

Based on Data set D, the dietary exposure was estimated for all dietary sources of sulfur dioxide, including analytical data that exceeded the MPL. The analytical levels of sulfur dioxide–sulfites (E 220–228) are more representative of the actual concentration of sulfur dioxide in foods and beverages at the time of consumption than use levels.

4.3.5.2. Results of the exposure assessment

Table 8 summarises the estimated dietary exposure to sulfur dioxide from the use of sulfur dioxide–sulfites (E 220–228), from carry-over and through natural occurrence in six population groups (Table 7) according to the different exposure scenarios explained above. Detailed results per population group and survey are presented in Annex C (Table C.6).

Table 8: Summary of dietary exposure to sulfur dioxide from the use of sulfur dioxide–sulfites (E 220–228) in the regulatory maximum level exposure assessment scenario and in the refined exposure scenarios based on Data set A, and in addition from carry-over and through natural occurrence in the refined exposure scenarios based on Data sets B, C and D, in six population groups (minimum–maximum) across the dietary surveys in mg SO_2 equivalents/kg bw per day

	Infants (12 weeks to 11 months)		Toddlers (12–35 months)		Children (3–9 years)		Adolescents (10–17 years)		Adults (18–64 years)		The elderly (≥ 65 years)	
	Min	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
DATA SET MPL: Regulatory maximum level exposure assessment scenario												
Mean	0.05	0.59	0.26	3.58	0.25	2.53	0.08	0.93	0.17	0.77	0.16	0.82
95th percentile	0.16	3.46	0.80	11.88	0.83	7.81	0.37	3.43	0.53	2.12	0.60	1.89
DATA SET A: Refined estimated exposure assessment scenario (only authorised below MPL)												
Brand-loyal scenario												
Mean	0.002	0.30	0.14	3.03	0.13	2.17	0.05	0.76	0.12	0.59	0.10	0.64
95th percentile	0.09	1.62	0.33	10.51	0.39	7.51	0.20	3.28	0.43	1.77	0.32	1.63
Non-brand-loyal scenario												
Mean	0.002	0.11	0.05	1.63	0.06	1.20	0.02	0.42	0.05	0.31	0.03	0.32

	Infants (12 weeks to 11 months)		Toddlers (12–35 months)		Children (3–9 years)		Adolescents (10–17 years)		Adults (18–64 years)		The elderly (≥ 65 years)	
	Min	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
95th percentile	0.03	0.67	0.17	5.91	0.16	4.24	0.07	1.84	0.20	1.06	0.11	0.86
DATA SET B: Refined estimated exposure assessment scenario (authorised below MPL + carry over)												
Brand-loyal scenario												
Mean	0.002	0.30	0.14	3.03	0.13	2.17	0.05	0.76	0.12	0.59	0.10	0.64
95th percentile	0.09	1.62	0.33	10.51	0.39	7.51	0.20	3.28	0.43	1.77	0.32	1.63
Non-brand-loyal scenario												
Mean	0.002	0.11	0.05	1.63	0.06	1.20	0.02	0.42	0.05	0.31	0.03	0.32
95th percentile	0.03	0.67	0.17	5.91	0.16	4.24	0.07	1.84	0.20	1.06	0.11	0.86
DATA SET C: Refined estimated exposure assessment scenario (authorised with exceeding MPL + carry over)												
Brand-loyal scenario												
Mean	0.002	1.09	0.21	4.17	0.27	3.43	0.16	1.34	0.29	1.06	0.21	0.88
95th percentile	0.25	3.76	0.82	12.37	1.16	8.82	0.70	4.04	0.97	4.00	0.80	2.23
Non-brand-loyal scenario												
Mean	0.002	0.19	0.06	1.73	0.08	1.29	0.03	0.46	0.09	0.45	0.07	0.43
95th percentile	0.06	0.91	0.20	5.97	0.20	4.27	0.12	1.86	0.32	1.56	0.24	0.94
DATA SET D: Refined estimated exposure assessment scenario (authorised with exceeding MPL + carry over, preference for analytical data)												
Brand-loyal scenario												
Mean	0.001	1.07	0.18	2.00	0.21	1.85	0.15	0.82	0.25	0.96	0.19	0.70
95th percentile	0.22	3.46	0.79	6.54	0.63	5.07	0.68	2.68	0.94	3.72	0.79	2.08
Non-brand-loyal scenario												
Mean	0.001	0.12	0.03	0.32	0.05	0.25	0.03	0.13	0.08	0.31	0.06	0.31
95th percentile	0.05	0.64	0.11	0.77	0.16	0.54	0.11	0.38	0.27	1.17	0.22	0.77

The results of Data sets A and B showed that including concentration data reflecting carry-over (Data set B), next to the presence of sulfur dioxide in food and beverages from the use of sulfur dioxide–sulfites (E 220–228), barely affected the dietary exposure to sulfur dioxide–sulfites (E 220–228).

The highest exposure was estimated for the brand-loyal scenario using Data set C, which resulted in a maximum mean and P95 exposure estimates of 4.17 and 12.37 mg SO_2 equivalents/kg bw per day in toddlers, respectively. Compared to Data sets A and B, Data set C also included analytical levels that exceeded the MPL.

The dietary exposure results of Data set D (see Table 8), compared to those of Data set C, were based as much as possible on analytical results. These results were considered to represent the level of SO_2 equivalents in final products, because they take into account losses of SO_2 during processing, storage and the preparation stages (see above). Use levels were only included in this data set for those food categories for which no analytical data were available. The Panel considered Data set D to most realistically represent the dietary exposure to sulfur dioxide equivalents. Furthermore, the non-brand-loyal scenario was considered as the most appropriate for the risk assessment of sulfur dioxide–sulfites (E 220–228), because these additives are added to a wide range of foods and they do not impact taste or flavour of the final food. The highest mean exposure of 0.32 mg SO_2 equivalents/kg

bw per day was observed in toddlers and the highest P95 exposure estimate of 1.17 mg SO_2 equivalents /kg bw per day was found in adults.

4.3.5.3. Main food categories contributing to the dietary exposure to sulfur dioxide–sulfites (E 220–228)

Considering the contributors of the non-brand loyal scenario of Data set D, for both the adult and elderly population groups, the major contributors were FCs '14.1.2 Beer and malt beverages' and '14.2.2 Wine and other products'. For adolescents, other children and toddlers, FCs '14.1.4 Flavoured drinks' and '14.1.2 Fruit and vegetable juices' contributed most to the dietary exposure. In addition, for adolescents, FC '14.1.2 Beer and malt beverages', while for the younger population groups 'FC 4.2.1 Dried fruits and vegetables' was found as the third major contributor. In infants, different food categories were contributing for each of the surveys with most frequently FC '14.1.2 Fruit and vegetable juices' that contributed more than 10% of the exposure in seven of 11 surveys.

Detailed results on the contributing food categories for all the data sets, scenarios, population groups and surveys are presented in Annex C (Tables C.7–C.9).

The Panel noted that for the only survey with very high exposure results for toddlers, FC '14.1.4 Flavoured drinks' contributed more than 89% to the exposure in the brand-loyal scenario based on Data sets A, B and C.

4.3.6. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered for the refined scenarios of Data set D and summarised in Table 9.

Table 9: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate in the refined scenarios from Data set D

Sources of uncertainties	Direction [(a)]
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Methodology used to estimate high percentiles (95th) long-term (chronic) exposure based on data from food consumption surveys covering only a few days	+
Correspondence of reported use levels and analytical data to the food items in the EFSA Comprehensive Database: uncertainties to which types of food the levels refer	+/–
Uncertainty in possible national differences in use levels of food categories	+/–
Reported use levels/analytical levels: – Use levels and analytical levels in the authorised categories considered applicable to all foods within the entire food category, whereas on average, depending on the sulfite, from 0.01 to 1% of the foods, belonging to food categories with foods labelled with additive, were labelled with the additives – 11 reported use levels of E 223 and E 224 converted in SO_2 equivalent for four food categories – Use levels do not consider the possible loss of sulfites during processing – In categories where only use levels, but no analytical data were available, carry-over could not be considered – Analytical levels: uncertainty when the food products were analysed compared to their production date	 + +/– + – +/–
Food categories selected for the exposure assessment: exclusion of food categories due to missing FoodEx2 linkage	–
Food categories selected for the exposure assessment: inclusion of food categories without considering the restriction/exception	+
Food categories included in the exposure assessment: no occurrence data for certain food categories which were therefore not considered in the exposure estimates	–
Exposure assessment is based on analytical data with results below LOD/LOQ considered with middle-bound approach in some of the foods considered (of which some authorised categories)	+/–
Exposure calculations based on the P95 or maximum (in the brand-loyal scenario only) or mean levels (in both brand-loyal and non-brand-loyal scenario) of analytical data or reported use levels	+/–

(a): +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

Sulfur dioxide–sulfites (E 220–228) are authorised in 40 food categories (representing 85 different uses due to different restrictions and exceptions). Most of these uses (n = 57) were considered in the current exposure assessment.

Restrictions and exceptions were addressed by selecting the specific FoodEx2 codes that were mapped to an authorised food category. When the specific uses could not be captured by the FoodEx2 facets, either all the corresponding foods were included in the exposure assessment, resulting in an overestimation; or the food use was not included in the assessment at all, as it was considered to represent a limited, very specific consumption occasion.

The major food categories for which the exposure is probably overestimated due to case (1) are FCs '14.1.2 Fruit and vegetable juices', where the restriction on their consumption from bulk dispensing in catering establishments cannot be taken into account; and '14.1.4 Flavoured drinks' where, for the restriction 'only other concentrates based on fruit juice or comminuted fruit', the FoodEx2 system does not distinguish between fruit based and non-fruit based drink bases, thus all the consumed liquid- and powdered drink bases were considered. Food uses disregarded due to case (2) would result in only a small underestimation.

Similarly, some food categories were not considered in the exposure assessment, as they are not referenced in the FoodEx2 classification system, or no consumption data were available (e.g. '14.2.5 Mead' or '15.2 Processed nuts, only marinated nut'). In case of foods not referenced, this may have resulted in an underestimation of the exposure, but this underestimation was considered to be negligible.

The Panel noted that information from the Mintel GNPD (Annex C, Table C.3) indicated that sulfur dioxide–sulfites (E 220–228) were labelled on 3,303 foods belonging to 107 food subcategories, categorised according to the Mintel GNPD nomenclature in the period between January 2018 and May 2022. Most of these food subcategories were included in the current exposure assessment, and only 39 foods labelled with sulfites from Mintel were not taken into account in the exposure assessment. In addition, the percentage of foods per Mintel subcategory labelled to contain sulfur dioxide–sulfites (E 220–228) was on average of 2.7%. For two subcategories, the percentage of foods labelled with any sulfur dioxide–sulfites (E 220–228) additive was above 20% (cider and dressings and vinegar). In the assessment, it was assumed that 100% of the foods and food categories considered contained sulfur dioxide–sulfites. The Panel noted that the information from the Mintel GNPD indicated that sulfur dioxide–sulfites (E 220–228) are used in a large range of food subcategories in a low number of foods within each subcategory. Therefore, the assumption that use/analytical data considered applicable to all foods within the authorised food categories most probably resulted in an overestimation of the dietary exposure.

The Panel noted that foods which may contain sulfur dioxide–sulfites due to carry-over and natural occurrence were considered in the current exposure assessment (Data sets B, C and D).

Overall, the Panel considered that the uncertainties identified would, in general, result in an overestimation of the exposure to sulfur dioxide–sulfites (E 220–228) for the refined exposure scenarios estimated considering Data set D; the data set which was considered by the Panel to most realistically represent the concentrations expressed in sulfur dioxide equivalents in foods and beverages (see above).

4.4. Proposed revision to existing EU specifications for E 220-E 228

The potential exposure to impurities from the use of sulfur dioxide-sulfites (E 220–228) can be calculated by assuming that the impurity is present in the food additive up to a limit value, and then by calculation pro-rata to the estimates of exposure to the food additive itself.

Data set D was considered to most realistically represent the dietary exposure to sulfur dioxide-sulfite (E 220–228). However, it is expected that toxic elements in food containing sulfur dioxide-sulfites (E 220–228) will be present in the final food at amounts that are present in the food additive when added to the food (i.e. no loss would be expected in contrast with sulfur dioxide/sulfites). Hence, Data set C, taking into account use levels and analytical data, was considered the most appropriate scenario available for estimating the exposure to toxic elements from the use of these food additives.

Since the exposure to sulfur dioxide-sulfites (E 220–228) is expressed in mg SO_2 equivalents/kg bw per day, to calculate the exposure to impurities from the use of these food additives, the Panel converted the estimates to sulfite and considered two cases: (a) all exposure to sulfur dioxide-sulfites (E 220–228) expressed as sodium metabisulfite E 223, that was considered to be the sulfite

most typically used, based on the reported use and use levels as well as from the highest number of products labelled with E 223 in Mintel's database; (b) all exposure to sulfur dioxide-sulfites (E 220–228) expressed as sodium bisulfite E 222 which is considered to be the worst case for these calculations due to its low yield of sulfur dioxide (19.7%, Table 3).

The theoretical yield of sulfur dioxide from sodium metabisulfite (E 223) is 67.4% (Ough and Were, 2005; Table 3) and according to the EU specifications for E 223 the content of sulfur dioxide should be not less than 64%. From the analytical data submitted, the content of sulfur dioxide in analysed batches of E 223 ranged from 66.2% to 66.6% (Documentation provided to EFSA No 4). Therefore, the Panel considered the theoretical yield of 67.4% sulfur dioxide for the conversion of the exposure from an 'as sulfur dioxide' to an 'as E 223' basis. This theoretical yield value was also used for calculation of doses expressed in sulfur dioxide in the toxicological studies performed with sodium metabisulfite (E 223) (see Section 2.2).

According to Commission Regulation (EU) No 231/2012, E 222 is used as a food additive in solution of not less than 32% w/w. The content of toxic elements was analysed in 13 batches of E 222 described as 38–40% w/w solutions (Documentation provided to EFSA No 2). Considering the theoretical sulfur dioxide yield of 61.6% (Ough and Were, 2005) from sodium bisulfite and assuming that E 222 is presented as a 32% w/w solution of sodium bisulfite as foreseen in the EU specifications of E 222 (which, as the lowest permitted concentration, is the worst case assumption for these calculations) a sulfur dioxide yield of 19.7% from E 222 was used.

For the current assessment, the highest exposure levels for the mean and 95th percentile among the different population groups were, considering data set C refined non-brand loyal scenario, 1.73 mg SO_2 equivalent/kg bw per day and 5.97 mg SO_2 equivalent/ kg bw per day for toddlers (Table 8). Using the transformations described above, these estimates correspond to 2.57 and 8.86 mg E 223/kg bw per day, and to 8.78 and 30.30 mg E 222/kg bw per day, each for the mean and 95th percentile, respectively.

The levels of the impurities in E 223 and E 222 combined with the estimated intakes of E 223 or E 222, as calculated by the Panel, could result in an exposure which can be compared with the following reference points or health-based guidance values (HBGV) (Table 10) for the undesirable impurities potentially present in these food additives.

Table 10: Reference points/health-based guidance values for impurities potentially present in sulfur dioxide and sulfites (E 220-E 228) food additives

Impurity/HBGV/RP (μg/kg bw)	Basis/Reference
Lead (Pb)/ 0.5 ($BMDL_{01}$)	The reference point is based on a study demonstrating perturbation of intellectual development in children with the critical response size of 1 point reduction in IQ. The EFSA CONTAM Panel mentioned that a 1 point reduction in IQ is related to a 4.5% increase in the risk of failure to graduate from high school and that a 1 point reduction in IQ in children can be associated with a decrease of later productivity of about 2%. A risk cannot be excluded if the exposure exceeds the $BMDL_{01}$ (MOE lower than 1). EFSA CONTAM Panel (2010)
Mercury (Hg)/4 (TWI)	The HBGV was set using kidney weight changes in male rats as the pivotal effect. Based on the $BMDL_{10}$ of 0.06 mg/kg bw per day, expressed as mercury, and an uncertainty factor of 100 to account for inter- and intraspecies differences, with conversion to a weekly basis and rounding to one significant figure, a TWI for inorganic mercury of 4 μg/kg bw per week, expressed as mercury was established. EFSA CONTAM Panel (2012)
Cadmium (Cd)/2.5 (TWI)	The derivation of the reference point is based on a meta-analysis to evaluate the dose–response relationship between selected urinary cadmium and urinary beta-2-microglobulin as the biomarker of tubular damage recognised as the most useful biomarker in relation to tubular effects. A group-based BMDL5 of 4 μg Cd/g creatinine for humans was derived. A chemical specific adjustment factor of 3.9 was applied to account for human variability in urinary cadmium within each dose-subgroup in the analysis resulting in a reference point of 1.0 μg Cd per g creatinine. In order to remain below 1 μg Cd/g creatinine in urine in 95% of the population by age 50, the average daily dietary cadmium intake should not exceed 0.36 μg Cd/kg bw, corresponding to a weekly dietary intake of 2.5 μg Cd/kg bw. EFSA CONTAM Panel (2009a)

Impurity/HBGV/RP (μg/kg bw)	Basis/Reference
Arsenic (As)/0.3–8 ($BMDL_{01}$)	The reference point is based on a range of benchmark dose lower confidence limit ($BMDL_{01}$) values between 0.3 and 8 μg/kg bw per day identified for cancers of the lung, skin and bladder, as well as skin lesions. In general, the MOE should be at least 10,000 if the reference point is based on carcinogenicity in animal studies. However, as the BMDL for As is derived from human studies, an interspecies extrapolation factor (i.e. 10) is not needed. EFSA CONTAM Panel (2009b); EFSA Scientific Committee (2012b)

HBGV: Health based guidance value; RP: Reference point; $BMDL_{01}$: benchmark dose (lower confidence limit); bw: body weight; TWI: Tolerable Weekly Intake; MOE: margin of exposure.

The risk assessment of the undesirable impurities helps inform whether there could be a possible health concern if these impurities would be present at the limit values in the food additive. The assessment is performed by calculating the MOE (margin of exposure) by dividing the reference point (e.g. BMDL, Table 10) by the exposure estimate, or by estimating the contribution of the use of the food additive to the HBGV (expressed as percentage of the HBGV).

As maximum levels for Cd in different food commodities are stipulated in the EU and also taking into account that the mean Cd exposure for certain population groups across Europe is close to, or slightly exceeding, the TWI of 2.5 μg/kg bw (ref. CONTAM PANEL, 2009a, 2011), this toxic element was also considered by the Panel in this risk assessment.

The results of the analysis of toxic elements in commercial samples of E 221, E 222, E 223 and E 224 are reported in the respective Section 4.2. As indicated in Section 4.2, one IBO proposed lowest technologically achievable levels for Pb, Hg and As for E 222.

The Panel noted that the occurrence data on toxic elements submitted by the IBOs for E 221, E 222, E 223 and E 224 are substantially lower than the current limits in the EU specifications (Documentation provided to EFSA No 2, 4 and 5).

The Panel noted that the data provided by the IBOs varies in format as part of the data were submitted as a mean value determined in different years or over longer periods, some data were reported as below the LOQ with or without reporting the LOQ value, and other data were reported at exact analytical values determined. As indicated in Section 4.2, one IBO submitted the proposal for the lowest technologically achievable levels for Pb 0.01 mg/kg, Hg 0.01 mg/kg and As 0.05 mg/kg, in E 222. The Panel noted that the lowest technologically achievable levels proposed by the IBO are at the lower end of the reported analytical LOQs and therefore cannot be reliably enforced. Thus, they were not further considered for the risk assessment. The Panel performed the risk assessment that would result if these toxic elements were present in the sulfur dioxide - sulfites (E 220–228) at (i) the current maximum limit in the EU specification and (ii) the highest reported LOQ for Pb, Hg, Cd and As, and by applying a factor of 10 (Table 11). The outcome of the risk assessment for these two different scenarios is presented in Tables 12 and 13.

The Panel emphasised that the choice of the maximum limit values as well as other considerations, such as on multiple sources of exposure to conclude on the maximum limits for toxic elements in the specifications, is in the remit of risk management. The numbers used here are merely taken to support the risk assessment of these toxic elements as presented below.

Table 11: Different scenarios for the potential exposure to toxic elements from the use of sulfur dioxide – sulfites (E 220–228)

Source of the values (mg/kg) listed	Lead	Mercury	Cadmium	Arsenic
Current limits in the EU specifications for sulfites	2[(c)]	1	–	3
Range of LOQs reported by IBOs	0.01[(a)]–0.03[(b)]	0.01[(a)]–0.03[(b)]	0.01[(a)]–0.05[(b)]	0.05[(a)]–0.1[(b)]
Considering the range of LOQ multiplied by factor of 10	0.1–0.3	0.1–0.3	0.1–0.5	0.5–1
Values selected by the Panel for the risk assessment	0.3	0.3	0.5	1

(a): Documentation provided to EFSA No 2.
(b): Documentation provided to EFSA No 4, 5.
(c): For sulfur dioxide (E 220) the maximum levels in the Eu specifications is 5 mg/kg.

Table 12: Risk assessment for toxic elements performed for Case a (typical) - E 223

Exposure to E 223 (mg/kg bw/day)	Considering the presence of toxic elements at current limits of the EU specifications for E 223 (Commission Regulation (EU) No 231/2012)			
	MOE for Pb at 2 mg/kg	% of the TWI for Hg at 1 mg/kg	MOE for As at 3 mg/kg	
2.57[(a)]	97	0.45%	39–1,038	
8.86[(b)]	28	1.55%	11–301	
	Considering the presence of toxic elements at the values selected by the Panel			
	MOE for Pb at 0.3 mg/kg	% of the TWI for Hg at 0.3 mg/kg	% of the TWI for Cd at 0.5 mg/kg	MOE for As at 1 mg/kg
2.57[(a)]	649	0.13%	0.4%	117–3,113
8.86[(b)]	188	0.47%	1.2%	34–903

(a): Highest exposure level among the different population groups (refined non-brand-loyal scenario using Data set C – toddlers – mean (Table 8)) converted to E 223.
(b): Highest exposure level among the different population groups (refined non-brand-loyal scenario – using Data set C – toddlers – 95th percentile (Table 8)) converted to E 223.

Table 13: Risk assessment for toxic elements performed for Case b (worse case) - E 222.

Exposure to E 222 (mg/kg bw/day)	Considering the presence of toxic elements at current limits of the EU specifications for E 222 (Commission Regulation (EU) No 231/2012)			
	MOE for Pb at 2 mg/kg	% of the TWI for Hg at 1 mg/kg	MOE for As at 3 mg/kg	
8.78[(a)]	28	1.54%	11–304	
30.3[(b)]	8	5.3%	3–88	
	Considering the presence of toxic elements at the values selected by the Panel			
	MOE for Pb at 0.3 mg/kg	% of the TWI for Hg at 0.3 mg/kg	% of the TWI for Cd at 0.5 mg/kg	MOE for As at 1 mg/kg
8.78[(a)]	190	0.46%	1.2%	34–911
30.3[(b)]	55	1.59%	4.2%	10–264

(a): Highest exposure level among the different population groups (refined non-brand-loyal scenario using Data set C – toddlers – mean (Table 8)) converted to E 222.
(b): Highest exposure level among the different population groups (refined non-brand-loyal scenario – using Data set C – toddlers – 95th percentile (Table 8)) converted to E 222.

The potential exposure to these impurities from the use of sulfur dioxide-sulfites (E 220-E 228) based on the outcome of the evaluation for the typical (E 223) and worse case (E 222), was compared with the available HBGV and RP (Table 10). For As, in both scenarios i.e. (i) the maximum current limit in the EU specification and (ii) the values selected by the Panel, in particular the lower end of the range of calculated MOE values was considered to be insufficient.

For both scenarios, for Pb, Hg and Cd based on the outcome of the evaluation for the typical (E 223) and worse case (E 222), the presence of these toxic elements in sulfur dioxide-sulfites (E 220–228) either at the current specifications limit values or at the levels selected by the Panel would not give rise to concern.

The Panel considered that the maximum limits in the EU specifications for toxic elements should be established based on actual levels in the commercial food additive. If the European Commission decides to revise the current limits in the EU specifications, the estimates of toxic elements intake as above could be considered.

Based on the fact that (i) Cd was occasionally reported in the sulfur dioxide-sulfites (E 220–228), (ii) maximum levels for Cd are stipulated in various food commodities in EU and (iii) Cd exposure for certain population groups across Europe is close to, or exceeds the TWI of 2.5 μg/kg bw (EFSA CONTAM Panel, 2009a, 2011), it seems prudent to recommend that the European Commission consider introducing a specification for Cd for these inorganic food additives.

Summary of the proposed revisions to the specifications.

Overall, based on the information provided by the IBOs (Documentation provided to EFSA No 1, 2, 3, 4, 5 and 6) and the above considerations, the Panel recommended the following revisions of the existing EU specifications for sulfur dioxide-sulfites (E 220–228) as listed in Table 14. The Panel noted that the choice of maximum limits for impurities in the EU specifications is in the remit of risk management.

These recommendations for specifications apply to all the food additives from the group sulfur dioxide-sulfites (E 220–228). The Panel noted however, that analytical data were received only for E 221, E 222, E 223 and E 224.

In the case of sulfur dioxide itself, information was submitted that the food additive E 220 is produced and distributed only in pressurised, liquified form (Documentation provided to EFSA No 6). This is also indicated in the JECFA specifications (Appendix A, Table A.1). Sulfur dioxide can be corrosive towards metals, especially if it picks-up moisture and oxygen from the atmosphere as this can form traces of sulfuric acid. If any toxic elements were to be picked-up by the sulfur dioxide, the Panel has no information whether or not they would still contaminate the food additive once re-gasified. It may depend on exactly how the liquified/gasified sulfur dioxide is added by the end user (the food producer). For these reasons, the Panel considered that these recommendations on the revision of the specifications should also cover E 220.

Table 14: Proposal for a revised version of the existing EU specifications for toxic elements for sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228)

Purity	Commission Regulation (EU) No 231/2012	Comment/justification for revision
Arsenic	3 mg/kg	Maximum limit to be lowered on the basis of the information provided and on the considerations of the Panel
Lead	2[(a)] mg/kg	Maximum limit to be lowered on the basis of the information provided and on the considerations of the Panel
Mercury	1 mg/kg	Maximum limit to be lowered on the basis of the information provided and on the considerations of the Panel
Cadmium	–	Maximum limit to be introduced on the basis of the information provided and on the considerations of the Panel

(a): Except for sulfur dioxide (E 220) for which a maximum limit of 5 mg/kg is set.

The Panel is of the view that the existing maximum limit values for Fe and Se in the EU specifications could be expressed on the basis of the food additive, in line with the JECFA specifications, rather than on the 'SO_2 content' basis.

Furthermore, the Panel also recommended introducing the correct EINECS number i.e. 233-596-8 in the EU specifications for calcium sulfite (E 226).

4.5. Toxicity database

4.5.1. Data submitted to the call for data from the European Commission as follow-up of the re-evaluation of sulfur dioxide-sulfites (E 220–228)

Following the re-evaluation of sulfur dioxide-sulfites (E 220–228) and the setting of a temporary ADI (EFSA ANS Panel, 2016), the ANS Panel recommended that the toxicological database should be improved prior to a new assessment. Following an European Commission call for data, no new data, specifically addressing the data gaps described in the re-evaluation of sulfur dioxide-sulfites (E 220–228) (EFSA ANS Panel, 2016), were received from IBOs.

4.5.2. Toxicity and genotoxicity studies from the literature search and additional genotoxicity studies submitted to EFSA

A total of 4,483 publications retrieved according to the literature search were screened based on criteria predefined by the FAF WG sulfur dioxide-sulfites (E 220–228) (Annex B). After the first

screening, approximately 150 *in vivo* studies and 60 *in vitro* studies were identified as potentially relevant for the current assessment.

The relevance of the identified *in vivo* toxicity studies was assessed following the approach described in Annex B. Around 100 studies were subsequently considered relevant for hazard characterisation and/or for providing supporting evidence. In a second step, an assessment of the reliability of the relevant publications was conducted according to the criteria described in Annex B. Only publications considered sufficiently reliable with respect to their internal validity, i.e. the extent to which the design and conduct of a study are likely to have prevented bias (with only minor or some limitations) were included in this safety assessment of sulfur dioxide-sulfites (E 220–228).

For in vitro studies, results from the identified studies are described narratively if the study investigates relevant endpoints related to effects observed in the in vivo studies.

For genotoxicity, the criteria for the assessment of relevance and reliability of *in vitro* and *in vivo* studies are provided in Annex B.

For hypersensitivity and intolerance potentially induced by sulfur dioxide or sulfites, all relevant animal, human and in vitro studies providing information on hypersensitivity or pseudo-allergic reactions and their mechanisms, including cellular and/or humoral mediators, have been reported narratively.

Several studies identified in the literature search reported on endogenous sulfur dioxide as a signalling molecule and its effects. These physiological effects are not considered in the present opinion.

4.5.2.1. ADME

As indicated in the re-evaluation of sulfur dioxide-sulfites (EFSA ANS Panel, 2016), all sulfites, once ingested, may react with water to form bisulfite, sulfite and sulfur dioxide. The prevailing species found in the stomach are bisulfite and sulfur dioxide, and the balance between these is determined by the acidity of the stomach whereas in the pH neutral environment of the intestine sulfite and bisulfite will exist as a nearly equimolar mixture. Sulfur dioxide gas may therefore be liberated in the stomach and a fraction of an oral dose may then be absorbed via the lungs through its inhalation. In any case, in plasma the predominant molecular species will be sulfite and bisulfite irrespective of the route of absorption, given the pH of blood. JECFA (1987) reported several studies that have examined the fate of sulfites given orally in mice, rats and monkeys using sodium metabisulfite, sodium sulfite or sulfur dioxide. Based on the data reported in these studies, JECFA concluded that 70 to 97% of the sulfite dose was absorbed from the intestine. Once absorbed, sulfite is converted to sulfate, by the enzyme sulfite oxidase (SOX) which is ubiquitously expressed with the highest activity in the liver and high activity in small intestine, indicating high pre-systemic metabolism. Although the expression of SOX is 20 times lower in human compared to rat liver, the ratio of specific enzyme activity between human and rat homogenates varied between 0.39 and 0.52, depending on the reaction measured (Johnson and Rajagopalan, 1976).

The half-life of sulfites in humans is reported to be 15 min. Under high load, the formation of S-sulfonates, including protein S-sulfonates may occur (EFSA ANS Panel, 2016). In its 2016 opinion, the ANS Panel had noted the absence of specific ADME studies measuring reaction products with the different sulfites (EFSA ANS Panel, 2016).

The ANS Panel considered that once ingested, based on their capacity to form sulfite ions, read across between the different sulfites possible. The FAF Panel agreed to this consideration.

The FAF Panel reconsidered one publication available in 2016 (Gunnison et al., 1977) which reported data on sulfite clearance in rats (n = 5; 3–4 repetitive experiments) following intravenous administration. The mean clearance of sulfite was reported to be 76.3 $\pm$ 13.4 ml/min/kg bw. Assuming that this clearance was primarily due to liver metabolism to sulfate and using published data on the liver blood flow of 104.3 $\pm$ 17.1 mL/min/kg (Gibson et al., 2021), sulfite extraction by the liver was calculated by the Panel to be 0.73. Using these assumptions, the Panel therefore estimated that at least 73% of an oral dose of sulfite will be cleared at first pass metabolism while up to 27% of an oral dose could reach the systemic circulation. It is known that other organs, e.g. kidney, heart also contain SOX and that the SOX in these organs contributes to the metabolism and hence clearance of sulfite.

Presystemic metabolism by the gut wall is not a major determinant in the clearance of sulfite after intravenous administration. Therefore, the estimated percentage of 27% is a conservative estimate of oral sulfite reaching the systemic circulation.

From the literature search a publication was found in which sulfite concentrations were measured in rat brain tissue homogenates from the prefrontal cortex (Wang et al., 2016). Adult male Sprague

Dawley (SD) rats were dosed with 500 mg Na2SO3/kg bw by intraperitoneal injection. Sulfite concentrations were measured by an enzymatic assay in prefrontal cortex tissue homogenates from animals sacrificed at 30 min (n = 5), 1 h (n = 5), 1.5 h (n = 3) and 2 h (n = 3) after administration. Concentrations were 21.7 mg/L, 20.4 mg/L, 1.2 mg/L and 2 mg/L at 30 min, 1 h, 1.5 h and 2 h, respectively. Although the study details are poorly reported the findings indicate that sulfite, when systemically available, reaches the brain rapidly and has a half-life there of some minutes. However, brain sulfite concentrations as measured following intraperitoneal administration will not be reached if the same dose is given via oral administration.

Another study, Meng et al. (2005) investigated the presence of sulfite ('all forms of sulfite in organs, including protein-bound sulfonates and low-molecular-mass S-sulfonates such as cysteine S-sulfonate and free sulfite') in the lungs, heart and brain of Kunming albino male mice exposed to increasing doses of sulfur dioxide (14.00 ± 1.25 mg/m^3, 28.00 ± 1.98 mg/m^3 and 56.00 ± 3.11 mg/m^3) for 4 h/day for 7 days in whole-body inhalation chambers. A dose-dependent increase in sulfites was detected in the three organs. This study indicates that inhaled sulfur dioxide may be systemically available leading to a dose-dependent increase of all forms of sulfites in lung, heart and brain. However, as the exposure was by whole-body exposure in the inhalation chamber oral exposure by licking the fur and dermal absorption may have contributed to the concentrations measured in the organs.

No new data on ADME were submitted by IBOs. The available data show distribution of sulfites in brain and heart following intraperitoneal or inhalation exposure to sodium sulfite and sulfur dioxide, respectively. The Panel considered that sulfites undergo high first pass metabolism after oral exposure but that systemic exposure to sulfites may be up to around a quarter of the dose.

4.5.2.2. General toxicity

Toxicity studies considered sufficiently reliable with respect to their internal validity (with only minor or some limitations according to Annex B) were considered for the assessment and data reflecting the information provided in the publications is presented in Appendix B/General toxicity.

There are nine general toxicity studies that were found sufficiently reliable with respect to their internal validity, three oral studies in rats and six inhalation studies in mice and rats.

Oral studies

Dalefield and Mueller (2016) studied in rats the effect of sodium metabisulfite given in the diet at concentrations between 0.25 and 4% for 7 days on gastric mucosal irritation. The only treatment-related effect observed was decreased bodyweight gain at the highest dose. The Panel noted that there was no observed effect on body weight gain at 1%, corresponding to 478 mg SO_2 equivalents/kg bw per day. However, in the absence of individual animal data and daily data for food intake and body weight changes, the relevance of the observed effects is unclear.

Ercan et al. (2015) administered 100 mg sodium metabisulfite (corresponding to 67.4 SO_2 equivalents/kg bw per day) by gavage for 35 days to rats and studied the effect on xanthine oxidase activity, endoplasmic reticulum (ER) and caspase activation in the liver, kidney and heart. The results demonstrated that sodium metabisulfite treatment activated xanthine oxidase, triggered ER stress and induced caspase activation in the liver but not in the heart or kidney. The toxicological relevance of these effects is however not known, and a reference point could not be identified.

Mahmoud et al. (2015) investigated the effects of sodium sulfite on various biochemical, cytological and histopathological parameters in various tissues of Wistar rats. Sodium sulfite of unclear origin was given in drinking water at concentrations of 9.4, 23.6 and 94.5 mg SO_2 equivalents/kg bw per day for 12 weeks. Sodium sulfite exposure caused significant changes in several haematological, clinical chemistry and histopathological endpoints. Histopathological changes in liver and kidney were observed in the mid and high dose groups and there were effects on haematological and clinical chemistry parameters already in the low dose group. Given that the observed effects have not been reported in other oral repeat dose studies even at higher doses (EFSA ANS Panel, 2016) and that the sodium sulfite used in this study was not sourced from an established supplier and not fully characterised, the Panel considered that it is uncertain whether the effects reported are due to sodium sulfite.

Inhalation studies

In the inhalation studies, animals were exposed to SO_2 between 2 and 4 h per day for from 7 days to up to 13 weeks. In all studies, the animals were exposed in inhalation chambers.

In an inhalation study in mice, Meng and Liu (2007) demonstrated a number of ultrastructural changes in all organs tested (lung, liver, spleen, kidney, testes, heart and brain) following sulfur dioxide exposure for 7 days at exposures calculated to correspond to an internal dose of 7.8 and 16 mg/kg bw per day. The toxicological relevance of these effects is unclear.

In an inhalation study in mice (Qin et al., 2015), the effect of sulfur dioxide exposure for 4 weeks, at an exposure calculated to correspond to an internal dose of 2.9 mg/kg bw per day, on mRNA expression of enzymes involved in mitochondrial function and apoptosis in the liver was investigated. Only minor effects of questionable toxicological relevance were observed.

In rats, Zhang et al. (2018) demonstrated that sulfur dioxide inhalation at an exposure calculated to correspond to an internal dose of 141 mg/kg bw per day for 7 days resulted in histopathological changes in the lung and reduced number of CD19+ cells. However, the Panel considered that the study findings were not plausible (see Appendix C) and the observations were likely associated with localised high concentrations of sulfur dioxide. These findings were considered by the Panel to be of limited relevance to oral exposure of sulfur dioxide-sulfites when used as a food additive.

In a 30-day inhalation study (Zhang et al., 2015), rats were exposed to exposures calculated to correspond to an internal dose of 0.6, 1.3 and 2.5 mg SO_2/kg bw per day and the effect on the expression of the ATP-sensitive K+ channel and the L-type calcium channel in the heart was investigated. At the highest dose, myocardial myofibril disorder and myocardial 'gap expansion' were reported. However, the histopathological effects were not quantified, and the histopathological observations were not performed under blinded conditions.

In another 30-day inhalation study in rats, Qin et al. (2017) studied the effect of sulfur dioxide at exposures calculated to correspond to an internal dose of 0.6, 1.3 and 2.6 mg/kg bw per day on mitochondria isolated from homogenised lung. Sulfur dioxide exposure at the lowest dose resulted in depressed inner mitochondrial membrane potential, cytochrome c oxidase activity, mitochondrial DNA contents, mRNA expression of respiratory complexes and both mRNA transcript and protein levels of PGC-1a, NRF1 and TFAM. The Panel considered this study could be used to identify potential modes of action of sulfur dioxide. Given the histological changes observed in the above-mentioned study by Zhang et al. (2015), the Panel considered that these effects might be indicative of early pulmonary damage related to sulfur dioxide exposure.

In summary, no adverse effects were observed following oral sulfite administration or inhaled SO_2 in rodents.

4.5.2.3. Reproductive and developmental toxicity

Reproductive and developmental toxicity studies considered sufficiently reliable with respect to their internal validity (with only minor or some limitations according to Annex B) were considered for the assessment. The data reflecting the information provided in the publications is presented in Appendix B/Reproductive and developmental toxicity. Very few studies met the criteria for full reliability and none of them were conducted with oral administration. Three studies (Zhang et al., 2006a,b; Murray et al., 1979) exposed rats, mice or rabbits to sulfur dioxide in whole-body inhalation chambers. One study (Gunnison et al., 1987) examined the effects of endogenously produced sulfite in a rat model with different degrees of SOX deficiency.

Zhang et al. (2006a,b) published two papers on the effects of sulfur dioxide inhalation in male rats; one examined testicular protein content and enzyme activity and the other concentrated on oxidative stress and antioxidant defences in the testes. The Panel considered it likely that the tests were conducted on the same animals. Male Wistar rats (24 rats per group, age 12 weeks) were exposed by inhalation (whole body) to an exposure calculated to correspond to an internal dose of 7.1 mg/kg bw per day for 8 weeks. After 2, 4 and 6 weeks, interim sacrifices were performed on groups of 6 animals. The following parameters were measured in testicular tissue: total protein content, gamma-glutamyl transpeptidase, lactate dehydrogenase and ion-activated adenosine triphosphatase; glutathione peroxidase activity, superoxide dismutase activity and malondialdehyde as oxidative stress indicators. Sulfur dioxide exposure resulted in a statistically significantly decreased ($p < 0.05$) protein content after 2, 4 and 6 weeks, a statistically significantly decreased ($p < 0.01$) gamma-glutamyl transpeptidase after 4 and 6 weeks. In addition, lactate dehydrogenase was statistically significantly increased ($p < 0.01$) after 2 ($p < 0.05$) and 6 weeks ($p < 0.01$) and some statistically significant increases and decreases were observed on ion-activated adenosine triphosphatases. However, after 8 weeks, all the measured parameters in the sulfur dioxide group showed no changes compared to the control group.

Murray et al. (1979) studied the developmental effects of sulfur dioxide by inhalation in a prenatal developmental toxicity study in CF1 mice and New Zealand White rabbits. The mated mice were exposed to an exposure calculated to correspond to an internal dose of 32 mg/kg bw per day for 10 days from gestation days (GD) 6–15. A caesarean section was performed on GD 18. The mated rabbits were exposed to an exposure calculated to correspond to an internal dose of 57.8 mg/kg bw per day for 13 days from gestation days (GD) 6–18. A caesarean section was performed on GD 29. During the first days of exposure the food consumption of the dams of both species was statistically significantly decreased. At caesarean section, the ossification of the skeleton was delayed in both species; the fetal weight was statistically significantly decreased only in mice. No other maternal or developmental effects were reported.

Gunnison et al. (1987) examined the effects of endogenously produced sulfite in a rat model with different degrees of SOX deficiency. In this study, adult male Sprague–Dawley CD rats were made sulfite oxidase-deficient by feeding them a low molybdenum diet (AIN-76, ICN Nutritional Biochemicals, Cleveland, Ohio) while supplying different concentrations of sodium tungstate in drinking water (0, 50, 100, 200, 400 and 800 mg/L) for a maximum of 21 weeks. Animals were not administered any sulfur dioxide equivalents. The molybdenum/sodium tungstate treatment resulted in different degrees of hepatic SOX deficiency, lowering the SOX activity from 3,000–5,000 units/g wet weight to between 300 units in the group exposed to 50 mg/L and 10 units in the group given 800 mg/L. Plasma endogenous sulfite concentration increased from near to zero in the SOX-competent control group to about 40 and 140 nmol/mL, respectively, in the groups exposed to 400 and 800 mg/L. Body weight loss, increased mortality and clinical signs, decreased relative testicular weight and testis atrophy were observed at 800 mg/L group. The Panel noted that decreased relative testes weight and testicular atrophy were observed macroscopically and correlated with the reduction in hepatic sulfite oxidase activity and the increased plasma sulfite levels in the 800 mg sodium tungstate/L group.

In summary, data from two species, mouse and rabbit, indicate that sulfur dioxide exposure of pregnant animals may lead to foetal skeletal retardation. However, it is unclear whether this is a direct effect on the conceptus or whether it is a consequence of the decrease in food consumption observed in the mothers. The Panel considered that the testis could be a target of sulfite toxicity either when the detoxification of endogenously produced sulfite is inhibited or when exogenous exposure overwhelms the SOX capacity of the tissue. The Panel also noted that in several other studies, although they were considered of insufficient reliability with respect to their internal validity (important flaws or major limitations), adverse effects were consistently found in the testes. A compilation of the results and shortcoming identified is presented in Appendix C. The main findings reported in these studies consist of damage to the seminiferous tubules and impairment of spermatogenesis, leading to reduced sperm counts, abnormal sperm morphology and lower sperm motility. Similar effects have been observed in SOX deficient rats (Gunnison et al., 1987). In addition, similar testicular effects have also been observed in rats or mice kept on a thiamine-deficient diet (Onodera et al., 1980; Camacho et al., 2016) or carrying a testis-specific absence of the high-affinity thiamine transporter, Tht1 (Slc19a2) (Oishi et al., 2004). The Panel cannot exclude the possibility that exposure to sulfur dioxide or sulfites may result in testicular toxicity.

4.5.2.4. Neurotoxicity

Neurotoxicity studies considered sufficiently reliable with respect to their internal validity (with only minor or some limitations according to Annex B) were considered for the assessment and data reflecting the information provided in the publications is presented in Appendix B/Neurotoxicity.

Multiple studies reported that oral sulfite administration in rats (repeat dose daily for 4–8 weeks) had adverse effects on the CNS (hippocampal cell loss, impaired learning and memory, delayed visual evoked potentials), possibly induced by oxidative stress. Several studies reported that adverse effects were reversed by concurrent antioxidant dosing. Some in vitro studies report effects in terms of oxidative stress and cell loss in neuronal and other cell types (see Section 4.5.2.7).

No oral studies of sufficient reliability with respect to their internal validity were identified in other species. The studies in rats are summarised briefly below:

Evoked Potentials

Ozturk et al. (2011) (gavage; 7, 67 or 175 mg SO_2 equivalents/kg bw per day for 5 weeks) reported dose-related prolonged visual evoked potentials (VEP; flash stimulus to visual cortex) and increased whole brain and retina thiobarbituric acid reactive substances (TBARS) and 4-hydroxy-2-

nonenal (4-HNE) levels. VEP latencies and TBARS levels in retina and brain were positively correlated. VEP prolongation is normally a consequence of defective myelination, but the effects on retina may also contribute in this case. The VEP latency data from this study were used for BMD analysis (see results in Section 4.5.4).

Küçükatay et al. (2006) (drinking water, nominal 17 mg SO_2 equivalents/kg bw per day for 6 weeks) reported that ingested sulfite prolonged flash visual evoked potential (FVEP) latency (P1, N1P2 and P3 components), and increased a marker of brain and retina lipid peroxidation (TBARS) in normal and to a greater extent in SOX-deficient rats. Effects were attenuated by concurrent oral vitamin E.

The study by Derin et al. (2009) (gavage; 175 mg SO_2 equivalents/kg bw per day for 5 weeks) found all components of VEP to be prolonged. Peak-to-peak amplitudes did not differ from the control group. The levels of TBARS were increased, while antioxidant capacity (glutathione peroxidase (GSH-Px)) was decreased in retina and brain. These effects were abrogated by concurrent oral dosing with an antioxidant, alpha-lipoic acid.

Kencebay et al. (2013) (gavage; 67 mg SO_2 equivalents/kg bw per day for 5 weeks) reported increased markers of whole brain cell apoptosis (caspase-3 and TUNEL positive cells) and inflammation (PLA2), as well as a prolonged somatosensory evoked potential (SEP) latency.

No effect on flash VEP latency or on brain lipid peroxidation markers and antioxidant status in normal or SOX deficient aged rats were reported in Ozsoy et al. (2016) (gavage; 17 mg SO_2 equivalents/kg bw per day for 6 weeks). The authors noted that the lack of effects on VEP latency and brain/hippocampal oxidative stress markers was inconsistent with other reports that showed effects on these endpoints at comparable doses in younger animals. They suggested that the lack of effects might be due to the advanced age of the rats in this study (24 months, close to end of lifespan). The Panel agreed with the authors.

The effects of sulfite on evoked potentials that were observed in the oral studies are supported by the findings in sulfur dioxide inhalation studies with rats.

Ağar et al. (2000) reported that sulfur dioxide inhalation exposure, to an exposure calculated to correspond to an internal dose of 1.2 mg/kg bw per day, prolonged VEP latency, and induced markers of oxidative stress in brain and retina.

Yargicoğlu et al. (1999) reported that sulfur dioxide inhalation exposure, to an exposure calculated to correspond to an internal dose of 1.2 mg/kg bw per day, increased brain oxidative stress (increased lipid peroxidation and Cu/Zn superoxide dismutase (SOD), decreased GSH-Px activity) in young, middle-aged and old rats, and impaired nerve conduction velocity (prolonged SEP latency) in young animals only. The lack of effect of sulfur dioxide on SEPs in older animals is possibly a 'floor'[18] effect, since latencies were also significantly prolonged in older controls compared to young controls.

Kilic (2003) confirmed that sulfur dioxide inhalation exposure, to an exposure calculated to correspond to an internal dose of 1.2 mg/kg bw per day, age-dependently prolonged visual evoked potential latency (most pronounced in young animals) and induced oxidative stress biomarkers in brain, retina and lens. In control animals, there were age-dependent increases in oxidative stress and VEP latency, which possibly explains ('floor effect') why the effects of sulfur dioxide were most pronounced in young animals.

From the oral gavage studies, the Panel noted that no effects on evoked potentials were observed at 7 mg SO_2 equivalents/kg bw per day (Ozturk et al., 2011) whereas at higher doses effects were reported in Ozturk et al. (2011) and other studies. The study by Ozturk et al. (2011) was used for BMD modelling (see Section 4.5.4). For the exposure through drinking water which results in a more continuous intake throughout the day, an effect was seen at 17 mg SO_2 equivalents/kg bw per day in the single-dose study by Küçükatay et al. (2006).

Learning/Memory

Küçükatay et al., 2005 (drinking water, nominal 17 mg SO_2 equivalents/kg bw per day for 6 weeks) reported impaired active avoidance learning and memory as well as increased hippocampal oxidative stress marker (TBARS) in SOX-deficient but not in SOX-normal rats. Concurrent oral vitamin E attenuated the effects on hippocampal TBARS but was ineffective in diminishing the effects on learning and memory in SOX-deficient rats.

Noorafshan et al., 2013 (gavage; 17 mg SO_2 equivalents/kg bw per day for 8 weeks) reported learning and memory deficits (increased reference and working memory errors) in a partially baited eight-arm radial maze. These adverse effects were abrogated by concurrent oral curcumin.

[18] It has been interpreted by the Panel as age-related loss of latency modulation.

No effect on active avoidance learning or on hippocampal oxidative stress/inflammation markers (COX activity, caspase-3, PGE 2, nitrate/nitrite), regardless of SOX status were found by Ozsoy et al. (2017) (gavage; 17 mg SO_2 equivalents/kg bw per day for 6 weeks). The authors noted that the lack of effects (on active avoidance learning and brain/hippocampal oxidative stress markers) was inconsistent with other reports that showed effects on these endpoints at comparable doses. They suggested that the lack of effects might be due to the advanced age of the rats (24 months, close to end of lifespan). The Panel agreed with the authors.

The observations from the oral studies that sulfite exposure can impair learning and memory processes are supported by a sulfur dioxide inhalation study in rats.

Yargicoglu et al. (2007) reported that sulfur dioxide inhalation exposure, to an exposure calculated to correspond to an internal dose of 1.2 mg/kg bw per day, impaired active avoidance learning (light–dark shuttle box) in young animals and increased hippocampal oxidative stress (increased lipid peroxidation and Cu/Zn SOD, decreased GSH-Px activity) in young, middle-aged and old rats. The lack of effect of sulfur dioxide on active avoidance learning in older animals is possibly a 'floor' effect, since acquisition was slowest in older controls compared to young controls. Impaired active avoidance learning normally reflects increased anxiety, in which the septo-hippocampal formation plays an important role.

From the oral studies, the Panel identified an effect at 17 mg SO_2 equivalents/kg bw per day on learning and memory after gavage (Noorafshan et al. (2013)) and drinking water exposure (Küçükatay et al. (2005)). Since only single dose studies were available, for this endpoint, a dose–response evaluation was not possible.

Cellular/molecular changes

Karimfar et al. (2014) (gavage; 17 mg SO_2 equivalents/kg bw per day for 8 weeks) reported a decrease in volume of deep cerebellar nuclei and in number of neurons. These effects were abrogated by concurrent oral curcumin.

Noorafshan et al. (2015) (gavage; 17 mg SO_2 equivalents/kg bw per day for 8 weeks) reported reductions in the volume and number of neurons and glia, in neuronal dendritic length and in the total number of spines in the median prefrontal cortex (mPFC). All these effects were abrogated by concurrent oral curcumin.

Kocamaz et al. (2012) (drinking water; nominal 70 mg 'sulfite'/kg bw per day for 6 weeks) reported reduced hippocampal CA3-2 pyramidal cell counts in SOX-competent rats after sulfite ingestion. Similar findings were obtained in SOX-deficient animals without exposure to exogenous sulfite, indicating that endogenously produced sulfur dioxide can induce brain damage when detoxification processes are inhibited.

Öztürk et al. (2006) (drinking water; nominal 17 mg SO_2 equivalents/kg bw per day for 6 weeks) reported reduced levels of the NMDA sub-units NR2A and NR2B in the hippocampus of normal, SOX-competent rats after sulfite ingestion and in SOX-deficient (high-W/Mo-deficient diet) rats without additional sulfite exposure.

Two further studies examined effects of sulfite exposure on general indicators of oxidative stress either in whole brain or in the hippocampus:

Derin et al. (2006) (gavage; 350.5 mg SO_2 equivalents/kg bw per day for 3 weeks) examined brain homogenates for parameters indicative of lipid peroxidation (TBARS) and antioxidant capacity (CAT, Cu/Zn SOD and GSH-Px activity). The level of TBARS increased, whereas GSH-Px activity decreased. No effects were seen on CAT and Cu/Zn SOD.

Küçükatay et al. (2007) (drinking water; nominal 17 mg SO_2 equivalents/kg bw per day for 6 weeks) found significant increases in hippocampal antioxidant enzyme activity (SOD, CAT and GSH-Px) in SOX-competent but not in SOX-deficient rats, possibly indicating up-regulation of detoxifying enzymes in the presence of lower sulfur dioxide concentrations.

Several sulfur dioxide inhalation studies with rats also reported changes in biomarkers of adverse effects in the hippocampus or the cerebral cortex:

Sang et al. (2011) (sulfur dioxide inhalation in whole body exposure chamber; exposures calculated to correspond to an internal dose of 1.9, 3.8 and 7.6 mg SO_2/kg bw per day for 7 days) reported increased hippocampal oxidative stress/inflammation markers (NF-κB and caspase-3 activation, elevated COX-2 expression, increased release of PGE 2 and cAMP, upregulated EP2, EP4 and NMDAR2B expression). Effects were observed at all tested doses.

Yao et al. (2016) (sulfur dioxide inhalation in whole body exposure chamber; exposures calculated to correspond to an internal dose of 0.95 and 1.9 mg/kg bw per day for 4 weeks, and 3.8 and 7.6 mg/kg

bw per day for 1 week; same total mass dosed in the two posologies) reported changes in the mRNA or protein expression of synaptic plasticity markers in the hippocampus: Arc, glutamate receptors GRIA1, GRIA2, GRIN1, GRIN2A and GRIN2B, memory-related kinase p-CaMKIIα, presynaptic marker synaptophysin, postsynaptic density protein 95 (PSD-95), protein kinase A (PKA) and protein kinase C (PKC). Presynaptic vesicle density and morphology of postsynaptic densities correlated with the protein expression of PSD-95, PKA and PKC.

Yun et al. (2010) (sulfur dioxide inhalation in whole body exposure chamber; exposures calculated to correspond to an internal dose of 1.9, 3.8 and 7.6 mg SO_2/kg bw per day for 7 days) reported increased mRNA and protein expression of apoptosis-related genes (p53, bax, bcl-2, c-fos and c-jun) in rat hippocampus in a concentration-dependent manner. Activation of pro-apoptotic signalling pathways was observed at all tested doses.

Sang et al. (2010) (sulfur dioxide inhalation in whole body exposure chamber; exposures calculated to correspond to an internal dose of 1.9, 3.8 and 7.6 mg/kg bw per day for 7 days) found elevated levels of ET-1, iNOS, COX-2 and ICAM-1 mRNA and protein in the cerebral cortex in a concentration-dependent manner. Effects were observed at all tested doses.

From the oral studies of Karimfar et al. (2014) and Noorafshan et al. (2015), the Panel noted that there were effects at 17 mg SO_2 equivalents/kg bw per day on neurons, glia and synaptic plasticity. Since only single-dose studies were available, a dose–response could not be identified for these morphological endpoints.

In summary, oral sulfite administration in rats induced adverse effects on the CNS (hippocampal cell loss, impaired learning and memory, delayed visual and somatosensory evoked potentials).

4.5.2.5. Genotoxicity

The genotoxicity of sulfur dioxide and sulfites was evaluated by the EFSA ANS Panel (EFSA ANS Panel, 2016). More than 60 in vitro and in vivo studies on sulfur dioxide, sodium sulfite, sodium bisulfite, sodium metabisulfite and potassium metabisulfite were considered. No genotoxicity studies were available on potassium bisulfite, calcium sulfite and calcium bisulfite, however, the ANS Panel considered that a read-across approach could be applied for the assessment of genotoxicity. Based on the in vitro data available, the ANS Panel concluded that there was no concern with respect to the potential induction of gene mutations with sulfur dioxide and sulfites, while the induction of chromosomal aberrations and micronuclei had been observed in studies in vitro with sodium and potassium metabisulfite. Positive results in chromosomal aberrations, micronucleus and comet assays had also been reported in some in vivo studies, however, the ANS Panel considered that all these in vivo studies had shortcomings and were of limited relevance, while negative results had been reported in reliable micronucleus assays with sulfur dioxide and sodium sulfite. Overall, the ANS Panel concluded that the use of sulfur dioxide and sulfites as food additives did not raise a concern with respect to genotoxicity.

In addition to all newly identified studies, studies assessed in 2016 were re-evaluated according to the reliability and relevance criteria described in Annex B.

The extensive literature search retrieved some additional in vitro and in vivo genotoxicity studies with sulfur dioxide and sulfites (see section 2.2 on methodology). These new studies, together with unpublished and earlier published studies not considered in the previous EFSA evaluation, are summarised in data extraction forms in Appendix D. The evaluation of reliability and relevance led to the scoring of most of these studies as having low relevance and, therefore, they have not been considered in the risk assessment of these food additives.

High relevance was assigned to recently performed bacterial reverse mutation tests (Documentation provided to EFSA No 7 and 9) and *in vitro* micronucleus assays (Documentation provided to EFSA No 8 and 11) with sodium sulfite and sodium metabisulfite, and to a gene mutation test in mammalian cells with sodium sulfite (Documentation provided to EFSA No 10). In the bacterial reverse mutation tests, sodium sulfite and sodium metabisulfite were tested with and without metabolic activation, using both the plate incorporation and preincubation procedures, up to 5 mg/plate with the Salmonella Typhimurium strains TA1535, TA1537, TA98 and TA100 and E. coli WP2 uvrA pKM101. In the *in vitro* micronucleus tests, human lymphocytes were exposed using the cytokinesis-block protocol to sodium sulfite and metabisulfite for 3 h with and without S9 and for 28 h (only without S9) up to the 10 mM concentration. In the gene mutation assay at the hprt locus, Chinese hamster V79 cells were treated with sodium sulfite for 4 h with and without metabolic activation up to the 10 mM concentration. These studies, performed following the more recent OECD guidelines (TG 471 (2020) and TG 487

(2016)) and up to the highest recommended concentrations, all provided clearly negative results according to OECD guideline criteria (details described in Appendix D).

Limited relevance was given to the three studies reported below.

Meng and Zhang (1992) examined chromosomal aberrations, micronucleus and sister chromatid exchange in cultured human peripheral blood lymphocytes treated with sodium bisulfite. The study results report chromosomal damage (chromatid breaks and micronuclei) in human lymphocytes treated with a high concentration of sodium bisulfite in the absence of metabolic activation, which was accompanied by 40% cytotoxicity (as determined by a reduction in mitotic index). The Panel noted some deviations from OECD TG 473 and TG 487 guidelines in the study protocol, and considered the results of limited relevance. Low relevance was given to the results of the SCE assay, due to their unclear biological mechanism.

Qu et al. (2017) evaluated the DNA damaging activity of sodium sulfite in human hepatoma (HepG2) cells using an antibody-based method for the detection of phosphorylated H2AX histone. At the highest sulfite concentration, a slight (~ 30% above untreated control) but statistically significant increase of signal intensity for phosphorylated H2AX was observed, indicating the induction of primary DNA damage. Under the same treatment conditions sodium sulfite also induced a concentration-related increase (2 to 4-fold) in intracellular ROS levels, evaluated with a colorimetric assay (CellRox, Invitrogen, USA). While there is no specific guideline for the determination of DNA damage through the evaluation of H2AX histone phosphorylation, the study is considered of sufficient reliability, even though the experimental procedure is only briefly described. As an indicator endpoint, the results on histone H2AX phosphorylation are of limited relevance for evaluation of genotoxicity but suggest that in vitro exposure to a high toxic concentration of sulfite (that resulted in an inhibition of cell proliferation) is associated with ROS formation and DNA damage.

The results of a rat dominant lethal test with sodium metabisulfite are reported in an unpublished study (*Stanford Research Institute*, 1972) not considered in the previous EFSA re-evaluation. An examination of the reproductive organs in pregnant females did not show statistically significant effects of treatments on study parameters (total and dead implants, corpora lutea, pre- and post-implantation losses). Although the protocol applied in this study was essentially compliant with the more recent OECD TG 478 recommendations, some deviations were noted. Therefore, the study was considered of limited reliability.

A synopsis of all available in vivo studies on sulfur dioxide and sulfites, for information including also the studies with low relevance, is presented in Table 15. The Panel considered that the new evidence provided by the *Stanford Research Institute* (1972) report, and the re-evaluation of the study by Renner and Wever (1983) in the light of more recent EFSA Guidance (EFSA Scientific Committee, 2017), does not change the conclusion on the genotoxic potential of sulfur dioxide and sulfites expressed in the previous EFSA evaluation (EFSA ANS Panel, 2016).

Overall, also in the light of more recent available genotoxicity 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.

Table 15: *In vivo* genotoxicity studies on sulfur dioxide and sulfites

Study	Reference	Route	Test material	Result	Reliability[a]	Relevance of the result[b]
Chromosomal aberration	Stanford Research Institute, 1972	Oral	$Na_2S_2O_5$	Negative	Not evaluated in EFSA ANS Panel (2016)	
					Current evaluation	
					2 Low number of analysed metaphases per animal: 50 instead of 200 as recommended in OECD TG475_2014 (100 in OECD TG475_1997)	Limited
	Renner and Wever, 1983	Oral	$Na_2S_2O_5$	Negative	2 Not clear if bone marrow was exposed	Limited
				Inconclusive[c]	Current evaluation	
					3 No proof that target organ is exposed; results not reported as required in TG 475; MI not reported; no info reported on cytotoxicity; no positive control	Low
	Pal and Bhunya, 1992	Oral	$Na_2S_2O_5$	Negative	3 Major deviations from OECD TG 475; purity not reported.	Low
	Meng and Zhang, 2002	Inhalation	SO_2	Positive	2–3 No positive control group; the statistical method does not appear to be appropriate; historical control data were not reported	Limited to low
	Pal and Bhunya, 1992	Single i.p.	$Na_2S_2O_5$	Positive	3 Major deviations from OECD TG 475; purity not reported; the positive result obtained after single i.p. administration is not consistent with the negative result obtained in the micronucleus assay after twice i.p. administration	Low
	Yavuz-Kocaman et al., 2008	i.p.	$K_2S_2O_5$	Positive	3 Major deviations from OECD TG 475; Only two animals per sex and dose, historical control data not reported and not tested at 36–42 h after treatment.	Low
	Pal and Bhunya, 1992	Subcutaneous	$K_2S_2O_5$	Positive	3 Major deviations from OECD TG 475; purity not reported	Low
	Mahmoud et al., 2015	Oral	Na_2SO_3	Positive	3	Low

Study	Reference	Route	Test material	Result	Reliability[a]	Relevance of the result[b]
					Major deviations from OECD 475: only two animals per group analysed with less scored metaphases than recommended; no positive control.	
Micronucleus	Renner and Wever, 1983	Oral	$Na_2S_2O_5$	Negative	3 Not clear if the bone marrow was exposed; in addition, major deviations from the current version of OECD TG 474 with respect to the study design; no info on ratio PCE/NCE; No positive control	Low
	Carvalho et al., 2011	Oral	$Na_2S_2O_5$	Positive	3 The PCE/NCE ratio was 1.67 ± 0.67 which is uncommon (usually the ratio is close to 1); historical control data not reported	Low
	Meng et al., 2002	Inhalation	SO_2	Positive	2–3 Deviations from the current version of OECD TG 474, i.e. only 1,000 PCE/animal instead of 4,000 PCE/animal; historical control data not reported, no positive control	Limited to Low
	Ruan et al., 2003	Inhalation	SO_2	Positive	Not evaluated in EFSA ANS Panel (2016)	
					Current evaluation	
					3 PCE/NCE ratio not reported Only 1 dose tested	Low
	Ziemann et al., 2010	Inhalation	SO_2	Negative	1–2 Maximum dose not justified; bone marrow exposure not directly demonstrated, there are only data on oxidative stress indirectly indicating that the BM might have been exposed; historical controls data not reported	High to limited
	BASF, 2008 (As in EFSA ANS Panel, 2016)	Subcutaneous	Na_2SO_3	Negative	1 Consistent with OECD 474 Proof that bone marrow was exposed	High
	Pal and Bhunya, 1992	Twice i.p.	$Na_2S_2O_5$	Negative	3 Major deviations from OECD TG 475; purity not reported	Low

Study	Reference	Route	Test material	Result	Reliability[a]	Relevance of the result[b]
Comet	Meng and Zhang, 2005	Inhalation	SO_2	Positive	2–3 No concurrent positive control; only 50 cells per animal; historical control data were not reported	Limited to Low
					Current evaluation[c]	
					3 Use of OTM as the only parameter to express DNA damage; individual animal data evaluated as mean instead of median value	Low
	Carvalho et al., 2011	Oral	$Na_2S_2O_5$	Positive	3 Samples were taken only at 24 h after treatment but not at 3–6 h; additionally, the genotoxicity was investigated based on a 'damage index' which is uncommon and not validated and 'clouds' and 'halos' were not investigated; unusual report of results (DI or DF); no report of % DNA in tail or OTM	Low
	Meng et al., 2004	i.p.	Na_2SO_3 $NaHSO_3$	Positive	3 No historically control data and no concurrent positive control; sampling time 24 h after last administration; interpretation of the results is difficult in the absence of an earlier sampling time (2–6 h); cell viability was generally >95% but other cytotoxicity parameters (clouds and halos) were not investigated; use of OTM as the only parameter to express DNA damage; individual animal data evaluated as mean instead of median value	Low
	Liang et al., 2018	Inhalation	SO_2	Positive	3 Only one dose tested Only 100 cells scored/group No positive control data reported Unusual parameters used to measure DNA damage (% tail DNA not measured).	Low
	Wang et al., 2018	Inhalation	SO_2	Positive	3 Only one dose tested Uncertainty about the number of cells scored per group No positive control group Unusual parameters used to measure DNA damage (% tail DNA not measured).	Low
	Gao et al., 2018	Inhalation	SO_2	Positive	3 Only one dose tested Only 100 cells scored/group	Low

Study	Reference	Route	Test material	Result	Reliability[a]	Relevance of the result[b]
					No positive control data reported Unusual parameters used to measure DNA damage (% tail DNA not measured).	
Dominant lethal	*Stanford Research Institute*, 1972	Oral	$Na_2S_2O_5$	Negative	Not evaluated in EFSA ANS Panel (2016)	
					Current evaluation	
					2 Insufficient number of total analysable implants (approx. 250, rather than 400 recommended as a minimum in OECD TG478_2015)	Limited
	Generoso et al., 1978	i.p.	$NaHSO_3$	Negative	3 Reporting deficiencies, source and purity of NaHSO3 not reported, not clear if target tissue was exposed, no positive control	Low
SCE	Renner and Wever, 1983	Oral	$Na_2S_2O_5$	Negative	2 Not clear if the bone marrow was exposed	Low
DNA-protein crosslinks	Xie et al., 2007	Inhalation	SO_2	Positive	2 Method not validated, no historical control data reported	Low
Cytologic investigation of chromosomal damage in oocytes during meiosis	Jagiello et al., 1975	Intravenous	Na_2SO_3	Negative	Not evaluated in EFSA ANS Panel (2016)	
					Current evaluation	
					3 Method not validated. Evaluation criteria not standardised. No positive control used. No historical control data reported. The number of oocytes investigated was not the same for different doses and was rather low.	Low

MI: i.p.: intraperitoneal; PCE: polychromatic erythrocytes; NCE: normochromatic erythrocytes; OTM: Olive tail moment; DI: damage index; DF: damage frequency.
(a): Reliability score based on Klimisch et al. (1997).
(b): Relevance of the study result is based on reliability and relevance of the test system: high, limited or low.
(c): Considering the EFSA Scientific Committee (2017).

4.5.2.6. Hypersensitivity and intolerance

In its 2016 opinion, the EFSA ANS Panel noted that 'Most sulfite sensitivities are not true allergic reactions and the mechanisms of sulfite sensitivity are unclear and are likely due to various biological reactions, depending on the individual genetic background'. Since this re-evaluation of sulfur dioxide-sulfites (EFSA ANS Panel, 2016), additional publications have confirmed that, in general, sulfur dioxide and sulfites exposure can induce hypersensitivity (immunologically initiated, allergy) and intolerance (non-immunologically triggered, pseudo allergy) reactions after oral or dermal exposure, or inhalation. As regards more specifically reactions reported in humans to sulfur dioxide/sulfites used as food additives, apart from dermatologic and gastrointestinal signs, respiratory manifestations are most often observed, in particular in asthmatic individuals, metabisulfites and sulfur dioxide being particularly effective agents (Fine et al., 1987). That asthma potentially represents a risk factor for bronchial hyperreactivity following sulfur dioxide exposure has been documented in studies using animal models.

Based on the evaluation of the available data, the following studies were considered for the assessment.

Li et al. (2014) investigated the effect of sulfur dioxide exposure in rats. The animals (male Wistar rats were challenged with ovalbumin (OVA) or SO_2 5 mg/m^3 (calculated to correspond to an internal dose of 0.23 mg/kg bw per day), one hour a day for seven consecutive days) alone or together, then mRNA and protein levels of some inflammatory and immune genes were measured in lung and trachea homogenates. Bronchoalveolar lavage (BAL), inflammatory cell counts and histopathologic examination were performed. The authors reported that, in this model, sulfur dioxide could increase the immune response to OVA, along with aggravating the inflammatory responses to OVA in lungs (increased activation of NF-kB; increased transcription and translation of TNF-alpha and IL-6, increased IL-4 and IgE levels and decreased IFN-gamma), which might contribute to the increased risk of asthma. According to the authors, the mechanism might be associated with an oxidative stress, increased pro-inflammatory cytokine expression, Th1/th2 imbalance and the altered regulation of NF-kB and Foxp3.

Li et al. (2018a) investigated the role of sulfur dioxide in asthma using a model in C57BL/6 mice treated with OVA then exposed or not to 10 mg/m^3 SO_2 (calculated to correspond to an internal dose of 0.7 mg/kg bw per day) during 30 min for 7 days, the animals were killed 24 h after the last treatment. Bronchoalveolar lavage (BAL), inflammatory cell counts and histopathologic examination were performed. Sulfur dioxide exposure alone caused only slight airway injury, increased H2O2 content and induced STAT6 expression in the lung of mice. In OVA-induced asthmatic mice, exposure to sulfur dioxide augmented the degree of pulmonary pathological injury and mucus production, significantly increased the inflammatory cell counts and markedly induced the expression of the mucin gene MUC5AC and the pro-inflammatory cytokine TNF-α. Up-regulated expression of Th2 cytokines and JAK/STAT6 pathway components (JAK1 and STAT6) were observed after OVA exposure and OVA+SO_2 exposure. These results provide evidence that sulfur dioxide enhances Th2 inflammatory responses in lungs of OVA-induced asthmatic mice by activating STAT6 pathway. These findings suggest a STAT6-mediated mechanism for the aggravation of asthma after sulfur dioxide exposure.

The Panel noted that the sulfur dioxide concentrations used in these studies were several orders of magnitude higher than the atmospheric concentrations currently provided by the WHO standards in cities i.e. from 5 to 50 μg/m^3 (WHO, 2021). However, they indicate that exposure to sulfur dioxide via inhalation has a potential to worsen pulmonary inflammation in asthmatics.

The Panel considered that these respiratory effects appear after both inhalation and ingestion, but that inhalation is the route of exposure which is the most frequently associated with symptoms. This is in particular evident in the case in occupational settings where the levels of exposure are high (Andersson et al., 2006). Respiratory reactions following ingestion of sulfites are mainly reported after consumption of beverages like wines (Wüthrich, 2018). As the ability of sulfur dioxide to produce airflow limitations is long recognised (Nadel et al., 1965), one hypothesis is that some sulfur dioxide may be produced in the stomach and re-inhaled; alternatively, some sulfites may be rapidly absorbed and reach the lungs through the general circulation. Numerous mechanisms are proposed to explain the respiratory effects and include: stimulation of effector cells such as airway smooth muscle cells, mucous-producing cells or neuronal cells, and of cells which produce inflammatory mediators (leukotrienes, prostaglandins, ROS,…) (Van Schoor et al., 2000). Sulfites may stimulate the parasympathetic system and provoke cholinergic-dependent bronchoconstriction, this being particularly prominent in individuals with low SOX activity (Skypala et al., 2015). Consequently, respiratory symptoms can be the result of a direct action on effector cells, or results from an action on immune system cells that act as intermediary between the sulfur dioxide/sulfites and the effector cells by

releasing soluble mediators. In this context, whatever the underlying mechanism(s), all of them result in airflow limitation.

The pathophysiology of these hypersensitivity/intolerance reactions remain unclear; for instance, even in case where prick tests, which are usually associated with a type 1 allergic reaction, were found positive, no IgE specific for sulfites could be detected. Obviously, the development of these reactions depends on numerous different factors that include genetic specificities of the population (atopy), different cell populations and soluble mediators as well as confounding factors coming from (e.g. allergens in wine) or formed after reaction with the food to which sulfites was added.

The possibility that sulfites induce adverse reactions such as hyperreactivity of the airways or other pseudo-allergic (intolerance) reactions is recognised by sulfites being included in the list of substances or products causing allergies or intolerances (Annex II to Regulation (EU) No 1169/2011). The Panel noted the lack of appropriate information and the absence of new data after the request made in the re-evaluation by the ANS Panel (EFSA ANS Panel, 2016). Therefore, the Panel supported their recommendation that studies on the origin and mechanisms (forms of sulfites involved) of the reactions of individuals who are sensitive or intolerant to sulfites should be conducted.

4.5.2.7. *In vitro* studies

In vitro toxicity studies identified in the literature search were selected for further consideration according to the criteria listed in Annex B.

Seven out of the 21 examined studies reported data on intracellular ROS formation following exposure to sulfur dioxide derivatives (Table 16). In two studies, the lowest-observed-adverse-effect concentration (LOAEC) identified by the Panel was 5 μM (Zhang et al., 2004) and 10 μM (Wu et al., 2022), respectively. However, in other cell models, increased ROS could be measured only when sulfur dioxide derivative concentrations were above 100 μM. In some of the studies, the authors reported evidence of ROS-dependent secondary effects, such as increases in malondialdehyde and glutathione oxidation (Wu et al., 2022) or inhibition of redox-sensing enzymes (Grings et al., 2013). The Panel noted that the wide range of LOAEC, values in different target cells may be due to both different responses by different cell types and different experimental conditions of incubation. The lowest LOAEC 5 μM (Table 16) was obtained with 2 different cell lines exposed to sulfite for only 0.5 h.

Table 16: Treatment-related increases in intracellular ROS levels in various in vitro test systems. Data are expressed as lowest observed effect concentration (μM)

In vitro model (cell line or tissue)	Related cell type/organ (species)	Test substance and exposure duration	LOAEC[(a)] (μM)	Reference
PC12	Neuronal adrenal medullary cells (rat)	Sodium sulfite for 0.5 h	5	Zhang et al. (2004)
HepG2	Hepatocytes (human)	Sodium sulfite for 0.5 h	5	Zhang et al. (2004)
NCM460	Colon enterocytes (human)	Sodium bisulfite for 24 h	10	Wu et al. (2022)
HaCaT	Skin keratinocytes (human)	Sodium bisulfite: sodium sulfite (1:3 M/M) for 24 h	100	Liang et al. (2020)
Cerebral cortex slices	Brain (rat)	'sulfite' for 1 h	100	Grings et al. (2013)
RGM1	Gastric mucosal cells (rat)	Sodium sulfite for 24 h	500	Oshimo et al. (2021)
RBL-2H3	Basophilic leukaemia cells (rat)	Sodium sulfite for 0.5 h	4,000	Liu et al. (2021)

(a): All concentrations refer to nominal applied concentrations, expressed as SO_2 equivalents.

Ten studies reported data on the effect of sulfite on cell viability (Table 17). In two studies (Li and Sang, 2009; Wu et al., 2022), using different models, the Panel identified a LOAEC of 10 μM. In three other studies the Panel identified LOAEC values were between 30–100 μM, whereas five studies with other cell models, including neurons, described cytotoxicity effects at concentrations ranging 300–4,000 μM.

Table 17: Treatment-related decreases in cell viability in various in vitro test systems. Data are expressed as lowest observed effect concentration (μM)

In vitro model (cell line or tissue)	Related cell type/ organ (species)	Test substance and exposure duration	LOAEC[(a)] (μM)	Reference
Primary hippocampal neurons	Brain hippocampus (rat)	Sodium bisulfite:sodium sulfite (3:1 M/M)	10	Li and Sang, (2009)
NCM460	Colon enterocytes (human)	Sodium bisulfite for 24 h	10	Wu et al. (2022)
HT22	Hippocampal neurons (mouse)	Sodium bisulfite:sodium sulfite (3:1 M/M)for 24 h	30	Guan et al. (2015)
BV2	Microglial cells (mouse)	Sodium bisulfite:sodium sulfite (3:1 M/M) for 24 h	50	Qiu et al. (2015)
L02	Fetal hepatocytes (human)	Sodium sulfite for 72 h	100	Han et al. (2020)
H9C2	Cardiomyocytes (rat)	Sodium bisulfite for 24 h	300	Qin et al. (2016)
RGM1	Gastric mucosal cells (rat)	Sodium sulfite for 24 h	5,00	Oshimo et al. (2021)
CSM14.1.4	Mesencephalic cells (rat)	'sulfite' for 24 h	1,000	Marshall et al. (1999)
CSM14.1.4	Mesencephalic cells (rat)	Sodium sulfite for 24 h	1,000	Reist et al. (1998)
RBL-2H3	Basophilic leukaemia cells (rat)	Sodium sulfite for 0.5 h	4,000	Liu et al. (2021)

The Panel considered the wide range of LOAEC values as indicative of differential responses to treatments of different duration on different target cells.

In a model of bronchial airway rings that contracted as an effect induced by metabisulfite treatment (Sun et al., 1995), the authors noted that their data showed 'no direct contractile responses but enhanced bronchoconstriction induced by activation of non-cholinergic neural pathways in the bronchus, probably through increased release of neuropeptides'. The Panel identified a LOAEC value of 0.1 μM.

A set of 7 studies (Li and Sang, 2009; Du and Meng, 2004a; Meng and Nie, 2005a; Meng and Nie, 2005b; Du et al., 2007; Du and Meng, 2006; and Du and Meng, 2004b) conducting electrophysiological measurements on rat primary hippocampal cells (Li and Sang, 2009; Du and Meng, 2004a; Meng and Nie, 2005a; Meng and Nie, 2005b) or on cultured post-natal dorsal root ganglion (DRG) neurons (Du et al., 2007; Du and Meng, 2006; and Du and Meng, 2004b) demonstrated a dose-dependent effect of sulfite or metabisulfite on ionic currents. The Panel identified LOAEC values spanning 1–10 μM. One of these studies (Meng and Nie, 2005b) showed that it was possible to abolish the treatment effects on neuronal excitability by adding antioxidant enzymes (catalase, superoxide dismutase or glutathione peroxidase) to the cell medium, suggesting a ROS-mediated mechanism of action of sulfur dioxide treatment. In one case (Li and Sang, 2009) sulfite-dependent activation of outward K+ current was directly related to cytotoxicity.

It is unclear whether effects on ion channels are direct or secondary to oxidative stress/ROS signalling. Wang et al. (2016) reported in vitro rapid induction of oxidative stress and intracellular calcium overload in primary cultures of rat cortical neurons after incubation with sulfite (Na2SO3 or NaHSO3). The effect was dose-related in the tested range of 250–1,000 μM, was detected at about 1–2 min after administration and rapidly reached a plateau within 4 min. The authors also reported a similar effect in vivo in rat prefrontal cortex within 30 min of intraperitoneal injection of sodium sulfite 500 mg/kg; Cmax in prefrontal cortex was 272 μM sulfite at 30 min post-dose. The authors reported increased levels of 'caspase 3' (presumably activated/cleaved caspase 3) in vitro and in vivo, a marker of apoptosis. Since these effects were blocked by antioxidants, the authors suggested that sulfite induced neurotoxicity is due to oxidative stress leading to redox-dependent calcium overload.

Some electrophysiological studies demonstrated that low concentrations of sulfite (in the range of 5–10 μM) modify potassium and sodium currents in hippocampal neurons, potentially altering their excitability (Li and Sang, 2009; Du and Meng, 2004a; Meng and Nie, 2005a).

The Panel considered the in vitro data as indicative of adverse effects of sulfite on neuronal and other cells.

4.5.3. Selected toxicity studies considered in the previous re-evaluation of sulfur dioxide-sulfites (E 220–228) (EFSA ANS Panel, 2016)

In addition to the new scientific evidence (Section 4.5.2), selected studies considered in the previous re-evaluation of these food additives (EFSA ANS Panel, 2016) were again reviewed in the current assessment.

As mentioned in Section 4.5.2.5, in addition to all newly identified genotoxicity studies, genotoxicity studies assessed in 2016 were again evaluated according to the reliability and relevance criteria considered in this assessment.

In particular, the Panel has re-assessed six oral prenatal developmental toxicity studies with sodium bisulfite, sodium metabisulfite and potassium metabisulfite that were conducted in 1972 and 1975 in rats and mice (FDRL, 1972a,b,c,d, 1975). These studies have been described in the previous EFSA opinion (EFSA ANS Panel, 2016); however, the individual litter data for the studies with potassium metabisulfite (FDRL, 1975) had not been available at that time but are now. The Panel decided to conduct BMD analyses of the combined data for post implantation loss from the different sulfites (sodium bisulfite, sodium metabisulfite and potassium metabisulfite) for each species, after conversion of the sulfite doses to SO_2 equivalents.

In line with the EFSA Guidance (EFSA Scientific Committee, 2017), a benchmark response (BMR) of 20% was selected based on biological considerations: the distribution of post-implantation losses in controls for which the most common value is 0, followed by 1 and 2 losses per litter; three or more losses were rarely seen in dams from control groups; depending on the overall litter size (between 11 and 12 in these studies), three dead implants would correspond to a loss of about 25%. Since the BMR describes the extra risk and controls are not at a zero risk of losing implants, a value of 20% was chosen for this analysis.

Two different approaches for BMD analysis were performed: (i) study year as covariate (tested substances were combined) (see Annex D for rats and Annex E for mice); (ii) study year and the tested substance as covariates (see Annex F for rats and Annex G for mice). Selecting only one of the studies (despite all having the same reliability) and presenting its analysis as representative result for the substance group was considered not appropriate since this does not take into account all the data available for the sulfites. As indicated in the EFSA Guidance on the use of the benchmark dose approach in risk assessment (EFSA Scientific Committee, 2017), the reason for combining data sets in a dose–response analysis with a covariate is to maximise the statistical power when examining and quantifying potential differences in dose–responses between subgroups or to improve the precision of the estimated BMD. The fact that the studies used the same protocol, investigated the same endpoints, were conducted in the same laboratory with the same strains of animals and, when performed in the same year, shared vehicle control and positive control groups, supported performing a covariate analysis for the BMD estimation.

The full reports and the results of the BMD analyses are presented in Annexes D–G and revealed no developmental effects within the dose-ranges tested (0.84–107.8 mg SO_2 equivalents/kg bw per day in mice and 0.62–89.3 mg SO_2 equivalents/kg bw per day in rats).

4.5.4. BMD estimation for visual evoked potential

The data used for a lower confidence limit of the benchmark dose (BMDL) estimation and possible derivation of reference value were taken from Ozturk et al. (2011), because this study was rated as reliable with only minor or no limitations, the effects were similar to those reported in other more recent studies (see Section 4.5.2.4), and there were sufficient dose groups in an appropriate dose range to establish a dose–response.

In the study performed by Ozturk et al. (2011), adult male rats were dosed by gavage with sodium metabisulfite at 0, 7, 67 or 175 mg SO_2 equivalents/kg bw per day daily for 5 weeks (n = 13/dose group). VEP latencies were then recorded over visual cortex area 17 under ether anaesthesia after repetitive ocular stimuli (1 Hz, flash duration 10 μsec, energy 0.1 J). The reported data were the average of 100 VEP responses per animal (both eyes). The VEP waveform presents successive positive and negative deflections designated as P1, N1, P2, N2 and P3.

VEP latency data from Table 4 of Ozturk et al. (2011) were used for BMD estimation (see full report in Annex H).

For the selection of BMR, the data of the study of You et al. (2011) were used. You et al. (2011) tabulated VEP latencies for components P1, N1 and P2, before and 6 days after optic nerve microinjection with lysolecithin, a major component of oxidised low-density lipoproteins which has a

detergent effect on myelin and myelinating cells and produces a partial demyelination lesion in the injected nerve, confirmed by histopathology. You et al. (2011) reported that lysolecithin dose-relatedly prolonged VEP latencies. Latencies of VEP components P1, N1 and P2 were significantly prolonged by 12, 13 and 24% after 0.4 μL lysolecithin, and by 28, 32 and 37% after 0.8 μL lysolecithin (Table 1 from You et al. 2011). Linear regression analysis demonstrated a strong correlation between latency prolongation and optic nerve bundle lesion volume. Hence, prolongation of the latency of VEP is a biomarker for adverse histopathological lesions. Based on the smallest significant effect reported by You et al. (2011) (≥ 12% latency prolongation after 0.4 μL lysolecithin, the lowest tested dose, with concomitant neurohistopathology), the Panel considered that a VEP latency prolongation of 10% can be considered adverse. Therefore, a BMR of 10% was used for derivation of BMDL from the VEP latency data of Ozturk et al. (2011).

The lowest BMDL estimated from these data was the BMDL for VEP component N2, i.e. 38 mg SO_2 equivalents/kg bw per day (Annex H).

4.5.5. Possible toxicity mechanisms

The mechanisms involved in sulfite induced toxicity are not fully understood. Two possible mechanisms have been proposed, one involving free radical formation and oxidative stress and one indirect mechanism involving sulfite induced thiamine destruction.

Free radical mechanism: There are many studies both in vitro and in vivo which implicate involvement of free radicals and oxidative stress in the toxicity of sulfite. In these studies, various effects indicating oxidative damage have been observed. These include effects on glutathione and protein thiol states and lipid peroxidation as well as on 'antioxidant' enzymes like superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase. The protective effect of different kinds of antioxidants also supports the involvement of oxidative damage in sulfite toxicity. A key step in the free radical mechanism appears to be the initial formation of sulfite radicals. Velayutham et al. (2016) proposed that sulfite is oxidised by mitochondrial cytochrome c (Fe3+) to the sulfite radical ($SO3^{\cdot -}$) which further reacts with oxygen to form peroxymonosulfate and other oxidants.

Sulfite-induced thiamine destruction: Inactivation of thiamine by sulfite is known to occur in stored food and drinks; EFSA ANS Panel (2016) also referred to inactivation of thiamine in ingested food in the stomach. There remains uncertainty around whether dietary thiamine could be reduced to an extent which would lead to a systemic thiamine deficiency sufficient to affect visual evoked potentials.

Thiamine deficiency is known to induce neurotoxicity and neurocognitive deficits (Spencer and Palmer, 2012; Whitfield et al., 2018), and the neurotoxic effects of thiamine deficiency in rodents (including hippocampal cell loss, impaired learning/memory and oxidative stress) are strikingly similar to those of sulfites. Further background information on findings in animals is provided in Appendix E.

Investigation in humans with thiamine deficiency have been performed to detect even subtle decreases of information processing using the tool of visually evoked potentials, whereby changes in the EEG pattern following a visual stimulus are recorded. The P100 component of VEP response, which is the positive peak with a delay about 100 ms after the stimulus, has major clinical importance. In alcoholic patients without Wernicke–Korsakoff syndrome, the latency of the P100 component, corresponding to P 1 in rats, was prolonged compared to the normal population (Chan et al., 1986). In those patients, the thiamine and thiamine–diphosphate blood/serum levels were found to be reduced to about 50% of that in healthy controls (Tallaksen et al., 1992; Mancinelli et al., 2003). Thus, in humans, changes of VEPs are observed in subjects with general thiamine deficiency.

The question whether destruction of thiamine by oral sulfite could exacerbate thiamine deficiency was addressed in a study reported in several publications (Hötzel et al., 1969; Bitsch et al., 1969). The authors reported that healthy volunteers (6/sex) consuming a thiamine-deficient diet for 15 days showed no clinical signs of thiamine deficiency and that the subsequent ingestion of 0.5 mg SO_2 equivalents/kg bw per day, for 25 days did not elicit adverse effects.[19] Given the low dose of added sulfite and the relatively short study duration, these data cannot be considered as conclusive.

[19] After 15 days on a thiamine-deficient diet, healthy adult volunteers (n = 6/sex) received a daily oral dose (in 500 mL grape juice and 300 mL wine) of 50 mg sodium bisulfite, equivalent to 30.8 mg SO2, and 350 mg sodium glucose sulfonate (not a source of SO2); assuming body weight 60 kg, this is a dose of 0.5 mg SO2 equivalent/kg bw per day, daily for 25 days. The thiamine-deficient diet induced relatively moderate ['relativ geringen'] biochemical signs of thiamine deficiency (lower thiamine levels in blood and urine, and lower activity of transketolase, lactic dehydrogenase LDH, G-6-PDH and NADH-Diaphorase in hemolysed erythrocytes), but no clinical signs and no effect on motor nerve conduction velocity. The subsequent dosing with sulfite had no additional effects on any endpoint.

In animals the Panel noted that in the oral studies showing effects on VEPs, administration was per gavage or in drinking water, thus no influence of sulfite on thiamine content in the animal diet was possible. Effects of VEPs seen in inhalation studies are also obviously not due to a thiamine deficiency in the feed.

However, inactivation of thiamine by sulfite could occur at the level of organs, e.g. in the brain. Experimentally administered sulfite reaches the brain as shown by Wang et al. (2016).

In summary, there is evidence that sulfite can generate radicals which may directly and/or indirectly disrupt mitochondria and other cell components. However, given that sulfite chemically inactivates thiamine, and that the adverse effects of thiamine deficiency on nervous tissue and testis have similarities with those of sulfites, it is possible that sulfite neurotoxicity and testis toxicity are at least partly due to local thiamine deficiency.

4.6. Uncertainties

Due to large amount of information that needed to be reviewed in the limited time available, a structured uncertainty analysis in line with the EFSA Guidance on Uncertainty Analysis in Scientific Assessment (EFSA Scientific Committee, 2018) was not possible. However, the Panel took a conservative approach in reaching the final conclusions.

In relation to the dietary exposure assessment (Section 4.3), the Panel considered that the uncertainties identified would, in general, result in an overestimation of the dietary exposure to sulfur dioxide–sulfites (E 220–228) from their use as food additives for the refined estimated exposure scenarios considering Data set D.

During the re-evaluation of these food additives (EFSA ANS Panel, 2016), the Panel noted the following uncertainties as regards their chemistry and fate:

- *Differences in stability and reactivity of sulfites when used either in beverages, such as water, soft drink or wines, or in solid foods may exist*.
- *The reaction products of sulfites appearing in various foods and beverages are not well characterised and information on their absorption and/or toxicity was limited*.

Among the uncertainties from the biological and toxicological data, the Panel considered that:

- *many data were obtained from toxicity studies with possible confounding factors, which were not adequately evaluated*: *diet with thiamine supplementation, which may induce formation of complexes with sulfites and a resulting modification of their biological effects*; *or sulfites administered in solution in water, which might modify their stability and/or reactivity;*
- *numerous publications, from non-regulatory studies, have reported biological effects of SO_2, sulfites, bisulfites in various cell models and in vivo, which may indicate the possibility of adverse effects. Although knowledge of the biological effects of sulfites has improved since their last evaluations, further research is needed to determine the mode of action and relative contributions of the different forms and their different metabolic pathways*.

In the absence of any additional data from IBO and limited data from the literature review, these uncertainties remain.

The Panel, identified the following additional data gaps that lead to further uncertainties:

- Lack of reliable studies on the extent of systemic exposure to sulfites in animal models and humans after ingestion of E 220–228, in order to improve the extrapolation of VEP findings.
- Lack of reliable studies on dietary SO_2-sulfites investigating the potential adverse effects on the testis.
- Lack of reliable studies on thiamine status in animals displaying sulfite-induced neurotoxicity and potential testis toxicity, so the possible role of systemic thiamine deficiency in these effects is unknown.

5. Discussion

The re-evaluation of sulfur dioxide-sulfites (E 220–228) was completed by EFSA ANS Panel in 2016 (EFSA ANS Panel, 2016). The ANS Panel noted several uncertainties and limitations in the database and concluded that the current group ADI of 0.7 mg SO_2 equivalents/kg bw per day (derived using a default uncertainty factor of 100) would remain adequate but should be considered temporary while the database was improved. In addition, the ANS Panel issued several recommendations.[1]

The present opinion deals with the assessment of the data provided by IBOs and additional evidence identified in the publicly available literature.

Dietary exposure to sulfur dioxide-sulfites (E 220–228), expressed in sulfur dioxide equivalents, was calculated using five data sets, taking into account different considerations on the available concentration data (MPLs, reported uses and use levels and analytical data) (see Section 4.3.5). The Panel considered Data set D to most realistically represent the dietary exposure to sulfur dioxide equivalents.

Data set D, based mainly on analytical data, includes the presence of sulfur dioxide in foods and beverages due to the addition of sulfur dioxide-sulfites (E 220–228), carry-over and other sources, such as natural occurrence. Furthermore, if analytical data were available for a certain authorised food category, these data were used in the assessment instead of use level data, even if the use levels were higher. It is known that sulfur dioxide may be lost during processing, storage and preparation stages. The dietary exposure results based on Data set D (see Table 8) were based as much as possible on analytical results. These analytical results were considered to best represent the level of sulfur dioxide equivalents in final products, because they take into account losses of sulfur dioxide during processing, storage and the preparation stages (see above). Use levels were only included, even if they were higher than the corresponding analytical levels, in the assessment based on this data set for those food categories for which no analytical data were available.

Furthermore, the non-brand-loyal scenario was considered as the most appropriate for risk assessment of sulfur dioxide–sulfites (E 220–228), because these food additives are added to a wide range of foods, and they do not impact on taste or flavour. The mean dietary exposure in this scenario (Data set D) ranged from < 0.01 mg SO_2 equivalents/kg bw per day in infants to 0.32 mg SO_2 equivalents/kg bw per day in toddlers. The 95th percentile of dietary exposure ranged from 0.05 mg SO_2 equivalents/kg bw per day in infants to 1.17 mg SO_2 equivalents/kg bw per day in adults.

Overall, the Panel considered that the uncertainties identified in the dietary exposure assessment would, in general, result in an overestimation of the dietary exposure to sulfur dioxide–sulfites (E 220–228) from their use as food additives for the refined estimated exposure scenarios considering data set D.

In response to the European Commission call for data, analytical data on toxic elements in commercial samples of E 221 E 222, E 223 and E 224 were provided by three IBOs. The potential exposure to these toxic elements from the use of sulfur dioxide -sulfites (E 220-E 280) was calculated by assuming that they may be present in the food additive up to a certain limit value and then by calculation pro-rata to the estimates of exposure to the food additive (Table 8) itself. Since the exposure to sulfur dioxide-sulfites (E 220–228) is expressed in mg SO_2 equivalents/kg bw per day, to calculate the exposure to impurities from the use of these food additives, the Panel converted the estimates to sulfite and considered two cases: (a) all the exposure to sulfur dioxide-sulfites (E 220–228) was expressed as sodium metabisulfite E 223, that was considered to be the sulfite most typically used, based on the reported use and use levels as well as from the highest number of products labelled with E 223 in Mintel's database; (b) all the exposure to sulfur dioxide-sulfites (E 220–228) was expressed as sodium bisulfite E 222 which is considered to be the worst case for these calculations due to its low yield of SO_2 (19.7%, Table 3).

It is expected that toxic elements in food containing SO_2-sulfites (E 220–228) will be present in the final food at amounts that are present in the food additive when added to the food (i.e. no loss would be expected in contrast with sulfur dioxide-sulfites). Hence, Data set C, taking into account use levels and analytical data, was considered the most appropriate scenario available for estimating the exposure to toxic elements from the use of these food additives.

Although currently there is no maximum limit for Cd included in the EU specifications for E 220–E 228, the Panel considered that based on the fact that maximum levels for Cd in different food commodities are stipulated in the EU and also taking into account that the mean Cd exposure for certain population groups across Europe is close to, or exceeds the TWI of 2.5 μg/kg bw (EFSA CONTAM PANEL, 2009a, 2011), this toxic element was also considered in this risk assessment.

The Panel estimated the potential exposure (i) to Pb, Hg and As based on the maximum limits specified in Regulation (EU) No 231/2012 and (ii) to Pb, Hg, Cd and As at the highest reported LOQ and by applying a factor of 10.

For both scenarios, in particular the lower end of the range of calculated MOE values for As was considered to be insufficient. For Pb, Hg and Cd based on the outcome of the evaluation for the typical (E 223) and worse case (E 222), the presence of these toxic elements in sulfur dioxide-sulfites

(E 220–E 228) either at the current specifications limit values or at the levels selected by the Panel would not give rise to concern (Tables 12 and 13).

The Panel noted that the maximum limits in the EU specifications for toxic elements should be established based on actual levels in the commercial food additive. Therefore, the Panel recommended that the maximum limits to be lowered on the basis of the information provided and on the considerations of the Panel (see Table 14). Moreover, the Panel recommends that the European Commission considers introducing a maximum limit for cadmium for these food additives.

The Panel considered that inhalation studies cannot be used to identify a reference point that could be used for deriving an oral health-based guidance value due to the uncertainty around the assumptions used when estimating internal doses resulting from inhalation exposure.

An extensive literature search has been performed as requested in the European Commission mandate (Section 1.1.2) according to the strategy described in Annex A and Section 2.2. Genotoxicity and toxicological studies retrieved in the literature search were screened and assessed for their relevance and reliability considering the criteria described in Annex B.

As indicated in the re-evaluation of sulfur dioxide-sulfites (EFSA ANS Panel, 2016), sulfites, once ingested, may react with water to form bisulfite, sulfite and sulfur dioxide. The prevailing species found in the stomach are bisulfite and sulfur dioxide and the balance between these is determined by the acidity of the stomach. In the neutral pH environment of the intestine, sulfite and bisulfite will exist as a near equimolar mixture. The EFSA ANS Panel noted the absence of both ADME studies with sulfur dioxide and sulfites, and data on reaction products from sulfur dioxide or the different sulfites.

The Panel considers that, based on chemical considerations for all the sulfite-based food additives, the predominant species in aqueous fluids will be bisulfite and sulfite ions. It is not expected that the cation (i.e. sodium, potassium, calcium) contributes to toxicity. Therefore, chemical and biological properties of the 'sulfite' anion are considered as being the relevant determinants of the toxic effects of these food additives. Once the food additive (sulfur dioxide or sulfite) is ingested, they form sulfite ions, and therefore, read-across between sulfur dioxide and the different sulfites is considered acceptable.

No new data on ADME or reaction products were submitted by IBOs following the European Commission call for additional data. The Panel considered that sulfites undergo high first pass metabolism after oral exposure but that systemic exposure to sulfites may be up to around a quarter of the dose. The available data show distribution of sulfites in brain following intraperitoneal administration of sodium sulfite and both brain and heart after inhalation of sulfur dioxide. Other tissues have not been examined.

Taking into consideration the genotoxicity studies previously evaluated in the re-evaluation (EFSA ANS Panel, 2016) and the more recent available genotoxicity 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 (see Section 4.5.2.5).

The Panel considered that oral sulfite administration in rodents did not produce adverse effects in the area of general toxicity (see Section 4.5.2.2). Local effects in lung tissue were observed at high sulfur dioxide inhalation doses. Other inhalation studies did not study apical endpoints and the toxicological relevance of effects observed remained unclear.

The Panel considered that inhalational sulfur dioxide exposure of pregnant animals (mouse and rabbit) may lead to fetal skeletal retardation. However, it is unclear whether this is a direct effect on the conceptus or whether it is a consequence of the decrease in food consumption observed in the mothers (see Section 4.5.2.3).

The Panel noted that studies, though individually of insufficient reliability with respect to their internal validity (important flaws or major limitations), consistently reported adverse effects in the testes (Appendix C). The main findings reported were damage to the seminiferous tubules and impairment of spermatogenesis, leading to reduced sperm counts, abnormal sperm morphology and lower sperm motility. Similar effects have been observed in SOX deficient rats (Gunnison et al., 1987). In addition, similar testicular effects have also been observed in rats or mice fed on a thiamine-deficient diet (Onodera et al., 1980; Camacho et al., 2016) or carrying a testis-specific absence of the high-affinity thiamine transporter, Tht1 (Slc19a2) (Oishi et al., 2004). The Panel therefore considered that the testis may be a target organ for sulfite toxicity, possibly secondary to sulfite-induced testicular thiamine deficiency.

In the previous opinion, EFSA ANS Panel (2016) concluded that '*numerous in vitro and animal studies reported that sulfites have a neurotoxic potential, however, the relevance of these studies for*

the interpretation of the health consequence of the use of sulfites as food additive is not demonstrated. This is because the doses used were high, and the consequence of exposure to sulfites used as food additives on the possible alteration of sulfites concentration in situ, in cells and organs, is not well known. However, these indications suggest that more data may be needed before a clear conclusion on the possible neurotoxic effects of sulfites used as food additives can be reached.'

The animal toxicity data reviewed in the present opinion indicate that oral sulfite exposure can induce adverse effects on CNS (hippocampal cell loss, impaired learning and memory, delayed visual evoked potentials) (see Section 4.5.2.4). It is noted that learning/memory deficits and delayed visual evoked potential latency reported in rodents will not be detectable in standard repeat-dose toxicity testing protocols.

Based on the available information, two possible mechanisms for neurotoxicity are discussed, generation of sulfite radicals and sulfite-induced thiamine destruction. There is evidence that sulfite can generate radicals which may directly and/or indirectly disrupt mitochondria and other cell components. However, given that sulfite chemically inactivates thiamine, and that the adverse effects of thiamine deficiency on nervous tissue and testis have similarities with those of sulfites, it is possible that sulfite neurotoxicity and testis toxicity are at least partly due to local thiamine deficiency.

The Panel noted the lack of appropriate information on hypersensitivity and intolerance and the absence of new data after the request made in the re-evaluation by the ANS Panel (EFSA ANS Panel, 2016). Therefore, the Panel supported their recommendation that studies on the origin and mechanisms (forms of sulfites involved) of the reactions of individuals who are sensitive or intolerant to sulfites should be conducted.

In summary, following the European Commission call for data, no new biological and toxicological data specifically addressing the data gaps described in the re-evaluation of sulfur dioxide-sulfites (E 220–228) (EFSA ANS Panel, 2016), were received from IBOs. In addition, only limited new data were identified from the literature search. Overall, the Panel considered that there was no substantial reduction in the uncertainties previously identified in the re-evaluation. From the literature search, there are no new data on adverse effects following oral and inhalation exposure in the area of general toxicity. However, there were consistent reports that oral sulfite administration produced adverse effects on the CNS and there were reports in studies of insufficient reliability with respect to their internal validity for adverse effects on the testis at lower doses than for CNS. 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. However, the Panel considered that the available toxicity database was inadequate to derive an ADI. The Panel therefore considered a margin of exposure (MOE) approach appropriate to assess the risk for these food additives at the current exposure levels.

In the re-evaluation in 2016, the temporary group ADI was based on gastrointestinal effects in a long-term rat study with a NOAEL of 70 mg SO_2 equivalents/kg bw per day (Til et al., 1972). At the time of the re-evaluation, it was also noted that numerous in vitro and animal studies reported that sulfites had a neurotoxic potential, however it was indicated at that time that more data would be needed before a clear conclusion on the possible neurotoxic effects of sulfites could be made, when used as food additives. The ANS Panel considered that the Ozturk et al. (2011) study 'was suggestive of a potential toxic effect for the eyes but needs further supporting data before being possibly used for identifying a NOAEL' (EFSA ANS Panel, 2016). The new evidence from the literature search (see Section 4.5.2.4) support sulfite-induced neurotoxic effects (e.g. prolonged VEP latency) which justifies using data from Ozturk et al. (2011) study.

Although other neurotoxicity endpoints are also affected by oral sulfite (hippocampal cell loss, impaired learning and memory, delayed somatosensory evoked potentials; see Section 4.5.2.4), the only available study which tested several dose levels and therefore enabled reliable BMD analysis was Ozturk et al. (2011) who reported VEP latency data. This endpoint was therefore used to identify a reference point.

In the present opinion, a BMDL of 38 mg SO_2 equivalents/kg bw per day, which is lower than the previous reference point of 70 mg SO_2 equivalents/kg bw per day, was estimated based on prolonged VEP latency reported in the Ozturk et al. (2011) study (see Section 4.5.4). This effect is a relevant endpoint for neurotoxicity, not only in rats but also in humans and is related to the apical endpoint 'demyelination'. Predictability of findings in the rat for humans has been demonstrated in the publication of Boyes (1994).

The Panel decided to use the BMDL of 38 mg SO_2 equivalents/kg bw per day as the reference point to calculate the MOE. Performing the quantitative extrapolation from the rat data to humans, the

Panel considered whether the available data would allow modifying the default assessment factor for the MoE approach of 100. The assessment factor for the MoE considers aspects of interspecies toxicokinetics and -dynamics as well as intraspecies toxicokinetics and –dynamics and also the duration of the study (WHO, 2005).

Data for the toxicodynamics were available (Dyer, 1985, Otto et al., 1988), which, however, did not allow the quantification of the respective interspecies differences.

It was noted that from an unpublished study (Gunnison and Jacobsen, 1983, as cited in JECFA (1987)), the half-life of sulfites in humans is reported as 15 min. However, since this study was not available to the Panel, this information was not further considered in the current assessment. Gunnison et al. (1977) have compared the sulfite clearance in monkeys with that of rats. Using the median clearances, the ratio of monkey to rat was 4.1. Thus, assuming monkey sulfite clearance is similar to human, there is no justification for modifying the default interspecies toxicokinetics factor of 4. Taking sulfite clearance into account when deriving the interspecies toxicokinetic factor was considered by the Panel as superior to comparing half-lives or comparison of specific SOX activities between human and rat liver homogenates. Although the expression of SOX is 20 times lower in human compared to rat liver, the ratio of specific enzyme activity between human and rat homogenates varied between 0.39 and 0.52, depending on the reaction measured (Johnson and Rajagopalan, 1976). Considering toxicodynamic intra-individual human variability of the specific endpoint used for deriving a reference point, the study of Chan et al. (1986) reported VEP P100 latency in 42 healthy volunteers of 97.2 ± 3.3 ms (mean ± standard deviation). From the standard deviation the distribution was derived. Calculating the ratio between the 0.3 percentile (mean minus 3 SDs) and the 99.7 percentile (mean plus 3 SDs) resulted in a factor of 1.23. This justifies reducing the default toxicodynamic factor of 3.2–1.23, resulting in a total assessment factor of 40 (2.5 x 4 x 3.16 x 1.23).

With regard to the duration of the study, it could be argued that the default factor of 2 for time extrapolation from short-term to long-term study would not be applicable as the half-life of sulfite is only several minutes in the rat and accumulation will not occur. However, the Panel considered the toxicokinetic argument of limited relevance, but could not exclude the possibility that the prolongation of VEP is associated with adverse neurotoxic effects that persist and may aggravate after repeated exposure over the long term. Accordingly, the Panel included the additional default extrapolation factor of 2 for sub-chronic to chronic exposure (EFSA Scientific Committee, 2012a).

The Panel therefore considered that an overall assessment factor of 80 should be applied for the assessment of the MOE. This means that the MOE, specific for the reference point identified, should be at least 80 for no safety concern to be raised.

Given the BMDL of 38 mg SO_2 equivalents/kg bw per day and taking into account the dietary exposure estimates from the non-brand-loyal scenario based on Data set D (Table 8), that was considered most representative of the dietary exposure to sulfur dioxide-sulfites (E 220–228), the resulting MOEs for all population groups at the mean and the 95th percentile of exposure have been calculated (Table 18). Ranges of MOEs using the MPL scenario are also presented in Table 18.

Table 18: Resulting ranges of MOEs for all population groups considering the dietary exposure estimates of sulfur dioxide-sulfites (E 220–228) (calculated as mg SO_2 equivalents/kg bw per day) from the non-brand-loyal refined exposure scenario based on Data set D and from the MPL exposure scenario

	Refined exposure (Data set D)		MPL exposure	
	Mean exposure	95th percentile exposure	Mean exposure	95th percentile exposure
	Min-Max	Min-Max	Min-Max	Min-Max
Infants	37,600–313	752–59	752–64	251–11
Toddlers	1,253–117	342–40	145–11	47–3
Children	752–150	235–70	150–15	45–5
Adolescents	1,253–289	342–99	470–40	102–11
Adults	470–121	193–32	221–49	71–18
The elderly	626–121	171–49	235–46	63–10

The Panel noted that, using the refined dietary exposure estimates (Data set D), MOEs at the maximum of the 95th percentile ranges were below 80 for all population groups, except for adolescents. The dietary exposure estimated using the MPLs would result in MOEs below 80 in all population groups at the maximum of the ranges of the mean, and for most of the population groups at both minimum and maximum of the ranges at the 95th percentile of exposure. This raises a safety concern for both dietary exposure scenarios.

During this assessment, as required by the European Commission mandate, discussions have taken place between FAF WG on sulfur dioxide–sulfites and ECHA's Human Health Working Group regarding the risk assessment of sulfur dioxide–sulfites (E 220–228) and sulfur dioxide as biocide. The Panel noted that the data set of open literature underpinning the conclusions of this scientific opinion is not the same as the one considered by ECHA BPC assessment on sulfur dioxide released from sodium metabisulfite and on sulfur dioxide generated from sulfur by combustion (ECHA, 2022a,b).

6. Conclusions

The Panel considered that the shortcomings in the toxicity database highlighted by the ANS Panel at the time of the 2016 re-evaluation, have not led to the generation of adequate new data that could have addressed these shortcomings. Accordingly, due to the absence of new biological and toxicological data from IBOs and following an assessment of the literature database, the Panel concluded that the available toxicity database was not adequate to derive an ADI, and consequently withdraws the current temporary group ADI for these food additives.

The Panel concluded that the MOE calculated based on the dietary exposure to sulfur dioxide-sulfites (E 220–228) as food additives should be at least 80. At the estimated dietary exposure to sulfur dioxide-sulfites (E 220–228), when using the refined exposure scenario (Data set D), MOEs at the maximum of the 95th percentile ranges were below 80 for all population groups, except for adolescents. The dietary exposure estimated using the maximum permitted levels would result in MOEs below 80 in all population groups at the maximum of the ranges of the mean, and for most of the population groups at both minimum and maximum of the ranges at the 95th percentile of exposure. This raises a safety concern for both dietary exposure scenarios.

The Panel also concluded that the technical data provided by the interested business operator support an amendment of the specifications for sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228) laid down in Commission Regulation (EU) No 231/2012, as presented by the recommendations made in Table 14.

7. Recommendation

The Panel recommended to the European Commission:

- exploring the technological need to maintain calcium sulfite (E 226) and calcium bisulfite (E 227) on the EU positive list of authorised food additives, considering the low number of labelled uses in the Mintel's GNPD (n = 0 for E 226 and n = 1 for E 227) and information provided by an IBO.
- introducing the correct EINECS number i.e. 233-596-8 in the calcium sulfite (E226) specifications.
- introducing the CAS numbers in the EU specifications for sulfur dioxide and sulfites as listed in Table 2.
- considering expressing the existing maximum limit values for iron and selenium in the EU specifications on the basis of the food additive rather than on the 'SO_2 content' basis, in line with the JECFA specifications.

8. Documentation provided to EFSA

1) BASF. Submission of data in response to the European Commission call for technical data on the permitted food additives sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228). Submitted by BASF to the EC.

2) LANXESS. Submission of data in response to the European Commission call for technical data on the permitted food additives sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228). Submitted by LANXESS to the EC.
3) SDIOC (Sulfur dioxide based chemicals REACH consortium). Submission of data in response to the European Commission call for technical data on the permitted food additives sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228). Submitted by SDIOC to the EC.
4) Additional information submitted in response to a request from EFSA. Submitted by SDIOC October 2021.
5) Additional information submitted in response to a request from EFSA. Submitted by BASF December 2021.
6) Additional clarification request submitted in response to a request from EFSA. Submitted by SDIOC March 2022.
7) ICCR-Roβdorf, 2022. Disodium disulfite: Salmonella Typhimurium and *Escherichia coli* reverse mutation assay. Study No 2177501. Submitted by Sulfur Dioxide Based Chemicals REACH Consortium (SDIOC) April 2022.
8) ICCR-Roβdorf, 2022. Disodium disulfite: Micronucleus Test in Human Lymphocytes *in vitro*. Study No 2177502. Submitted by SDIOC April 2022.
9) ICCR-Roβdorf, 2022. Sodium sulfite: Salmonella Typhimurium and *Escherichia coli* reverse mutation assay. Study No 2177503. Submitted by SDIOC April 2022.
10) ICCR-Roβdorf, 2022. Sodium sulfite: Gene Mutation Assay in Chinese Hamster V79 Cells *in vitro* (V79/HPRT). Study No 2177504. Submitted by SDIOC April 2022.
11) ICCR-Roβdorf, 2022. Sodium sulfite: Micronucleus Test in Human Lymphocytes *in vitro*. Study No 2177505. Submitted by SDIOC April 2022.
12) FRUCOM. Submission of data in response to the European Commission call for technical data on the permitted food additives sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228). Submitted by FRUCOM to the EC.
13) Aegean Dried Fruits Exporters' Association. Submission of data in response to the European Commission call for technical data on the permitted food additives sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228). Submitted by Aegean Dried Fruits Exporters' Association to the EC.
14) FDE (Food Drink Europe). Submission of data in response to the European Commission call for technical data on the permitted food additives sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228). Submitted by FoodDrinkEurope to the EC.
15) AIJN (European Fruit Juice Association). Submission of data in response to the European Commission call for technical data on the permitted food additives sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228). Submitted by AIJN European Fruit Juice Association to the EC.
16) Clitravi. Submission of data in response to the European Commission call for technical data on the permitted food additives sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228). Submitted by Clitravi to the EC.
17) Tessara (Pty) LTD. Submission of data in response to the European Commission call for technical data on the permitted food additives sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228). Submitted by Tessara (Pty) LTD to the EC.

18) BSDA (British Soft Drinks Association). Submission of data in response to the European Commission call for technical data on the permitted food additives sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228). Submitted by BSDA British Soft Drinks Association to the EC.
19) UNESDA (Union of European Soft Drinks Associations). Submission of data in response to the European Commission call for technical data on the permitted food additives sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228). Submitted by UNESDA Soft Drink Europe to the EC.
20) CEEV (Comité Européen des Entreprises Vins). Submission of data in response to the European Commission call for technical data on the permitted food additives sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228). Submitted by CEEV Comité Européen des Entreprises Vins to the EC.

References

Afadlal S, Labetoulle R and Hazell AS, 2014. Role of astrocytes in thiamine deficiency. Metabolic Brain Disease, 29, 1061–1068. https://doi.org/10.1007/s11011-014-9571-y

Ağar A, Küçükatay V, Yargiçoğlu P, Aktekin B, Kipmen-Korgun S, Gümüşlü D and Apaydin C, 2000. The effect of sulfur dioxide inhalation on visual evoked potentials, antioxidant status, and lipid peroxidation in alloxan-induced diabetic rats. Archives of Environmental Contamination and Toxicology, 39, 257–264.

Andersson E, Knutsson A, Hagberg S, Nilsson T, Karlsson B, Alfredsson L and Torén K, 2006. Incidence of asthma among workers exposed to sulphur dioxide and other irritant gases. The European Respiratory Journal, 27, 720–725.

BASF, 2008. Report Natriumsulfit wasserfrei food grade (E 221)/ (PRD 30042389). Micronucleus test in bone marrow cells of the mouse. Project No.: 26M0250/084046. As cited in Documentation provided to EFSA in EFSA ANS Panel, 2016.

Bettendorff L, 2021. Update on thiamine triphosphorylated derivatives and metabolizing enzymatic complexes. Biomolecules, 11, 1645. https://doi.org/10.3390/biom11111645

Bitsch I, Schiele K, Kunsemüller E and Benthien I, 1969. Thiaminmangel und Unbedenklichkeit von Sulfit für den Menschen. 2. Der Thiaminstatus und die Aktivität einiger Dehydrogenasen in den Erythrozyten. Internationale Zeitschrift für Vitaminforschung, 39, 384–400.

Boyes WK, 1994. Rat and human sensory evoked potentials and the predictability of human neurotoxicity from rat data. Neurotoxicology, 15, 569–578.

Brown RP, Delp MD, Lindstedt SL, Rhomberg LR and Beliles RP, 1997. Physiological parameter values for physiologically based pharmacokinetic models. Toxicology and Industrial Health, 13, 407–484.

Butterworth RF, 2003. Thiamin deficiency and brain disorders. Nutrition Research Reviews, 16, 277–284. https://doi.org/10.1079/NRR200367

Camacho L, Lewis SM, Vanlandingham MM, Juliar BE, Olson GR, Patton RE, Gamboa da Costa G, Woodling K, Sepehr E, Bryant MS, Doerge DR, Basavarajappa MS, Felton RP and Delclos KB, 2016. Comparison of endpoints relevant to toxicity assessments in 3 generations of CD-1 mice fed irradiated natural and purified ingredient diets with varying soy protein and isoflavone contents. Food and Chemical Toxicology, 94, 39–56.

Carvalho IM, Melo Cavalcante AA, Dantas AF, Pereira DL, Costa Rocha FC, Andrade TJ and Da Silva J, 2011. Genotoxicity of sodium metabisulphite in mouse tissues evaluated by the comet assay and the micronucleus test. Mutation Research/Genetic Toxicology, 720, 58–61.

Chan YW, McLeod JG, Tuck RR, Walsh JC and Feary PA, 1986. Visual evoked responses in chronic alcoholics. Journal of Neurology, Neurosurgery & Psychiatry, 49, 945–950.

Chatterton BJ, Nunes PT and Savage LM, 2020. The effect of chronic ethanol exposure and thiamine deficiency on myelin-related genes in the cortex and the cerebellum. Alcoholism, Clinical and Experimental Research, 44, 2481–2493. https://doi.org/10.1111/acer.14484

Chauhan A, Srivastva N and Bubber P, 2018. Thiamine deficiency induced dietary disparity promotes oxidative stress and neurodegeneration. Indian Journal of Clinical Biochemistry, 33, 422–428. https://doi.org/10.1007/s12291-017-0690-1

Dalefield RR and Mueller U, 2016. Gastric mucosal irritation following oral exposure to sodium metabisulphite: a reproducible effect? Regulatory Toxicology and Pharmacology, 80, 277–282.

Derin N, Yargiçoğlu P, Aslan M, Elmas O, Agar A and Aicigüzel Y, 2006. The effect of sulfite and chronic restraint stress on brain lipid peroxidation and anti-oxidant enzyme activities. Toxicology and Industrial Health, 22, 233–240.

Derin N, Akpinar D, Yargicoglu P, Agar A and Aslan M, 2009. Effect of alpha-lipoic acid on visual evoked potentials in rats exposed to sulfite. Neurotoxicology and Teratology, 31, 34–39.
Desjardins P and Butterworth RF, 2005. Role of mitochondrial dysfunction and oxidative stress in the pathogenesis of selective neuronal loss in Wernicke's encephalopathy. Molecular Neurobiology, 31, 17–25. https://doi.org/10.1385/MN:31:1-3:017
Doerge DR, McNamee MG and Ingraham LL, 1982. Some neurochemical properties of thiamin. Annals of the New York Academy of Sciences, 378, 422–434. https://doi.org/10.1111/j.1749-6632.1982.tb31215.x
Du Z and Meng Z, 2004a. Effects of derivatives of sulfur dioxide on transient outward potassium currents in acutely isolated hippocampal neurons. Food and Chemical Toxicology, 42, 1211–1216.
Du Z and Meng Z, 2004b. Modulation of sodium currents in rat dorsal root ganglion neurons by sulfur dioxide derivatives. Brain Research, 1010, 127–133.
Du Z and Meng Z, 2006. Sulfur dioxide derivatives modulation of high-threshold calcium currents in rat dorsal root ganglion neurons. Neuroscience Letters, 405, 147–152.
Du Z, Zhou Y and Yang P, 2007. Sulfur dioxide derivatives increase a hyperpolarization-activated inward current in dorsal root ganglion neurons. Toxicology, 239, 180–185.
Dyer RS, 1985. The use of sensory evoked potentials in toxicology. Fundamental and Applied Toxicology, 5, 24–40.
ECHA (European Chemicals Agency), 2012. Guidance on information requirements and chemical safety assessment Chapter R. 8: Characterisation of dose [concentration]-response for human health.
ECHA (European Chemicals Agency), 2021. RAC opinion proposing harmonised classification and labelling at EU level of Sulfur dioxide; EC Number: 231–195-2; CAS Number: 7446-09-5. Adopted 26 November 2021. Available online: https://echa.europa.eu/documents/10162/5ea9c21b-8f7c-a6f0-6852-866fc7887902
ECHA (European Chemicals Agency), 2022a. Opinion on the application for approval of the active substance: sulfur dioxide released from sodium metabisulfite. Product type: 9. Available online: https://echa.europa.eu/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/1419/PT09
ECHA (European Chemicals Agency), 2022b. Opinion on the application for approval of the active substance: sulfur dioxide generated from sulfur by combustion. Product type: 4. Available online: https://echa.europa.eu/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/1394/PT04
EFSA (European Food Safety Authority), 2007. Scientific opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. EFSA Journal 2007;5(1):438, 54 pp. https://doi.org/10.2903/j.efsa.2007.438
EFSA (European Food Safety Authority), 2010. Management of left-censored data in dietary exposure assessment of chemical substances. EFSA Journal 2010;8(3):1557, 96 pp. https://doi.org/10.2903/j.efsa.2010.1557
EFSA (European Food Safety Authority), 2011. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097
EFSA (European Food Safety Authority), 2015. The food classification and description system FoodEx2 (revision 2). EFSA supporting publication 2015:EN-804, 90 pp.
EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2012. Guidance for submission for food additive evaluations. EFSA Journal 2012;10(7):2760, 53 pp. https://doi.org/10.2903/j.efsa.2012.2760
EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources Added to Food), 2016. Scientific Opinion on the re-evaluation sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228) as food additives. EFSA Journal 2016;14(4):4438, 151 pp. https://doi.org/10.2903/j.efsa.2016.4438
EFSA ANS Panel (EFSA Panel on Food Additives and Nutrients Sources added to Food), 2017. Approach followed for the refined exposure assessment as part of the safety assessment of food additives under re-evaluation. EFSA Journal 2017;15(10):5042, 9 pp. https://doi.org/10.2903/j.efsa.2017.5042
EFSA CONTAM Panel (Panel on Contaminants in the Food Chain), 2009a. Scientific opinion on Cadmium in food. EFSA Journal 2009;7(10):1351, 199 pp. https://doi.org/10.2903/j.efsa.2009.1351
EFSA CONTAM Panel (Panel on Contaminants in the Food Chain), 2009b. Scientific opinion on Arsenic in food. EFSA Journal 2009;7(3):980, 139 pp. https://doi.org/10.2903/j.efsa.2009.980
EFSA CONTAM Panel (Panel on Contaminants in the Food Chain), 2010. Scientific opinion on Lead in food. EFSA Journal 2010;8(4):1570, 151 pp. https://doi.org/10.2903/j.efsa.2010.1570
EFSA CONTAM Panel (Panel on Contaminants in the Food Chain), 2011. Statement on tolerable weekly intake for cadmium. EFSA Journal 2011;9(2):1975, 19 pp. https://doi.org/10.2903/j.efsa.2011.1975
EFSA CONTAM Panel (Panel on Contaminants in the Food Chain), 2012. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 2012;10(12):2985, 15 pp. https://doi.org/10.2903/j.efsa.2012.2985
EFSA Scientific Committee, 2009. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessment carried out by EFSA. Part 2: general principles. EFSA Journal 2009;7(5):1051, 22 pp.
EFSA Scientific Committee, 2012a. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. https://doi.org/10.2903/j.efsa.2012.2579

EFSA Scientific Committee, 2012b. Statement on the applicability of the Margin of Exposure approach for the safety assessment of impurities which are both genotoxic and carcinogenic in substances added to food/feed. EFSA Journal 2012;10(3):2578, 5 pp. https://doi.org/10.2903/j.efsa.2012.2578

EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger MJ, Knutsen KH, More S, Mortensen A, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Silano V, Solecki R, Turck D, Aerts M, Bodin L, Davis A, Edler L, Gundert-Remy U, Sand S, Slob W, Bottex B, Abrahantes JC, Marques DC, Kass G and Schlatter JR, 2017. Update: Guidance on the use of the benchmark dose approach in risk assessment. EFSA Journal 2017;15 (1):4658, 41 pp. https://doi.org/10.2903/j.efsa.2017.4658

EFSA Scientific Committee, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V, Solecki R, Turck D, Younes M, Craig P, Hart A, Von Goetz N, Koutsoumanis K, Mortensen A, Ossendorp B, Martino L, Merten C, Mosbach-Schulz O and Hardy A, 2018. Guidance on uncertainty analysis in scientific assessments. EFSA Journal 2018;16(1):5123, 39 pp. https://doi.org/10.2903/j.efsa.2018.5123

Ercan S, Kencebay C, Basaranlar G, Derin N and Aslan M, 2015. Induction of xanthine oxidase activity, endoplasmic reticulum stress and caspase activation by sodium metabisulfite in rat liver and their attenuation by Ghrelin. Food and Chemical Toxicology, 76, 27–32.

FAO, WHO, 2009. Principles and methods for the risk assessment of chemicals in food. Environmental Health Criteria, 240.

Fathabad AA, Shekarforoush S, Hoseini M and Ebrahimi Z, 2018. Attenuation of Sulfite-Induced Testicular Injury in Rats by Zingiber officinale Roscoe. Journal of dietary supplements, 15, 398–409.

FDRL (Food and Drug Research Laboratories), 1972a. Teratology evaluation of FDA 71–20 in rats. FDA 71–20 (Sodium bisulfite). Laboratory No. 0894 j.

FDRL (Food and Drug Research Laboratories), 1972b. Teratology evaluation of FDA 71–22 in rats. FDA 71–22 (Sodium meta-bisulfite). Laboratory No. 0894 k.

FDRL (Food and Drug Research Laboratories), 1972c. Teratology evaluation of FDA 71–20 in mice. FDA 71–20 (Sodium bisulfite). Laboratory No. 0893 j.

FDRL (Food and Drug Research Laboratories), 1972d. Teratology evaluation of FDA 71–22 in mice. FDA 71–22 (Sodium meta-bisulfite). Laboratory No. 0893 k.

FDRL (Food and Drug Research Laboratories), 1975. Teratology evaluation of FDA 71–21 in rats. FDA 71–20 (Potassium metabisulfite). Laboratory No. 2143.

Fine JM, Gordon T and Sheppard D, 1987. The roles of pH and ionic species in sulfur dioxide- and sulfite- induced bronchoconstriction. The American Review of Respiratory Disease, 136, 1122–1126.

Gangolf M, Wins P, Thiry M, El Moualij B and Bettendorff L, 2010. Thiamine triphosphate synthesis in rat brain occurs in mitochondria and is coupled to the respiratory chain. The Journal of Biological Chemistry, 285, 583–594. https://doi.org/10.1074/jbc.M109.054379

Gao Y, Liang C, Zhang J, Ma J, Wang J, Niu R, Tikka C, Wang Y and Wang J, 2018. Combination of fluoride and SO_2 induce DNA damage and morphological alterations in male rat kidney. Cellular Physiology and Biochemistry, 50, 734–744.

Generoso WM, Huff SW and Cain KT, 1978. Tests on induction of chromosome aberrations in mouse germ cells with sodium bisulphite. Mutation Research, 56, 363–365.

Gibson CR, Gleason A and Messina E, 2021. Measurement of total liver blood flow in intact anesthetized rats using ultrasound imaging. Pharmacology Research & Perspectives, 9, e00731.

Grings M, Moura AP, Parmeggiani B, Marcowich GF, Amaral AU, de Souza Wyse AT, Wajner M and Leipnitz G, 2013. Disturbance of brain energy and redox homeostasis provoked by sulfite and thiosulfate: potential pathomechanisms involved in the neuropathology of sulfite oxidase deficiency. Gene, 531, 191–198.

Guan DF, Ren PY, Hu W and Zhang YL, 2015. The mGluR5 positive allosteric modulator CDPPB inhibits SO_2-induced protein radical formation and mitochondrial dysfunction through activation of Akt in mouse hippocampal HT22 cells. Cellular and Molecular Neurobiology, 35, 573–583.

Gunnison AF and Jacobsen DW, 1983. Hypersensitivity to sulfites: a reappraisal of sulfite toxicity. Prepared for International Life Sciences Institute, Washington, DC, USA. 92 p. As cited in JECFA (1987) https://inchem.org/documents/jecfa/jecmono/v21je15.htm

Gunnison AF, Bresnahan CA and Palmes ED, 1977. Comparative sulfite metabolism in the rat, rabbit, and rhesus monkey. Toxicology and Applied Pharmacology, 42, 99–109.

Gunnison AF, Sellakumar A, Currie D and Snyder EA, 1987. Distribution, metabolism and toxicity of inhaled sulfur dioxide and endogenously generated sulfite in the respiratory tract of normal and sulfite oxidase-deficient rats. Journal of Toxicology and Environmental Health, Part A Current Issues, 21, 141–162.

Han X, Zhu F, Chen L, Wu H, Wang T and Chen K, 2020. Mechanism analysis of toxicity of sodium sulfite to human hepatocytes L02. Molecular and Cellular Biochemistry, 473, 25–37.

Hazell AS, Todd KG and Butterworth RF, 1998. Mechanisms of neuronal cell death in Wernicke's encephalopathy. Metabolic Brain Disease, 13, 97–122. https://doi.org/10.1023/a:1020657129593

Hötzel D, Muskat E, Bitsch I, Aign W, Althoff JD and Cremer HD, 1969. Thiaminmangel und Unbedenklichkeit von Sulfit für den Menschen. I. Problemstellung, Versuchsplanung, Klinische Untersuchungen. Internationale Zeitschrift für Vitaminforschung, 39, 372–383.

Hrubša M, Siatka T, Nejmanová I, Vopršalová M, Kujovská Krčmová L, Matoušová K, Javorská L, Macáková K, Mercolini L, Remião F, Máťuš M, Mladěnka P and On Behalf Of The Oemonom, 2022. Biological Properties of Vitamins of the B-Complex, Part 1: Vitamins B1, B2, B3, and B5. Nutrients, 14, 484. https://doi.org/10.3390/nu14030484

Ikarashi Y, Iizuka S, Imamura S, Yamaguchi T, Sekiguchi K, Kanno H, Kawakami Z, Yuzurihara M, Kase Y and Takeda S, 2009. Effects of yokukansan, a traditional Japanese medicine, on memory disturbance and behavioral and psychological symptoms of dementia in thiamine-deficient rats. Biological & Pharmaceutical Bulletin, 32, 1701–1709. https://doi.org/10.1248/bpb.32.1701

Inaba H, Kishimoto T, Oishi S, Nagata K, Hasegawa S, Watanabe T and Kida S, 2016. Vitamin B1-deficient mice show impairment of hippocampus-dependent memory formation and loss of hippocampal neurons and dendritic spines: potential microendophenotypes of Wernicke-Korsakoff syndrome. Bioscience, Biotechnology, and Biochemistry, 80, 2425–2436. https://doi.org/10.1080/09168451.2016.1224639

Jagiello GM, Lin JS and Ducayen MB, 1975. SO_2 and its metabolite: Effects on mammalian egg chromosomes. Environmental Research, 9, 84–93.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1987. Evaluation of certain food additives and contaminants (Thirtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 751, 1987. Available online: https://apps.who.int/iris/bitstream/handle/10665/41306/WHO_TRS_751.pdf?sequence=1&isAllowed=y. Monograph: https://inchem.org/documents/jecfa/jecmono/v21je15.htm

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000. Guidelines for the preparation of toxicological working papers for the Joint FAO/WHO Expert Committee on Food Additives. Geneva, Switzerland.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006. Monograph 1. Combined compendium of food additive specifications. Available online: http://www.fao.org/ag/agn/jecfa-additives/search.html

Johnson JL and Rajagopalan KV, 1976. Human sulphite oxidase deficiency. Characterization of the molecular defect in a multicomponent system. Journal of Clinical Investigation, 58, 551–556.

Karimfar MH, Noorafshan A, Rashidiani-Rashidabadi A, Poostpasand A, Asadi-Golshan R, Abdollahifar MA and Karbalay-Doust S, 2014. Curcumin prevents the structural changes induced in the rats' deep cerebellar nuclei by sodium metabisulfite, a preservative agent. Asian Pacific Journal of Tropical Medicine, 7, S301–S305.

Kencebay C, Derin N, Ozsoy O, Kipmen-Korgun D, Tanriover G, Ozturk N, Basaranlar G, Yargicoglu-Akkiraz P, Sozen B and Agar A, 2013. Merit of quinacrine in the decrease of ingested sulfite-induced toxic action in rat brain. Food and Chemical Toxicology, 52, 129–136.

Kilic D, 2003. The effects of ageing and sulfur dioxide inhalation exposure on visual-evoked potentials, antioxidant enzyme systems, and lipid-peroxidation levels of the brain and eye. Neurotoxicology and Teratology, 25, 587–598.

Klimisch HJ, Andreae M and Tillmann U, 1997. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory Toxicology and Pharmacology, 25, 1–5.

Kocamaz E, Adiguzel E, Buket ER, Gundogdu G and Küçükatay V, 2012. Sulfite leads to neuron loss in the hippocampus of both normal and SOX-deficient rats. Neurochemistry International, 61, 341–346.

Küçükatay V, Savcıoğlu F, Hacıoğlu G, Yargıçoğlu P and Ağar A, 2005. Effect of sulfite on cognitive function in normal and sulfite oxidase deficient rats. Neurotoxicology and Teratology, 27, 47–54.

Küçükatay V, Hacıoğlu G, Savcıoğlu F, Yargıçoğlu P and Ağar A, 2006. Visual evoked potentials in normal and sulfite oxidase deficient rats exposed to ingested sulfite. Neurotoxicology, 27, 93–100.

Küçükatay V, Bor-Kucukatay M, Atsak P and Ağar A, 2007. Effect of ingested sulfite on hippocampus antioxidant enzyme activities in sulfite oxidase competent and deficient rats. International Journal of Neuroscience, 117, 971–983.

Langlais PJ and Zhang SX, 1997. Cortical and subcortical white matter damage without Wernicke's encephalopathy after recovery from thiamine deficiency in the rat. Alcoholism, Clinical and Experimental Research, 21, 434–443. https://doi.org/10.1111/j.1530-0277.1997.tb03788.x

Li G and Sang N, 2009. Delayed rectifier potassium channels are involved in SO_2 derivative-induced hippocampal neuronal injury. Ecotoxicology and Environmental Safety, 72, 236–241.

Li R, Kou X, Tian J, Meng Z, Cai Z, Cheng F and Dong C, 2014. Effect of sulfur dioxide on inflammatory and immune regulation in asthmatic rats. Chemosphere, 112, 296–304.

Li X, Huang L, Wang N, Yi H and Wang H, 2018a. Sulfur dioxide exposure enhances Th2 inflammatory responses via activating STAT6 pathway in asthmatic mice. Toxicology Letters, 285, 43–50.

Li X, Yi H and Wang H, 2018b. Sulphur dioxide and arsenic affect male reproduction via interfering with spermatogenesis in mice. Ecotoxicology and Environmental Safety, 165, 164–173.

Liang C, Gao Y, Zhao Y, Manthari RK, Ma J, Niu R, Wang J, Zhang J and Wang J, 2018. Effects of fluoride and/or sulfur dioxide on morphology and DNA integrity in rats' hepatic tissue. Biological Trace Element Research, 183, 335–341.

Liang J, Liu L, Kang X, Hu F and Mao L, 2020. Mechanism underlying the effect of SO_2-induced oxidation on human skin keratinocytes. Medicine, 99, e23152.

Liu M, Lu J, Chen Y, Shi X, Li Y, Yang S, Yu J and Guan S, 2021. Sodium sulfite-induced mast cell pyroptosis and degranulation. Journal of Agricultural and Food Chemistry, 69, 7755–7764.

Mahmoud AAT, Hassan GM, Hassan AMS, Latif AKMA and Ramadan MF, 2015. Demonstrating adverse effects of a common food additive (sodium sulfite) on biochemical, cytological and histopathological parameters in tissues of albino Wister rats. European Journal of Integrative Medicine, 7, 234–242.
Mahmoudi R, Honarmand Z, Karbalay-Doust S, Jafari-Barmak M, Nikseresht M and Noorafshan A, 2017. Using curcumin to prevent structural impairments of testicles in rats induced by sodium metabisulfite. EXCLI Journal, 16, 583.
Makino M, Takahashi-Ito K, Murasawa H, Pawlak A, Kashimoto Y and Kitano Y, 2019. Memantine ameliorates learning and memory disturbance and the behavioral and psychological symptoms of dementia in thiamine-deficient mice. Pharmacology, Biochemistry, and Behavior, 183, 6–13. https://doi.org/10.1016/j.pbb.2019.05.008
Mancinelli R, Ceccanti M, Guiducci MS, Sasso GF, Sebastiani G, Attilia ML and Allen JP, 2003. Simultaneous liquid chromatographic assessment of thiamine, thiamine monophosphate and thiamine diphosphate in human erythrocytes: a study on alcoholics. Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences, 789, 355–363. https://doi.org/10.1016/s1570-0232(03)00139-9
Marshall KA, Reist M, Jenner P and Halliwell B, 1999. The neuronal toxicity of sulfite plus peroxynitrite is enhanced by glutathione depletion: Implications for Parkinson's disease. Free Radical Biology and Medicine, 27, 515–520.
Meng Z and Liu Y, 2007. Cell morphological ultrastructural changes in various organs from mice exposed by inhalation to sulfur dioxide. Inhalation Toxicology, 19, 543–551.
Meng Z and Nie A, 2005a. Effects of sodium metabisulfite on potassium currents in acutely isolated CA1 pyramidal neurons of rat hippocampus. Food and Chemical Toxicology, 43, 225–232.
Meng Z and Nie A, 2005b. Enhancement of sodium metabisulfite on sodium currents in acutely isolated rat hippocampal CA1 neurons. Environmental Toxicology and Pharmacology, 20, 35–41.
Meng Z and Zhang L, 1992. Cytogenetic damage induced in human lymphocytes by sodium bisulfite. Mutation Research/Genetic Toxicology, 298, 63–69.
Meng Z and Zhang B, 2002. Induction effects of sulfur dioxide inhalation on chromosomal aberrations in mouse bone marrow cells. Mutagenesis, 17, 215–217.
Meng Z and Zhang B, 2005. DNA damage in mice treated with sulfur dioxide by inhalation. Environmental and Molecular Mutagenesis, 46, 150–155.
Meng Z, Zhang B, Ruan A, Sang N and Zhang J, 2002. Micronuclei induced by sulfur dioxide inhalation in mouse bone marrow cells in vivo. Inhalation Toxicology, 14, 303–309.
Meng Z, Qin G, Zhang B and Bai J, 2004. DNA damaging effects of sulfur dioxide derivatives in cells from various organs of mice. Mutagenesis, 19, 465–468.
Meng Z, Li R and Zhang X, 2005. Levels of sulfite in three organs from mice exposed to sulfur dioxide. Inhalation Toxicology, 17, 309–313.
Murray FJ, Schwetz BA, Crawford AA, Henck JW, Quast JF and Staples RE, 1979. Embryotoxicity of inhaled sulfur dioxide and carbon monoxide in mice and rabbits. Journal of Environmental Science and Health. Part C: Environmental Health Sciences, 13, 233–250.
Nadel JA, Salem H, Tamplin B and Tokiwa Y, 1965. Mechanism of bronchoconstriction: during inhalation of sulfur dioxide; reflex involving vagus nerves. Archives of Environmental Health: An International Journal, 10, 175–178.
Noorafshan A, Asadi-Golshan R, Karbalay-Doust S, Abdollahifar MA and Rashidiani-Rashidabadi A, 2013. Curcumin, the main part of turmeric, prevents learning and memory changes induced by sodium metabisulfite, a preservative agent, in rats. Experimental Neurobiology, 22, 23–30.
Noorafshan A, Asadi-Golshan R, Abdollahifar MA and Karbalay-Doust S, 2015. Protective role of curcumin against sulfite-induced structural changes in rats' medial prefrontal cortex. Nutritional Neuroscience, 18, 248–255.
Oishi K, Barchi M, Au AC, Gelb BD and Diaz GA, 2004. Male infertility due to germ cell apoptosis in mice lacking the thiamin carrier, Tht1. A new insight into the critical role of thiamin in spermatogenesis. Developmental Biology, 266, 299–309.
Onodera K, Kisara K, Okabe H and Ogura Y, 1980. Studies on the effects of thiamine deficiency on rat testes. Folia Pharmacologica Japonica, 76, 143–152. https://doi.org/10.1254/fpj.76.143
Oshimo M, Nakashima F, Kai K, Matsui H, Shibata T and Akagawa M, 2021. Sodium sulfite causes gastric mucosal cell death by inducing oxidative stress. Free Radical Research, 55, 606–618.
Otto D, Hudnell K, Boyes W, Janssen R and Dyer R, 1988. Electrophysiological measures of visual and auditory function as indices of neurotoxicity. Toxicology, 49, 205–218.
Ough CS and Were L, 2005. Sulfur dioxide and sulfites. FOOD SCIENCE AND TECHNOLOGY-NEW YORK-MARCEL DEKKER, 145, 143.
Ozsoy O, Aras S, Ozkan A, Parlak H, Aslan M, Yargicoglu P and Agar A, 2016. The effect of ingested sulfite on visual evoked potentials, lipid peroxidation, and antioxidant status of brain in normal and sulfite oxidase-deficient aged rats. Toxicology and Industrial Health, 32, 1197–1207.
Ozsoy O, Aras S, Ozkan A, Parlak H, Gemici B, Uysal N, Aslan M, Yargicoglu P and Agar A, 2017. The effect of ingested sulfite on active avoidance in normal and sulfite oxidase-deficient aged rats. Toxicology Mechanisms and Methods, 27, 81–87.
Öztürk OH, Küçükatay V, Yönden Z, Ağar A, Bağci H and Delibaş N, 2006. Expressions of N-methyl-D-aspartate receptors NR2A and NR2B subunit proteins in normal and sulfite-oxidase deficient rat's hippocampus: effect of exogenous sulfite ingestion. Archives of Toxicology, 80, 671–679.

Ozturk N, Yargicoglu P, Derin N, Akpinar D, Agar A and Aslan M, 2011. Dose-dependent effect of nutritional sulfite intake on visual evoked potentials and lipid peroxidation. Neurotoxicology and Teratology, 33, 244–254.
Pal BB and Bhunya SP, 1992. Genotoxic effect of a preservative, sodium meabisulphite as revealed by mammalian in vivo bioassays. Cytologia, 57, 455–461.
Qin G, Wang J, Huo Y, Yan H, Jiang C, Zhou J, Wang X and Sang N, 2012. Sulfur dioxide inhalation stimulated mitochondrial biogenesis in rat brains. Toxicology, 300, 67–74.
Qin G, Wu M and Sang N, 2015. Sulfur dioxide and benzo (a) pyrene trigger apoptotic and anti-apoptotic signals at different post-exposure times in mouse liver. Chemosphere, 139, 318–325.
Qin G, Wu M, Wang J, Xu Z, Xia J and Sang N, 2016. Sulfur dioxide contributes to the cardiac and mitochondrial dysfunction in rats. Toxicological Sciences, 151, 334–346.
Qin G, Wang J and Sang N, 2017. Sulfur dioxide inhibits expression of mitochondrial oxidative phosphorylation genes encoded by both nuclear DNA and mitochondrial DNA in rat lungs. Environmental Science and Pollution Research, 24, 2527–2534.
Qiu JL, Zhu WL, Lu YJ, Bai ZF, Liu ZG, Zhao P, Sun C, Zhang YB, Li H and Liu W, 2015. The selective mGluR5 agonist CHPG attenuates SO_2-induced oxidative stress and inflammation through TSG-6/NF-κB pathway in BV2 microglial cells. Neurochemistry International, 85, 46–52.
Qu D, Gu Y, Feng L and Han J, 2017. High content analysis technology for evaluating the joint toxicity of sunset yellow and sodium sulfite in vitro. Food Chemistry, 233, 135–143.
Qureshi IZ, Din NU, Khadilla G, Shahzadi A, Rafiq B and Afaqi H, 2022. Adverse physiologic effects of a common food additive potassium metabisulfite (E224) in laboratory rats. Journal of Food Processing and Preservation, 46, e16393.
Reist M, Marshall KA, Jenner P and Halliwell B, 1998. Toxic effects of sulphite in combination with peroxynitrite on neuronal cells. Journal of Neurochemistry, 71, 2431–2438.
Renner HW and Wever J, 1983. Attempts to induce cytogenetic effects with sulphite in sulphite oxidase-deficient Chinese hamsters and mice. Food and Chemical Toxicology, 21, 123–127.
Ruan A, Min H, Meng Z and Lü Z, 2003. Protective effects of seabuckthorn seed oil on mouse injury induced by sulfur dioxide inhalation. Inhalation Toxicology, 15, 1053–1058.
Sang N, Yun Y, Li H, Hou L, Han M and Li G, 2010. SO_2 inhalation contributes to the development and progression of ischemic stroke in the brain. Toxicological Sciences, 114, 226–236.
Sang N, Yun Y, Yao GY, Li HY, Guo L and Li GK, 2011. SO_2-induced neurotoxicity is mediated by cyclooxygenases-2-derived prostaglandin E2 and its downstream signaling pathway in rat hippocampal neurons. Toxicological Sciences, 124, 400–413.
Shekarforoush S, Ebrahimi Z and Hoseini M, 2015. Sodium metabisulfite-induced changes on testes, spermatogenesis and epididymal morphometric values in adult rats. International Journal of Reproductive BioMedicine, 13, 765–770.
Skypala IJ, Williams M, Reeves L, Meyer R and Venter C, 2015. Sensitivity to food additives, vaso-active amines and salicylates: a review of the evidence. Clinical and Translational Allergy, 5, 1–11.
Spencer PS and Palmer VS, 2012. Interrelationships of undernutrition and neurotoxicity: food for thought and research attention. Neurotoxicology, 33, 605–616. https://doi.org/10.1016/j.neuro.2012.02.015
Stanford Research Institute, 1972. Study of the Mutagenic Effects of Sodium meta-bisulfite (71–22). FDA 71–267. Report No. FDABF-GRAS-091.
Sun J, Sakamoto T and Chung KF, 1995. Effects of sodium metabisulphite on Guinea pig contractile airway smooth muscle responses in vitro. Thorax, 50, 875–879.
Tallaksen CME, Bøhmer T and Bell H, 1992. Blood and serum thiamin and thiamin phosphate esters concentrations in patients with alcohol dependence syndrome before and after thiamin treatment. Alcoholism: Clinical and Experimental Research, 16, 320–325.
Til HP, Feron VJ, De Groot AP and Van der Wal P, 1972. Toxicity of sulphite. II. Short- and long-term feeding studies in pigs. Food and Cosmetics Toxicology, 10, 463–473.
Van Schoor J, Joos GF and Pauwels RA, 2000. Indirect bronchial hyperresponsiveness in asthma: mechanisms, pharmacology and implications for clinical research. European Respiratory Journal, 16, 514–533.
Velayutham M, Hemann CF, Cardounel AJ and Zweier JL, 2016. Sulfite oxidase activity of cytochrome c: role of hydrogen peroxide. Biochemistry and Biophysics Reports, (5), 96–104. https://doi.org/10.1016/j.bbrep.2015.11.025
Wang X, Cao H, Guan XL, Long LH, Hu ZL, Ni L, Wang F, Chen JG and Wu PF, 2016. Sulfite triggers sustained calcium overload in cultured cortical neurons via a redox-dependent mechanism. Toxicology Letters, 258, 237–248.
Wang C, Liang C, Ma J, Manthari RK, Niu R, Wang J, Wang J and Zhang J, 2018. Co-exposure to fluoride and sulfur dioxide on histological alteration and DNA damage in rat brain. Journal of Biochemical and Molecular Toxicology, 32, e22023.
Whitfield KC, Bourassa MW, Adamolekun B, Bergeron G, Bettendorff L, Brown KH, Cox L, Fattal-Valevski A, Fischer PR, Frank EL, Hiffler L, Hlaing LM, Jefferds ME, Kapner H, Kounnavong S, Mousavi MPS, Roth DE, Tsaloglou MN, Wieringa F and Combs GF Jr, 2018. Thiamine deficiency disorders: diagnosis, prevalence, and a roadmap for global control programs. Annals of the New York Academy of Sciences, 1430, 3–43. https://doi.org/10.1111/nyas.13919

WHO (World Health Organization), 2009. Principles and Methods for the Risk Assessment of Chemicals in Food. Environment Health Criteria 240. Available online: http://www.who.int/foodsafety/publications/chemical-food/en/

World Health Organization (WHO), 2005. International Programme on Chemical Safety & IPCS Workshop on Incorporating Uncertainty and Variability into Risk Assessment (2000: Berlin, Germany). Chemical-specific adjustment factors for interspecies differences and human variability: guidance document for use of data in dose/concentration-response assessment. Available online: https://apps.who.int/iris/handle/10665/43294

World Health Organization (WHO), 2021. WHO global air quality guidelines: particulate matter (PM2.5 and PM10), ozone, nitrogen dioxide, sulfur dioxide and carbon monoxide. Available online: https://apps.who.int/iris/handle/10665/345329

Wu W, Fu G, Xuan R, Zhai L, Lu Y, Tang M, Liu J, Zhang C, Chen H and Wang F, 2022. Food additive sodium bisulfite induces intracellular imbalance of biothiols levels in NCM460 colonic cells to trigger intestinal inflammation in mice. Toxicology Letters, 359, 73–83.

Wüthrich B, 2018. Allergic and intolerance reactions to wine. Allergologie Select, 2, 80–88.

Xie J, Fan R and Meng Z, 2007. Protein oxidation and DNA-protein crosslink induced by sulfur dioxide in lungs, livers, and hearts from mice. Inhalation Toxicology, 19, 759–765.

Yao G, Yun Y and Sang N, 2016. Differential effects between one week and four weeks exposure to same mass of SO_2 on synaptic plasticity in rat hippocampus. Environmental Toxicology, 31, 820–829.

Yargicoğlu P, Ağar A, Gümüşlü S, Bilmen S and Oğuz Y, 1999. Age-related alterations in antioxidant enzymes, lipid peroxide levels, and somatosensory-evoked potentials: effect of sulfur dioxide. Archives of Environmental Contamination and Toxicology, 37, 554–560.

Yargicoglu P, Şahin E, Gümüşlü S and Ağar A, 2007. The effect of sulfur dioxide inhalation on active avoidance learning, antioxidant status and lipid peroxidation during aging. Neurotoxicology and Teratology, 29, 211–218.

Yavuz-Kocaman A, Rencuzogullari E, Ila HB and Topaktas M, 2008. The genotoxic effect of potassium metabisulphite using chromosome aberration, sister chromatid exchange, micronucleus tests in human lymphocytes and chromosome aberration test in bone marrow cells of rats. Environmental and Molecular Mutagenesis, 49, 276–282.

You Y, Klistorner A, Thie J and Graham SL, 2011. Latency delay of visual evoked potential is a real measurement of demyelination in a rat model of optic neuritis. Investigative Ophthalmology & Visual Science, 52, 6911–6918. https://doi.org/10.1167/iovs.11-7434

You Y, Gupta VK, Chitranshi N, Reedman B, Klistorner A and Graham SL, 2015. Visual Evoked Potential Recording in a Rat Model of Experimental Optic Nerve Demyelination. Journal of Visualized Experiments, 101, e52934. https://doi.org/10.3791/52934

Yun Y, Li H, Li G and Sang N, 2010. SO_2 inhalation modulates the expression of apoptosis-related genes in rat hippocampus via its derivatives in vivo. Inhalation Toxicology, 22, 919–929.

Zaki EI, El-Mahdy AR, El-Gamal HM and Ayman S, 2021. Cytogenetic and testicular histological alterations induced by sulphur dioxide in dried apricot leather. Cellular and Molecular Biology, 67, 66–75.

Zare S, Hossein Dabbaghmanesh M, Noorafshan A, Koohpeyma F, Bakhshayeshkaram M and Montazeri-Najafabady N, 2019. Protective effect of vitamin E and vitamin C alone and in combination on testicular damage induced by sodium metabisulphite in rats: a stereological study. Andrologia, 51, e13193.

Zera K and Zastre J, 2018. Stabilization of the hypoxia-inducible transcription Factor-1 alpha (HIF-1α) in thiamine deficiency is mediated by pyruvate accumulation. Toxicology and Applied Pharmacology, (355), 180–188. https://doi.org/10.1016/j.taap.2018.07.004

Zhang X, Vincent AS, Halliwell B and Wong KP, 2004. A mechanism of sulfite neurotoxicity: direct inhibition of glutamate dehydrogenase. Journal of Biological Chemistry, 279, 43035–43045.

Zhang J, Liang C, Ma J, Zhou B and Wang J, 2006a. Changes in testis protein and metabolic enzyme activities in rats induced by sodium fluoride and sulfur dioxide. Fluoride, 39, 179–184.

Zhang J, Liang C, Ma J, Zhou B and Wang J, 2006b. Effects of sodium fluoride and sulfur dioxide on oxidative stress and antioxidant defenses in rat testes. Fluoride, 39, 185–190.

Zhang Q, Bai Y, Yang Z, Tian J and Meng Z, 2015. Effect of sulfur dioxide inhalation on the expression of KATP and L-Ca^{2+} channels in rat hearts. Environmental Toxicology and Pharmacology, 39, 1132–1138.

Zhang J, Zheng F, Liang C, Zhu Y, Shi Y, Han Y and Wang J, 2016a. Sulfur dioxide inhalation lowers sperm quality and alters testicular histology via increasing expression of CREM and ACT proteins in rat testes. Environmental Toxicology and Pharmacology, 47, 47–52.

Zhang J, Li Z, Qie M, Zheng R, Shetty J and Wang J, 2016b. Sodium fluoride and sulfur dioxide affected male reproduction by disturbing blood-testis barrier in mice. Food and Chemical Toxicology, 94, 103–111.

Zhang Z, Ma Z, Sun W, Ma D and Cao J, 2018. The effect of exposure of SO_2 in high concentrations on CD19+ cells in reactive airway dysfunction syndrome in rat. European Journal of Inflammation, 16, 2058739218791905.

Ziemann C, Hanse T, Pohlmann G, Farrar D, Pohlenz-Michel C, Tillmann T and Mangelsdorf I, 2010. Genotoxicity testing of sulfur dioxide (SO_2) in a mouse bone marrow micronucleus test complemented with haematological endpoints. Mutation Research/Genetic Toxicology and Environmental Mutagenicity, 697, 38–46.

Abbreviations

4-HNE	4-hydroxy-2-nonenal
AAF	European Starch Industry Association
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AIJN	European Fruit Juice Association
ANS Panel EFSA	Panel on Food Additives and Nutrient Sources added to Food
ATP	adenosine triphosphate
BL	brand-loyal
BMD	benchmark dose
BMDL	lower confidence limit of the benchmark dose
BMPA	British Meat Processors Association
BMR	benchmark response
bw	body weight
CA	chromosomal aberrations
CAS	Chemical Abstract Service
CAT	catalase
CBPI	cytokinesis block proliferation index
CNS	central nervous system
CONTAM	Panel EFSA Panel on Contaminants in the Food Chain
COX	Cyclooxygenase
cPGES	cytosolic prostaglandin E synthases
DAAS	Direct Atomic Absorption Spectroscopy
DI	damage index
DF	damage frequency
DMSO	dimethyl sulfoxide
DNA	Deoxyribonucleic Acid
ECHA	European Chemicals Agency
EEG	Electroencephalography
EINECS	European Inventory of Existing Commercial chemical Substances
ER	endoplasmic reticulum
ET-1	endothelin-1
FAF	Food Additives and Flavourings
FC	food category
FCS	Food Categorisation System
FDE	FoodDrinkEurope
FDRL	Food and Drug Research Laboratories
FVEP	flash visual evoked potential
GD	gestation day
GLP	good laboratory practices
GME	Gelatine Manufacturers of Europe
GSH-Px	glutathione peroxidase
GSH	glutathione
GSSG	oxidised glutathione
HBGV	health-based guidance value
HPRT	hypoxanthine-guanine phosphoribosyl transferase
IBO	interested business operator
ICAM-1	intercellular adhesion molecule 1
ICP-MS	inductively coupled plasma-mass spectrometry
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectroscopy
IgE	Immunoglobulin E
iNOS	inducible nitric oxide synthase
i.p.	Intraperitoneal
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD50	lethal dose, 50%
LOAEC	lowest-observed-adverse-effect concentration

LOAEL	Lowest Observed Adverse Effect Level
LOD	limit of detection
LOQ	limit of quantification
MB	medium-bound
MI	mitotic index
Mintel's GNPD	Mintel's Global New Products Database
MN	micronucleus
MOE	margin of exposure
mPFC	median prefrontal cortex
mPGES	microsomal prostaglandin E synthases
MPL	maximum permitted level
NCE	normochromatic erythrocytes
NBL	non-brand-loyal
NMDA	N-methyl-D-aspartate
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
OIV	International Organisation of vine and wine
OTM	olive tail moment
OVA	ovalbumin
P95	95th percentile
PCE	polychromatic erythrocytes
PKA	protein kinase A
PKC	protein kinase C
PSD	postsynaptic density
RAC	Committee for Risk Assessment
RMPI	Roswell Park Memorial Institute
ROS	reactive oxygen species
SCE	Sister Chromatid Exchange
SDIOC	sulfur dioxide-based chemicals REACH consortium
SEP	somatosensory evoked potential
SOD	superoxide dismutase
SOX	sulfite oxidase enzyme
SPSS	Statistical Package for Social Sciences
TBARS	thiobarbituric acid reactive substances
TD	thiamine-deficient
TEM	transmission electron microscopy
TG	test guideline
TWI	Tolerable Weekly Intake
UNESDA	Union of European Soft Drinks Associations
VEP	visual evoked potential
WG	Working Group
WHO	World Health Organization

Appendix A – Specifications for sulfur dioxide (E 220), sodium sulfite (E 221), sodium bisulfite (E 222), sodium metabisulfite (E 223), potassium metabisulfite (E 224), calcium sulfite (E 226), calcium bisulfite (E 227) and potassium bisulfite (E 228) E 220–228

Table A.1: Specifications for sulfur dioxide (E 220) according to Commission Regulation (EU) No 231/2012 and to JECFA (2006)

Purity	Commission Regulation (EU) No 231/2012	JECFA (2006)
Description	Colourless, non-flammable gas with strong pungent suffocating odour	Colourless, non-flammable gas, with strong, pungent, suffocating odour. Its vapour density is 2.26 times that of air at atmospheric pressure and 0°C. The specific gravity of the liquid is about 1.436 at 0°/4°. At 20°C, the solubility is about 10 g of SO_2 per 100 g of solution. It is normally supplied under pressure in containers in which it is present in both liquid and gaseous phases.
Assay	Content not less than 99%	Not less than 99.9% SO_2 by weight
Water content	Not more than 0.05%	Not more than 0.05%
Sulfur trioxide	Not more than 0.1%	–
Selenium	Not more than 10 mg/kg	Not more than 20 mg/kg
Other gases not normally present in the air	No trace	–
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 5 mg/kg	Not more than 5 mg/kg
Mercury	Not more than 1 mg/kg	–
Non-volatile residue	–	Not more than 0.05%

Table A.2: Specifications for sodium sulfite (E 221) according to Commission Regulation (EU) No 231/2012 and to JECFA (2006)

Purity	Commission Regulation (EU) No 231/2012	JECFA (2006)
Description	White crystalline powder or colourless crystals	White powder with not more than a faint odour of sulfur dioxide
Assay	Anhydrous: Not less than 95% of Na_2SO_3 and not less than 48% of SO_2 Heptahydrate: Not less than 48% of Na_2SO_3 and not less than 24% of SO_2	Not less than 95.0%
Thiosulfate	Not more than 0.1% based on the SO_2 content	Not more than 0.1%
Iron	Not more than 10 mg/kg based on the SO_2 content	Not more than 10 mg/kg
Selenium	Not more than 5 mg/kg based on the SO_2 content	Not more than 5 mg/kg
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–
pH	pH of a 10% solution (anhydrous) or a 20% solution (heptahydrate) between 8.5 and 11.5	8.5–10.0 (1 in 10 soln)

Table A.3: Specifications for sodium bisulfite (E 222) according to Commission Regulation (EU) No 231/2012 and to JECFA (2006)

Purity	Commission Regulation (EU) No 231/2012	JECFA (2006)
Description	A clear, colourless to yellow solution	White crystals or granular powder having an odour of sulfur dioxide
Assay	Content not less than 32% w/w $NaHSO_3$	Not less than 58.5% and not more than 67.4% of SO_2
Iron	Not more than 10 mg/kg of Na_2SO_3 based on the SO_2 content	A clear, colourless to yellow solution
Selenium	Not more than 5 mg/kg based on the SO_2 content	Not more than 5 mg/kg
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–
pH	pH of a 10% aqueous solution between 2.5 and 5.5	2.5–4.5 (1 in 10 soln)

Table A.4: Specifications for sodium metabisulfite (E 223) according to Commission Regulation (EU) No 231/2012 and to JECFA (2006)

Purity	Commission Regulation (EU) No 231/2012	JECFA (2006)
Description	White crystals or crystalline powder	White crystals or crystalline powder having an odour of sulfur dioxide
Assay	Content not less than 95% $Na_2S_2O_5$ and not less than 64% of SO_2	Not less than 90.0%
Thiosulfate	Not more than 0.1% based on the SO_2 content	Not more than 0.1%
Iron	Not more than 10 mg/kg based on the SO_2 content	Not more than 10 mg/kg
Selenium	Not more than 5 mg/kg based on the SO_2 content	Not more than 5 mg/kg
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–
pH	pH of a 10% aqueous solution between 4.0 and 5.5	4.0–4.5 (1 in 10 soln)

Table A.5: Specifications for potassium metabisulfite (E 224) according to Commission Regulation (EU) No 231/2012 and to JECFA (2006)

Purity	Commission Regulation (EU) No 231/2012	JECFA (2006)
Description	Colourless crystals or white crystalline powder	Colourless free-flowing crystals, crystalline powder or granules, usually having an odour of sulfur dioxide
Assay	Content not less than 90% of $K_2S_2O_5$ and not less than 51.8% of SO_2, the remainder being composed almost entirely of potassium sulfate	Not less than 90%
Thiosulfate	Not more than 0.1% based on the SO_2 content	Not more than 0.1%
Iron	Not more than 10 mg/kg based on the SO_2 content	Not more than 10 mg/kg
Selenium	Not more than 5 mg/kg based on the SO_2 content	Not more than 5 mg/kg
Arsenic	Not more than 3 mg/kg	–

Purity	Commission Regulation (EU) No 231/2012	JECFA (2006)
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–

Table A.6: Specifications for calcium sulfite (E 226) according to Commission Regulation (EU) No 231/2012

Purity	Commission Regulation (EU) No 231/2012
Description	White crystals or white crystalline powder
Assay	Content not less than 95% of $CaSO_3 \cdot 2H_2O$ and not less than 39% of SO_2
Iron	Not more than 10 mg/kg based on the SO_2 content
Selenium	Not more than 5 mg/kg based on the SO_2 content
Arsenic	Not more than 3 mg/kg
Lead	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg

Table A.7: Specifications for calcium bisulfite (E 227) according to Commission Regulation (EU) No 231/2012

Purity	Commission Regulation (EU) No 231/2012
Description	Clear greenish-yellow aqueous solution having a distinct odour of sulfur dioxide
Assay	6–8% (w/v) of sulfur dioxide and 2.5–3.5% (w/v) of calcium dioxide corresponding to 10–14% (w/v) of calcium bisulfite $[Ca(HSO_3)_2]$
Iron	Not more than 10 mg/kg based on the SO_2 content
Selenium	Not more than 5 mg/kg based on the SO_2 content
Arsenic	Not more than 3 mg/kg
Lead	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg

Table A.8: Specifications for potassium bisulfite (E 228) according to Commission Regulation (EU) No 231/2012

Purity	Commission Regulation (EU) No 231/2012
Description	Clear colourless aqueous solution
Assay	Content not less than 280 g $KHSO_3$ per litre (or 150 g SO_2 per litre)
Iron	Not more than 10 mg/kg based on the SO_2 content
Selenium	Not more than 5 mg/kg based on the SO_2 content
Arsenic	Not more than 3 mg/kg
Lead	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg

Appendix B – Summary of Toxicity studies considered sufficiently reliable with respect to their internal validity (with only minor or some limitations according to Annex B)

Guideline studies: i.e. use of EPA, OECD, FDA or other guideline for study design.

Overview of the study: duration, species, route of exposure,...

Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available: based on analytical data and measured feed (water) intake (μg, mg or g/kg bw per day, mean for a whole study period, when the test compound is given in the diet (water)). In gavage studies 'achieved doses' are based on analytical data for a concentration of a test compound. When the doses were not explicitly reported by the authors as mg/kg bw per day and was not possible to be calculated from the analytical data and measured feed (water) intake, default factors were applied (see Section 2.2 Methodologies) and equivalence to mg/kg bw per day was reported.

Measured endpoints: In case of guideline studies, state only if there were any deviation from the guideline (e.g. missing and/or additional endpoints).

Time of measurement/observation period: For reproductive and developmental toxicity studies, please indicate the life stage at which the measurement/observations were done i.e. pre-mating, mating, gestation, lactation, adult. For short-term, subchronic, chronic and carcinogenicity study, it should be indicated in which week of the study or if at the beginning or the end of the treatment measurements/observations were done. In case of guideline studies, state only if there were any deviation from the guideline.

Methods to measure the endpoints: State 'established methodology' or describe deviation or new methodology. In case of guideline studies, state only if there were any deviation from the guideline.

No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound: If and as reported by the study author.

The Panel identified a LOAEL or NOAEL from studies which were included in the assessment. However, it should be noted that any identification does not take into account additional issues with the data (e.g. plausibility, variability, effect size) which may result in any LOAEL or NOAEL not being used for further consideration.

In studies reporting effect of multiple substances, only the results from the sulfur dioxide and/or sulfites, and the control groups are presented.

General toxicity

Mice

Oral

No studies sufficiently reliable with respect to their internal validity were identified.

Inhalation

Reference	Meng and Liu, 2007. Cell morphological ultrastructural changes in various organs from mice exposed by inhalation to sulfur dioxide
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	
Animal model	
Species and strain	Mice
Disease models (e.g. diabetes, allergy, obesity)	No

Housing conditions	
Housing condition	Metallic cages
Diet name and source (if reported)	Not known
Treatment	
Test material	Sulfur dioxide
Provider	Beijing gas company
Compound purity	Not reported
Vehicle used	Air
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	Two dose levels: 28 and 56 mg/m^3 Calculated to correspond to an internal dose of 7.8 and 16 mg/kg bw per day
Route of administration (diet, drinking water, gavage)	Inhalation, whole body exposure
Period of exposure (pre-mating, mating, gestation, lactation, adult)	4 h/day
Duration of the exposure	7 days
Study design	
Sex and age at the start of the treatment	Male mice
Number animals/sex/group	6
Measured endpoints	Ultrastructural changes
Time of measurement/observation period	At sacrifice after 7 days
Methods to measure the endpoints	Morphology
Statistical analysis	
Statistical methods	None
Results	
Findings reported by the study author/s	Ultrastructural changes in all organs tested; lung, liver, spleen, brain, kidney, testis and heart following 7 days inhalation exposure
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted that SO_2 at both doses tested (7.8 and 16 mg/kg bw per day) induced ultrastructural changes of varying degree in lung, liver, spleen, testis heart brain and kidney. The Panel considered that no RP could be identified.
Further information	
Test substance purity was not reported, reducing confidence in the results of this study	

Results from this study demonstrated that inhaled sulfur dioxide at doses of 28 and 56 mg/m^3 (calculated to correspond to an internal dose of 7.8 and 16 mg/kg bw per day) for 7 days resulted in morphological changes in all organs investigated. The effects seen were generally more severe at the high dose. The lung was the most sensitive organ and effects were seen in Type 2 alveolar cells including vacuolation, changes in the structure of the nucleus and chromatin as well as changes in the mitochondrial compartment. In liver several ultra-structural changes were also observed including necrosis, swelling of the nucleus and dilatation of the endoplasmic reticulum. Ultrastructural changes of varying degree were also observed in spleen, testis heart brain and kidney.

The Panel noted that high concentrations of inhaled sulfur dioxide using whole body exposure in mice caused a number of ultrastructural changes in several organs after 7 days. The Panel noted that the downstream adversity of the observed effects cannot be predicted with any certainty.

Reference	**Meng et al., 2005. Levels of sulfite in three organs from mice exposed to sulfur [corrected] dioxide**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	7 days repeat dose by inhalation

Animal model	
Species and strain	Mouse (Kunming albino)
Disease models (e.g. diabetes, allergy, obesity)	n/a
Housing conditions	
Housing condition	Housed in groups of three mice in metal cages under 'standard conditions'.
Diet name and source (if reported)	Not reported
Treatment	
Test material	Sulfur dioxide
Provider	Beijing He-Pu-Bei-Fen Gas Company
Compound purity	99.99%
Vehicle used	Air
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	SO_2-exposed groups in 1-m^3 exposure chambers for 4 h/day for 7 days with either 14.00 ± 1.25, 28.00 ± 1.98 and 56.00 ± 3.11 mg/m^3 Calculated to correspond to an internal dose of 3.9 ± 0.35, 7.8 ± 0.55 and 16 ± 0.87 mg/kg bw per day The control group was exposed to filtered air in the other 1-m^3 chamber for the same period of time.
Route of administration (diet, drinking water, gavage)	Inhalation
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult (18–22 g bw)
Duration of the exposure	7 days
Study design	
Sex and age at the start of the treatment	Male, age not stated
Number animals/sex/group	Not explicitly stated. Likely 3/group ('They [the mice] were housed in groups of three mice in metal cages under standard conditions')
Measured endpoints	Contents of sulfite in different organs from mice exposed to SO_2
Time of measurement/observation period	7 days (though not explicitly stated), 18 h after last dose.
Methods to measure the endpoints	High performance liquid chromatography with fluorescence detection
Statistical analysis	
Statistical methods	All values were expressed as mean ± standard deviation, and the data were analysed using one-way analysis of variance (ANOVA) for significant differences between the sulfur dioxide-exposed groups and the control groups.
Results	
Findings reported by the study author/s	Statistically significant increases in brain, heart and lung homogenate sulfite levels at all sulfur dioxide dose levels.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	Not applicable, No toxicity endpoints examined, only toxicokinetics.
Further information	
No	

Meng et al. (2005) administered sulfur dioxide by repeated daily inhalation (4 h/day) for 7 days at 14.00 ± 1.25 mg/m^3, 28.00 ± 1.98 mg/m^3 or 56.00 ± 3.11 mg/m^3 to male (18–22 g bw) kunming albino mice (3/group, though not explicitly stated). Control mice were exposed to filtered air in for the same period of time. Eighteen hours after the last exposure, mice were killed and tissue homogenates prepared from brain heart and lung. Using an HPLC method with fluorescence detection, the authors reported, with increasing dose, statistically-significant increases in sulfite levels of brain (1.6-, 1.7- and 2.0-fold compared to control), heart (1.6-, 2.5- and 2.7-fold compared to control) and lung (1.8-,

1.9- and 2.7-fold compared to control) homogenate. The Panel noted that no toxicological endpoints were examined in this study.

Reference	**Qin et al., 2015. Sulfur dioxide and benzo(a)pyrene trigger apoptotic and anti-apoptotic signals at different post-exposure times in mouse liver**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat dose (4-week exposure, 13-week recovery) mice inhalation
Animal model	
Species and strain	C57BL16 mice
Disease models (e.g. diabetes, allergy, obesity)	No
Housing conditions	
Housing condition	Stainless steel cages under standard conditions (24 ± 2°C and50 ± 5% humidity) with a 12-h light–dark cycle
Diet name and source (if reported)	Ad libitum
Treatment	
Test material	Sulfur dioxide
Provider	Not stated
Compound purity	Not stated
Vehicle used	Not stated
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	Mice in the SO_2 and SO_2+ BaP groups were exposed to 7 mg/m^3 SO_2 in 1 m^3 exposure chambers for 4 weeks (6 h/day) (calculated to correspond to an internal dose of 2.9 mg/kg bw per day), while those in the control and BaP groups were exposed to filtered air in another 1 m^3 chamber. On the first 5 days of SO_2 exposure, the mice in the BaP and SO_2+ BaP groups were intraperitoneally injected with 40 mg/kg bw of BaP, which was dissolved in olive oil, while the mice in the control and SO_2 groups were injected with olive oil only.
Route of administration (diet, drinking water, gavage)	Inhalation
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	4 weeks: 20 mice from each group were sacrificed at 4 weeks (identified by the author as the first day of post-exposure (p.e. 1 d)). The remaining mice were sacrificed at 13 weeks post-exposure
Study design	
Sex and age at the start of the treatment	Age not stated but body weight 180–200 g
Number animals/sex/group	40 males group; groups: control, SO_2, BaP and SO_2+ BaP
Measured endpoints	Liver morphological changes Liver markers of mitochondrial dysfunction (MMP, CO1&4, ATP6) Liver expression of mRNA apoptosis-related genes (bcl-2, bax and p53) Liver expression of apoptosis-related gene proteins (caspase-3, caspase-9, protein expression of bcl-2, bax and the bcl-2/bax ratio, protein levels of p53 and p53 phosphorylation)
Time of measurement/observation period	At sacrifice

Methods to measure the endpoints	Liver tissue was prepared and stained with haematoxylin and eosin. Real time PCR for RNA apoptosis-related genes and genes of mitochondrial dysfunction Western blot for apoptosis-related proteins
Statistical analysis	
Statistical methods	The data were analysed using one-way ANOVA ($p < 0.05$)
Results	
Findings reported by the study author/s	• Liver morphological changes Cell morphology was altered in SO_2 and BaP groups. The cells detached from the subsurface, and cell-to-cell attachments were lost; the injury in the BaP group was worse than the SO_2 group. Co-exposure of SO_2 and BaP changed the cell morphology more significantly than each single exposure. • Liver markers of mitochondrial dysfunction MMP depression was significant at 1d post-exposure of SO_2 and BaP compared to the control. The co-exposure of SO_2 and BaP led to significant decreases of CO1, CO4 and ATP6 mRNA expression at 1 d post-exposure. No detectable effects on mRNA levels of the three mitochondrial respiratory complex subunits were observed after 13-week post-exposure. No changes in these complex subunits in SO_2 or BaP groups. • Liver expression of mRNA apoptosis-related genes (bcl-2, bax and p53) Co-exposure of SO_2 and BaP significantly decreased the mRNA levels of bcl-2 and the bcl-2/bax ratio at 1-d post-exposure and increased the mRNA levels of p53 compared with the control groups. Significant increases of bcl-2mRNA and the bcl-2/bax ratio were observed after 13-week post-exposure in mouse livers in the SO_2 or/and BaP group; decreases of the bax andp53 mRNA levels, except for bax mRNA expression in SO_2 group were also observed • Liver expression of apoptosis-related gene proteins After exposure for 4 weeks, BaP treatment alone was able to activate caspase-3, and significant synergies were observed in the cleaved caspase-3 protein levels after SO_2 + BaP 1-d post-exposure. Cleaved caspase-9 was observed after SO_2 inhalation alone and co-exposure of SO_2 and BaP after both 1-d and 13-w post-exposure. Bcl-2 expression and the bcl-2/bax ratio were reduced after SO_2 and/or BaP 1-d post-exposure, while they were elevated after 13-w post-exposure. Changes in protein expression were more evident after SO_2 and BaP co-exposure than SO_2 or BaP exposure alone. Bax protein expression was increased after SO_2 or SO_2 + BaP 1-d post-exposure but decreased after 13-w post-exposure. BaP treatment significantly increased the protein expression of p53 and phosphorylation of Ser15 after 1-d post-exposure. The accumulation and phosphorylation of p53 were enhanced in SO_2 and the BaP co-exposure group compared with the SO_2 or BaP-alone group after 1-d post-exposure. Significant depression of p53 protein expression and phosphorylation were detected in the SO_2 and BaP co-exposure group after 13-w post-exposure.

No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted that a single dose (calculated to correspond to an internal dose of 2.9 mg SO_2/kg bw per day) was tested and SO_2 exposure induced only minor liver effects of questionable toxicological relevance. The Panel considered that no RP could be identified.
Further information	
Test substance purity and provider were not reported, reducing confidence in the results of this study	

Qin et al. (2015) investigated the effects of sulfur dioxide and benzo(a)pyrene (BaP) on the induction of apoptosis-related genes in mouse liver. Mice were exposed to sulfur dioxide via inhalation for 4 weeks. Animals were sacrificed after 4 exposure and after 13-week post-exposure and mRNA expression of enzymes involved in mitochondrial function and apoptosis in the liver was investigated. Exposure of sulfur dioxide alone via inhalation induced only minor liver effects of questionable toxicological relevance.

Rats

Oral

Reference	**Dalefield and Mueller, 2016. Gastric mucosal irritation following oral exposure to sodium metabisulfite: A reproducible effect?**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat dose (7 days) rat oral (diet)
Animal model	
Species and strain	Male Sprague–Dawley rats
Disease models (e.g. diabetes, allergy, obesity)	No
Housing conditions	
Housing condition	Not described in detail
Diet name and source (if reported)	Not reported
Treatment	
Test material	Sodium metabisulfite ($Na_2S_2O_5$)
Provider	Sigma-Aldrich New Zealand
Compound purity	Purity 98%
Vehicle used	Feed
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	0.25%, 0.5%, 1% or 4% (w/w) (equal to 90, 198, 390 and 1,478 mg SO_2 equivalents/kg bw per day)
Route of administration (diet, drinking water, gavage)	Diet
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	7 days
Study design	
Sex and age at the start of the treatment	Male, 10 weeks
Number animals/sex/group	4 male/group
Measured endpoints	Haematocrit (Hct), haemoglobin (Hb), red blood count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC), platelet count, total protein, serum albumin, serum globulin, albumin:globulin ratio, white blood cell morphology, white blood cell differential count, erythrocyte morphology; in the highest dose group:

	histopathology of oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon Body weight gain, feed and water consumption
Time of measurement/observation period	Day 8
Methods to measure the endpoints	Not detailed
Statistical analysis	
Statistical methods	Not done because of the low number of animals
Results	
Findings reported by the study author/s	Water intake unaffected, small decrease of feed intake (7%) in the highest dose group; bodyweight gain for the 4% SM (equal to 1,478 mg SO_2 -equivalents/kg bw per day) group was profoundly depressed to only 19%of that observed in the week prior to treatment. Slightly lower group mean values for RBC, Hb, Hct, total WBC and lymphocyte count were observed in the 4% SM (equal to1,478 mg SO_2 equivalents/kg bw per day) group relative to the control group (Table 3) although the decreased mean WBC and lymphocyte counts were attributable to markedly decreased lymphocyte count in one rat. no microscopic evidence of treatment-related mucosal damage, inflammation or other toxicity of any part of the gastro-intestinal tract in any of the 4% SM group rats
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted that there was no observed effect on body weight gain at 1% (equal to 390 mg SO_2 equivalents/kg bw per day)
Further information	
No	

The aim of the study of Dalefield and Mueller (2016) was to evaluate whether stomach lesions were the most sensitive toxicological endpoint following sodium metabisulfite exposure. In a 7-day dietary study rats (11 weeks, 4/group) were exposed to 0%, 0.25%, 0.5%, 1% or 4% (w/w) sodium metabisulfite (purity 98%) whereby the feed was prepared freshly every day. Clinical signs, feed and water intake, bodyweight gain, haematology, serum protein chemistry, necropsy findings and gastrointestinal histopathology were recorded. Mean bodyweight gain was markedly decreased (up to 81%) in the highest dose group, 4% sodium metabisulfite equal to 1,478 mg SO_2 equivalents/kg bw per day, whereas feed consumption was lowered only about 7%, not explaining the decreased body weight gain. In the highest dose group, lower mean values for RBC, Hb, Hct, total WBC and lymphocyte count were observed which were clinically not meaningful and without compensatory haematopoiesis. There were no treatment-related clinical signs or gastrointestinal lesions. In the absence of individual animal data and daily data for food intake and body weight changes and in view of the low number of animals used per group, the panel considered that there is a high degree of uncertainty regarding the dose level having no effect on body weight gain.

Reference	**Ercan et al., 2015. Induction of xanthine oxidase activity, endoplasmic reticulum stress and caspase activation by sodium metabisulfite in rat liver and their attenuation by Ghrelin**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat dose (35 days) rat oral (gavage)
Animal model	
Species and strain	Wistar rat
Disease models (e.g. diabetes, allergy, obesity)	No

Housing conditions	
Housing condition	Stainless steel cages, 12-h light–dark cycles and a constant temperature of 23 ± 1°C
Diet name and source (if reported)	Ad libitum
Treatment	
Test material	Sodium metabisulfite ($Na_2S_2O_5$)
Provider	Not stated
Compound purity	Not stated
Vehicle used	Not stated
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	$Na_2S_2O_5$ group: freshly prepared 100 mg/kg bw per day (corresponding to 67.4 mg SO_2 equivalents/kg bw per day), via gavage Ghrelin group: ghrelin given intraperitoneally at a dose of 20 μg/kg for 35 days Control group: 1 ml/kg bw per day distilled water via gavage and 1 ml/kg bw per day saline via intraperitoneal injection as vehicle for 35 days.
Route of administration (diet, drinking water, gavage)	Gavage
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	35 days
Study design	
Sex and age at the start of the treatment	Male, age not stated but body weight 350–450 g
Number animals/sex/group	8 or 10 male/4 groups: control(n = 8); rats treated with sodium metabisulfite ($Na_2S_2O_5$) (n = 10); rats treated with ghrelin (n = 10); rats treated with $Na_2S_2O_5$ + ghrelin (n = 10)
Measured endpoints	Measurement in liver, heart and kidney tissues of • Xanthine oxidase/xanthine dehydrogenase (XO/XDH) enzyme activity • Caspase-3, -8 and -9 activities • Nuclear factor Kappa-B protein levels • Endoplasmic reticulum (ER) stress markers (glucose-regulated protein 78 (GRP78) and C/EBP-homologous protein (CHOP))
Time of measurement/observation period	At sacrifice
Methods to measure the endpoints	XO/XDH: fluorimetric assay Endoplasmic reticulum stress markers: Western blot Caspase activity: colorimetric assay kits NF-κB protein: ELISA kit
Statistical analysis	
Statistical methods	Statistical analysis was performed by one-way analysis of variance and all pairwise multiple comparisons were via Tukey test ($p < 0.05$).
Results	
Findings reported by the study author/s	• XO/XDH enzyme activity XO activity significantly increased and XDH/XO ratio significantly decreased in the liver of $Na_2S_2O_5$ treated rats compared to control, ghrelin and $Na_2S_2O_5$ + ghrelin-treated groups. Significantly decreased liver XO levels and increased XDH/XO ratio in ghrelin group when compared to $Na_2S_2O_5$ treated rats. No significant difference was observed in XO and XDH activities in the heart and kidney of all experimental groups.

	• Induction of ER stress markers $Na_2S_2O_5$ treatment caused a significant increase in ER stress in the liver compared to control, ghrelin and $Na_2S_2O_5$ + ghrelin treated groups. Treatment with ghrelin significantly decreased the expression of the analysed ER stress markers in the liver when compared to $Na_2S_2O_5$ treated rats. No significant difference was observed in the heart and kidney of all experimental groups. • Caspase activity A significant increase in caspase-3, -8 and -9 activities were observed in the liver of $Na_2S_2O_5$-treated rats compared to control(C), ghrelin (G) and $Na_2S_2O_5$ + ghrelin-treated groups. Treatment with ghrelin significantly decreased liver caspase-3, -8 and -9 activities when compared to $Na_2S_2O_5$-treated rats. No significant difference was observed in the heart and kidney of all experimental groups. • Nuclear factor Kappa-B protein levels Increased phosphorylated NF-κBp65 in $Na_2S_2O_5$-treated groups. Protein levels of NF-κB measured in the liver, heart and kidney showed no significant difference among the experimental groups.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted that a single repeated oral dose of sodium metabisulfite (67.4 mg SO_2 equivalents/kg bw per day) administered as a bolus resulted in activation of various enzymes involved in oxidative and ER stress and apoptosis in the liver. The panel considered that no RP could be identified.
Further information	
No	

Ercan et al. (2015) studied the effect of sodium metabisulfite on xanthine oxidase activity, endoplasmic reticulum stress and caspase activation in rat liver, kidney and heart and the attenuation of any effects by the peptide hormone Ghrelin. Sodium metabisulfite was administered by gavage at a concentration of 100 mg/kg bw per day for 5 weeks. The results demonstrated that sodium metabisulfite treatment activated Xanthine oxidase, triggered endoplasmic reticulum stress and induced caspase activation in liver but not in kidney or heart.

The panel noted that sodium metabisulfite treatment for 5 weeks at 100 mg/kg bw per day (corresponding to 67.4 mg SO_2 equivalents/kg bw per day) given by bolus administration resulted in activation of various enzymes involved in oxidative and ER stress and apoptosis in the liver. The toxicological significance of these effects is however unknown. The panel considered that no RP could be identified.

Reference	**Mahmoud et al., 2015. Demonstrating adverse effects of a common food additive (sodium sulfite) on biochemical, cytological and histopathological parameters in tissues of albino Wister rats.**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat dose (12 weeks) rat oral (drinking water)

Animal model	
Species and strain	Rat, albino Wistar
Disease models (e.g. diabetes, allergy, obesity)	No
Housing conditions	
Housing condition	The animals were housed in cages and subjected to 1 week acclimatisation before starting of experiment at atmosphere of 12 h dark/light cycle, at 25 ± 2°C.
Diet name and source (if reported)	Standard laboratory chow.
Treatment	
Test material	Sodium sulfite (Na_2SO_3) (crystal, industrial grade).
Provider	Local market in Fayoum City (Egypt).
Compound purity	96% minimum purity
Vehicle used	Drinking water
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	Na_2SO_3 dissolved in drinking water at concentrations of 200, 500 and 1,000 ppm. Corresponding to 9.4, 23.6 and 94.5 mg SO_2 equivalents/kg bw per day
Route of administration (diet, drinking water, gavage)	Drinking water
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult (110 ± 3 g when obtain prior to 1 week acclimatisation)
Duration of the exposure	12 weeks
Study design	
Sex and age at the start of the treatment	Female
Number animals/sex/group	5
Measured endpoints	Body weight Body weight gain Relative liver weight Relative kidney weight Haemoglobin Haematocrit Red blood cells White blood cells mean corpuscular volume mean corpuscular haemoglobin mean corpuscular haemoglobin concentration. Platelets count Serum total protein Serum Albumin Serum AST(GOT) Serum ALT(GPT) Serum ALP (U/L) Serum Creatinine Serum Urea Serum Cholesterol Blood glucose Chromosomal aberrations assay (see Section 4.5.2.5) Histopathological examination of liver (H&E) Histopathalogical examination of kidney (H&E)
Time of measurement/observation period	At 12 weeks
Methods to measure the endpoints	Weighing animals Dissection and weighing organs Haematology Clinical chemistry Histology

Statistical analysis	
Statistical methods	Results expressed as mean ± SD, and the values of $p < 0.05$ were considered statistically significant. Data were statistically analysed by one-way analysis of variance (one-way ANOVA) and post-comparison was carried out with least significant difference test using SPSS (Statistical Package for Social Sciences) version 17.00.
Results	
Findings reported by the study author/s	Statistically significant decrease in percentage body weight gain No change in relative liver weight No change in relative kidney weight Statistically significant decrease in haemoglobin Statistically significant decrease in haematocrit No change in red blood cells Statistically significant decrease mean corpuscular volume Statistically significant decrease in mean corpuscular haemoglobin Statistically significant decrease in mean corpuscular haemoglobin concentration. Statistically significant decrease in platelets count Statistically significant decrease in white blood cells Statistically significant decrease in serum total protein Statistically significant decrease in serum albumin Statistically significant increase in serum AST(GOT) Statistically significant increase in serum ALT(GPT) Statistically significant increase in serum ALP (U/L) Statistically significant increase in serum creatinine Statistically significant increase in serum urea Statistically significant increase in serum cholesterol (but variable) Statistically significant decrease in blood glucose Histopathological changes in liver Histopathological changes in liver
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel identified an LOAEL from this study of 18.6 Na_2SO_3/kg bw per day (corresponding to 9.4 SO_2 equivalents/kg bw per day) based on dose-dependent changes (63% reduction in white blood cell count; 50% reduction in platelet count; 22% reduction in serum albumin; 52% increase in creatinine and a 31% drop in blood glucose levels).
Further information	
The Panel noted that such effects have not been reported in other repeat dose studies (see studies above) and considered that the effects reported were more likely due to contaminant(s) in the substance used.	

Mahmoud et al. (2015) investigated the effects of daily oral drinking water administration of sodium sulfite on female albino rats (five animals per group) at doses of 200, 500 and 1,000 ppm (equivalent to 18.6, 46.5, 186 mg/kg bw day [corresponding to 9.4, 23.6 and 94.5 mg SO_2 equivalents/kg bw per day]) for 12 weeks. The panel noted that the sodium sulfite was obtained from a local market. The authors reported that it had a minimum purity of 96%. Effects on body and organ weight changes, haematological, clinical chemistry endpoints and histopathological endpoints were examined.

Sodium sulfite exposure caused a statistically significant decrease in percentage body weight gain but no change in relative liver or kidney weights. There were statistically significant decreases in blood glucose, haemoglobin haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, platelets count, white blood cells, serum total protein and serum albumin but no change in red blood counts. There were also statistically significant increases in serum AST, ALT, ALP, creatinine, urea and cholesterol. Histopathological changes in liver (vacuolation, large sinusoidal dilatation, degenerative changes and cellular congestion) were reported in rats in the 46.5 and 186 mg/kg bw per day treatment groups. Histopathological changes were also reported in the kidney.

The panel noted dose-dependent changes were seen that were statistically and toxicologically significant at the lowest dose level of 18.6 mg/kg bw per day (corresponding to 9.4 SO_2 equivalents/kg bw per day) when compared to the control group (63% reduction in white blood cell count; 50% reduction in platelet count; 22% reduction in serum albumin; 52% increase in creatinine and a 31% drop in blood glucose levels). The Panel noted that although the substance used conformed to the specifications in terms of purity, the substance was not sourced from an established supplier and was not further characterised. Given that the observed effects have not been reported in other oral repeat dose studies even at higher doses (see studies above), the panel considered that it is uncertain whether the effects reported are due to sodium sulfite.

Inhalation

Reference	**Zhang et al., 2018. The effect of exposure of SO_2 in high concentrations on CD19(+) cells in reactive airway dysfunction syndrome in rat**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat dose (7 days) rat inhalation
Animal model	
Species and strain	Rats Sprague–Dawley
Disease models (e.g. diabetes, allergy, obesity)	No
Housing conditions	
Housing condition	When not being treated, all of the rats had free access to food and water ad libitum. No further details.
Diet name and source (if reported)	Not reported.
Treatment	
Test material	Sulfur dioxide
Provider	Beijing Ya-nan Gas Scientific and Technology Corporation Ltd.
Compound purity	99.9%
Vehicle used	Air
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	The rats of the SO_2 exposure group were placed in the exposure chamber described above and exposed to 600 ppm SO_2 for 2 h/day for 7 days consecutively. The rats of the control group were exposed to filtered air in another identical chamber for the same period of time. Calculated to correspond to an internal dose of 141 mg/kg bw per day
Route of administration (diet, drinking water, gavage)	Inhalation
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult (body weight of 180–200 g)
Duration of the exposure	2 h/day for 7 days consecutively
Study design	
Sex and age at the start of the treatment	Male, age not indicated.
Number animals/sex/group	10
Measured endpoints	Lung CD19 mRNA Lung CD19 protein Lung CD19+ cells Lung CD19+/CD23+ Serum IgG, IgA, and IgE Bronchoalveolar lavage fluid (BALF) IgG, IgA, and IgE. Lung histology (H&E) Lung Immunohistochemistry (CD3 and CD19)

Time of measurement/observation period	At day 7.
Methods to measure the endpoints	Microarray QRT-PCR Western blotting Flow cytometry. Enzyme-linked immunosorbent assay (ELISA). Histology Immunohistochemistry
Statistical analysis	
Statistical methods	All values were expressed as mean ± standard deviation (SD). Significance testing was performed using the unpaired student's t test. $P < 0.05$ was considered statistically significant.
Results	
Findings reported by the study author/s	The result of microarray analysis indicated that CD19 expression in the lungs of SO_2 exposed group was lower than in the control group (11.172 and 12.218, respectively). SO_2 reduced the expression of CD19 mRNA (relative to beta actin) in lung as confirmed by qRT-PCR. SO_2 reduced the expression of CD19 protein (relative to beta actin) in lung as determined by Western blotting. SO_2 exposure downregulated CD19+ and CD19+ CD23+ cells in lung tissues. Serum IgG, IgA and IgE were unchanged by exposure to SO_2. Bronchoalveolar lavage fluid (BALF) IgG, IgA and IgE were unchanged by exposure to SO_2. SO_2 exposure induced lung injury (bronchitis, local alveolar haemorrhage, lymphocytes infiltration) as determined by histopathological examination (H&E stained histological sections). Lung CD19+ cells were decreased and CD3+ cells were increased in the lungs of SO_2 exposed group.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted that a single dose (141 mg/kg bw per day) was tested and SO_2 exposure resulted in histopathological changes (bronchitis, local alveolar haemorrhage, lymphocytes infiltration) in the lung. However, the Panel considered that no RP could be identified.
Further information	
The Panel noted that the dose was very high	

Zhang et al. (2018) exposed male rats (10 per group) to sulfur dioxide by inhalation at a dose calculated to correspond to an internal dose of 141 mg/kg bw per day for 7 days (whole body exposure) and examined the expression of CD19, at the level of mRNA and protein by RT-PCR and Western blotting, respectively, in the lung at termination. The percentages of CD19+ and CD19+ CD23+ cells in the lung were also examined by flow cytometry. IgG, IgA and IgE levels were determined in serum and bronchoalveolar lavage fluid (BALF) by ELISA. Sulfur dioxide exposure resulted in statistically significantly lower percentages of CD19+ and CD19+ CD23+ cells and histopathological changes (bronchitis, local alveolar haemorrhage, lymphocytes infiltration) in the lung, in the absence of significant changes in serum or BALF levels of IgG, IgA and IgE. The albumin was not measured in BALF. Hence, the results cannot be taken as being robust. In addition, with respect to the histopathological changes, the results in BALF are also considered not plausible.

Reference	Zhang et al., 2015. Effect of sulfur dioxide inhalation on the expression of K-ATP and L-Ca^{2+} channels in rat hearts
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat dose (30 days) rat inhalation
Animal model	
Species and strain	Male Wistar rats weighing 220–250 g
Disease models (e.g. diabetes, allergy, obesity)	No
Housing conditions	
Housing condition	50% ± 5% humidity, 24 °C ± 2°C, 12 h light–dark cycle.
Diet name and source (if reported)	Not reported
Treatment	
Test material	Sulfur dioxide
Provider	Beijing He-Pu-Bei-Fen Gas (Beijing, China).
Compound purity	purity: 99.99%
Vehicle used	Air
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	3.5 ± 0.35, 7.0 ± 1.08 and 14.1 ± 2.04 mg/m^3 SO_2 Calculated to correspond to an internal dose of 0.63 ± 0.063, 1.3 ± 0.19 and 2.5 ± 0.37 mg/kg bw per day, respectively
Route of administration (diet, drinking water, gavage)	Inhalation
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	4 h/day, inhalation chamber, 30 days
Study design	
Sex and age at the start of the treatment	Male, 220–250 g
Number animals/sex/group	6 male/group
Measured endpoints	mRNA and protein expression of K ATP channel subunits mRNA and protein expression of L-Ca 2 + channel subunit, histology of heart, HE, light microscopy
Time of measurement/observation period	Day 31
Methods to measure the endpoints	QRT-PCR, Western blotting
Statistical analysis	
Statistical methods	One-way ANOVA for significant differences between the SO_2 groups and the control group, followed by a least significant difference post hoc test
Results	
Findings reported by the study author/s	The 3.5 and 7 mg/m^3 SO_2 groups showed normal histological features as did the control group. Myocardial myofibril disorder and myocardial gap expansion were observed in the 14 mg/m^3 SO_2 group. mRNA and protein expression of Kir6.2 and SUR2A increased and mRNA and protein expression of Cav 1.2 (A and B) and Cav 1.3 decreased in the highest dose group indicating activation of KATP channels and inhibition of L-Ca^{2+} channels
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel identified an NOAEL 1.3 mg/kg bw per day based on the myocardial myofibril disorder and myocardial gap expansion observed at the highest dose tested
Further information	
No	

The study of Zhang et al. (2015) investigated the effect of sulfur dioxide inhalation on the expression of the ATP-sensitive K+ (KATP) channel and the L-type calcium (L-Ca^{2+}) channel in rat hearts. Male Wistar rats (6/group), weighing 220–250 g, were exposed to doses of SO_2 calculated to correspond to an internal dose of 0.63 ± 0.063, 1.3 ± 0.19 and 2.5 ± 0.37 mg/kg bw per day by 4 h chamber inhalation per day over 30 days. Endpoints tested were histology of heart, after HE staining, examined by light microscopy and mRNA and protein expression of K ATP channel subunits mRNA as well as protein expression of L-Ca 2 + channel subunit. The 3.5 and 7 mg/m^3 SO_2 groups showed normal histological features compared to the control group. Myocardial myofibril disorder and myocardial gap expansion were observed in the 14 mg/m^3 SO_2 group. mRNA and protein expression of Kir6.2 and SUR2A increased and mRNA and protein expression of Cav 1.2 (A and B) and Cav 1.3 decreased in the highest dose group indicating activation of KATP channels and inhibition of L-Ca^{2+} channels. However, the histopathological effects were not quantified, and the histopathological observations were not performed under blind conditions.

Reference	**Qin et al., 2017. Sulfur dioxide inhibits expression of mitochondrial oxidative phosphorylation genes encoded by both nuclear DNA and mitochondrial DNA in rat lungs**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat dose (30 days) rat inhalation
Animal model	
Species and strain	Rat, Wistar
Disease models (e.g. diabetes, allergy, obesity)	No
Housing conditions	
Housing condition	Housed in stainless steel cages under standard conditions (24 ± 2°C and 50 ± 5% humidity) with a 12-h light–dark cycle.
Diet name and source (if reported)	Not stated
Treatment	
Test material	Sulfur dioxide, no further details
Provider	Not reported.
Compound purity	Not stated.
Vehicle used	Filtered air, though not explicitly stated.
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	Rats in three SO_2 groups were exposed to 3.5 ± 0.39, 7.1 ± 1.13 and 14.3 ± 2.07 mg/m^3 SO_2 (calculated to correspond to an internal dose of 0.63 ± 0.07, 1.3 ± 0.2 and 2.6 ± 0.37 mg/kg bw per day) in 1-m^3 exposure chambers, while those in the control group were exposed to filtered air in another 1-m^3 chamber.
Route of administration (diet, drinking water, gavage)	Inhalation
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	30 days (4 h/day)
Study design	
Sex and age at the start of the treatment	Male, age not stated but 180–200 g bw at start.
Number animals/sex/group	6
Measured endpoints	Lung inner mitochondrial membrane potential Lung cytochrome c oxidase activity. Lung mitochondrial DNA content. Lung complex IV mRNA. Lung complex V mRNA. Lung PGC-1α mRNA.

	Lung NRF1 mRNA. Lung TFAM mRNA. Lung PGC-1α protein. Lung NRF1 protein. Lung TFAM protein.
Time of measurement/observation period	At day 30
Methods to measure the endpoints	JC-1 fluorophore accumulation in mitochondrial preparations. QRT-PCR QPCR Western blotting
Statistical analysis	
Statistical methods	The results were expressed as the mean ± SE. The data were analysed by using one-way ANOVA for significant differences between the SO_2 groups and the control group. A level of $P < 0.05$ was accepted as statistically significant.
Results	
Findings reported by the study author/s	SO_2 inhalation statistically significantly depressed the lung inner mitochondrial membrane potential. SO_2 inhalation decreased statistically significantly depressed the lung cytochrome c oxidase activity. SO_2 inhalation statistically significantly reduced lung mtDNA contents (using Cyt b and CO_2 as proxies). SO_2 inhalation statistically significantly depressed lung mRNA expression of respiratory complex IV and V subunits SO_2 inhalation statistically significantly depressed the lung mRNA of PGC-1α, NRF1 and TFAM. SO_2 inhalation statistically significantly depressed the lung protein expression of PGC-1α, NRF1 and TFAM
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted that the reported effects were observed at the lowest dose level (0.63 mg/kg bw per day).
Further information	
No	

Qin et al. (2017) aimed to determine the effects of sulfur dioxide inhalation on mitochondria from homogenised whole lung. Male Wistar rats (six rats per group) were exposed to 3.5 ± 0.39, 7.1 ± 1.13 and 14.3 ± 2.07 mg/m^3 SO_2 (calculated to correspond to an internal dose of 0.63 ± 0.07, 1.3 ± 0.2 and 2.6 ± 0.37 mg/kg bw per day) for 30 days. Sulfur dioxide exposure resulted in statistically significantly depressed inner mitochondrial membrane potential, cytochrome c oxidase activity, mtDNA contents (using Cyt b and CO2 as proxies), mRNA expression of respiratory complex IV and V subunits and both mRNA transcript and protein levels of PGC-1α, NRF1 and TFAM.

The Panel considered this study is limited to identifying potential modes of action for sulfur dioxide.

Reproductive and developmental toxicity

Mice

Oral

No studies sufficiently reliable with respect to their internal validity were identified.

Inhalation

Reference	Murray et al., 1979. Embryotoxicity of inhaled sulfur dioxide and carbon monoxide in mice and rabbits
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	Comparable to older OECD guideline, this study was performed before the guideline was drafted
Overview of the study	Mated female mice were exposed to SO_2, 7 h/day from GD 6–15, C-section on GD 18
Animal model	
Species and strain	CF-1 mice Charles River, Portage, Michigan
Disease models (e.g. diabetes, allergy, obesity)	No
Housing conditions	
Housing condition	Wire mesh cages, room temperature 21 C, humidity 45%, light cycle (12 h light and dark)
Diet name and source (if reported)	Commercial chow, source not described
Treatment	
Test material	Sulfur dioxide
Provider	The Matheson Company, Joliet, Illinois
Compound purity	99.98%
Vehicle used	Air
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	0, 25 ppm SO_2, 7 h/day (calculated to correspond to an internal dose of 32 mg/kg bw per day)
Route of administration (diet, drinking water, gavage)	Inhalation (whole body)
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Gestation GD 6–15
Duration of the exposure	10 days
Study design	
Sex and age at the start of the treatment	Female
Number animals/sex/group	Mated/pregnant at C-section: 40/26 control and 32/21 SO_2 groups Mated/pregnant at C-section: 20/17 control and 20/17 SO_2 groups
Measured endpoints	Clinical signs, body weight, food and water consumption. At necropsy liver weight, nasal turbinates, trachea and lungs fixed of five mice and six to seven rabbits for microscopical examination. Number of live and dead fetuses, fetal weight and fetal length, sex, external alterations, one-third of the fetuses of each litter was examined immediately for soft tissue alterations and all fetuses for skeletal alterations. Pregnancy rate, staining of uterus with sodium sulfide of apparently non-pregnant females for evidence of early resorptions.
Time of measurement/observation period	Clinical signs daily from GD 6, body weight, food and water consumption at regular intervals. Liver weight, microscopical examination nasal turbinates, trachea and lungs and reproductive data at C-section: GD 18
Methods to measure the endpoints	Light microscopy

Statistical analysis	
Statistical methods	Fetal alterations: Wilcoxon test as modified by Haseman and Hoel (litter as experimental unit) Continuous data: one-way analysis pf variance and Dunnett's test
Results	
Findings reported by the study author/s	Food consumption was decreased during the first few days of exposure (data not shown). Statistically significant decrease in fetal weight (−5%). No effect on the number of visceral or skeletal malformations; in the SO_2 group delayed ossification of the sternebrae and occipital bone (data not shown)
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	Decreased fetal weight and delayed ossification were observed at 25 ppm (equivalent to 32 mg/kg bw per day), the only dose tested based on.
Further information	
Randomisation not described. Number of pregnant animals not clear The rabbit data are reported in a separate table (see below)	

Murray et al. (1979) studied the developmental effects of sulfur dioxide by inhalation in a prenatal developmental toxicity study in CF1 mice. The mated animals were exposed to 0 or 25 ppm SO_2 for 7 h/day (calculated to correspond to an internal dose of 32 mg/kg bw per day) from gestation day (GD) 6–15. A C-section was performed on GD 18. During the first days of exposure, the food consumption of the dams was statistically significantly decreased and at C-section, the fetal weight was statistically significantly decreased and the ossification was delayed. No other effects on maternal or developmental toxicity were reported.

Rats

Oral

No studies sufficiently reliable with respect to their internal validity were identified.

Inhalation

Reference	**Zhang et al., 2006a. Changes in testis protein and metabolic enzyme activities in rats induced by sodium fluoride and sulfur dioxide**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Exposure by inhalation for 8 weeks; Sacrifice after 8 weeks, interim sacrifices at 2, 4 and 6 weeks
Animal model	
Species and strain	Rat Wistar
Disease models (e.g. diabetes, allergy, obesity)	–
Housing conditions	
Housing condition	Normal conditions of temperature (22–25 C) 12/12-h light/dark cycle, ventilation and good hygiene
Diet name and source (if reported)	Standard diet (source not reported); approx. 23 mg NaF/kg, 10 mg F^-/kg diet; 0.8 mg/kg bw per day
Treatment	
Test material	Sulfur dioxide
Provider	Foshan Kedi Gas Chemical Industry, Co. Ltd., Guangdong, China
Compound purity	99.99%

Vehicle used	Air
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	15 ± 5 ppm during 4 h per day (calculated to correspond to an internal dose of 7.1 ± 2.4 mg/kg bw per day).
Route of administration (diet, drinking water, gavage)	Inhalation
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Premating (adult phase)
Duration of the exposure	2, 4, 6 or 8 weeks
Study design	
Sex and age at the start of the treatment	Male, age approximately 12 weeks
Number animals/sex/group	24/sex/group; 6/time of sacrifice
Measured endpoints	Testicular tissue: total protein content, gamma-glutamyl transpeptidase, lactate dehydrogenase and ion-activated adenosine triphosphatase
Time of measurement/observation period	After 2, 4, 6 or 8 weeks
Methods to measure the endpoints	Enzyme reagent kit provided by Nanjing Jianchen Biological Institute
Statistical analysis	
Statistical methods	Not described
Results	
Findings reported by the study author/s	Protein content: statistically significant decreased ($p < 0.05$) after 2, 4 and 6 weeks, not after 8 weeks. Gamma-glutamyl transpeptidase: statistically significant decreased ($p < 0.01$) after 4 and 6 weeks, not after 2 and 8 weeks. Lactate dehydrogenase, statistically significant increased ($p < 0.01$) after 2 ($p < 0.05$) and 6 weeks ($p < 01$), not after 2 and 8 weeks. Ion-activated adenosine triphosphatase: some statistically significant increases and decreases were observed in ion-activated adenosine triphosphatases; no effects after 8 weeks.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	After 8 weeks no effect of SO_2 on all measured endpoints at 15 +/− 5 ppm during 4 h per day (calculated to correspond to an internal dose of 7.1 ± 2.4 mg/kg bw per day) the only dose tested.
Further information	
–	

Zhang et al. (2006a) aimed to determine the effects of sulfur dioxide inhalation on testis protein and enzyme activity. Male Wistar rats (24 rats per group, age 12 weeks) were exposed to sulfur dioxide to a dose calculated to correspond to an internal dose of 7.1 mg/kg bw per day for 8 weeks. After 2, 4 and 6 weeks, interim sacrifices were performed on groups of six animals. The following parameters were measured in testicular tissue: total protein content, gamma-glutamyl transpeptidase, lactate dehydrogenase and ion-activated adenosine triphosphatase. Sulfur dioxide exposure resulted in a statistically significant decreased ($p < 0.05$) protein content after 2, 4 and 6 weeks, a statistically significant decreased ($p < 0.01$) gamma-glutamyl transpeptidase after 4 and 6 weeks. In addition, lactate dehydrogenase was statistically significant increased ($p < 0.01$) after 2 ($p < 0.05$) and 6 weeks ($p < 01$) and some statistically significant increases and decreases were observed in ion-activated adenosine triphosphatases. However, after 8 weeks, all the measured parameters in the sulfur dioxide group showed no treatment-related effect. The Panel considered that this study is limited to identifying potential modes of action for sulfur dioxide.

Reference	Zhang et al., 2006b. Effects of sodium fluoride and sulfur dioxide on oxidative stress and antioxidant defences in rat testes
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Exposure by inhalation for 8 weeks; Sacrifice after 8 weeks, interim sacrifices at 2, 4 and 6 weeks
Animal model	
Species and strain	Rats, Wistar
Disease models (e.g. diabetes, allergy, obesity)	–
Housing conditions	
Housing condition	Normal conditions of temperature (22–25 C) 12/12-h light/dark cycle, ventilation and good hygiene
Diet name and source (if reported)	Standard diet (source not reported); approx. 23 mg NaF/kg, 10 mg F^-/kg diet; 0.8 mg/kg bw/day
Treatment	
Test material	Sulfur dioxide
Provider	Foshan Kedi Gas Chemical Industry, Co. Ltd., Guangdong, China
Compound purity	99.9%
Vehicle used	Ambient air
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	15 ± 5 ppm during 4 h per day (calculated to correspond to an internal dose of 7.1 ± 2.4 mg/kg bw per day)
Route of administration (diet, drinking water, gavage)	inhalation
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Premating (adult phase)
Duration of the exposure	2, 4, 6 or 8 weeks
Study design	
Sex and age at the start of the treatment	Male, age approximately 12 weeks
Number animals/sex/group	24/sex/group; 6/time of sacrifice
Measured endpoints	Testicular tissue: glutathione peroxidase activity, superoxide dismutase activity and malondialdehyde
Time of measurement/observation period	After 2, 4, 6 or 8 weeks
Methods to measure the endpoints	Enzyme reagent kit provided by Nanjing Jianchen Biological Institute
Statistical analysis	
Statistical methods	Not described
Results	
Findings reported by the study author/s	Glutathione peroxidase activity: statistically significant increased ($p < 0.05$) after 2 and 6 weeks, not after 4 and 8 weeks. Superoxide dismutase activity: statistically significant increased ($p < 0.01$) after 2 and 6 weeks, not after 4 and 8 weeks. Malondialdehyde: statistically significant increased ($p < 0.01$) after 2 ($p < 0.05$) and 6 weeks ($p < 0.01$), not after 4 and 8 weeks
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	After 8 weeks, no effect of SO_2 on all measured endpoints at 15 ± 5 ppm during 4 h per day (calculated to correspond to an internal dose of 7.1 ± 2.4 m, the only dose tested).

Further information
–

Zhang et al. (2006b) aimed to determine the effects of sulfur dioxide inhalation on oxidative stress and antioxidant defences in the testes. Male Wistar rats (24 rats per group, age 12 weeks) were exposed to sulfur dioxide to a dose calculated to correspond to an internal dose of 7.1 mg/kg bw per day for 8 weeks. In addition, groups with sodium fluoride in drinking water and a group which was exposed to the combination of sodium fluoride and sulfur dioxide were tested. After 2, 4 and 6 weeks, interim sacrifices were performed on groups of six animals. The following parameters were measured in testicular tissue: glutathione peroxidase activity, superoxide dismutase activity and malondialdehyde. Sulfur dioxide exposure resulted in a statistically significant increase in glutathione peroxidase activity, superoxide dismutase activity and malondialdehyde after 2 and 6 weeks and no treatment effect was observed after 4 and 8 weeks. The Panel considered this study is limited to identifying potential modes of action for sulfur dioxide.

Rabbits

Inhalation

Reference	Murray et al., 1979. Embryotoxicity of inhaled sulfur dioxide and carbon monoxide in mice and rabbits
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	Comparable to older OECD guideline, this study was performed before the guideline was drafted
Overview of the study	Mated female rabbits were exposed to SO_2, 7 h/day from GD 6–18 C-section on GD 29
Animal model	
Species and strain	New Zealand White rabbits, Langshaws Rabbitery, Augusta, Michigan
Disease models (e.g. diabetes, allergy, obesity)	no
Housing conditions	
Housing condition	Wire mesh cages, room temperature 21°C, humidity 45%, light cycle (12 h light and dark)
Diet name and source (if reported)	Commercial chow, source not described
Treatment	
Test material	Sulfur dioxide
Provider	The Matheson Company, Joliet, Illinois
Compound purity	99.98%
Vehicle used	Ambient air
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	0, 70 ppm SO_2, 7 h/day (calculated to correspond to an internal dose of 57.8 mg/kg bw per day)
Route of administration (diet, drinking water, gavage)	Inhalation (whole body)
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Gestation GD 6–18
Duration of the exposure	13 days
Study design	
Sex and age at the start of the treatment	Virgin female; age not described
Number animals/sex/group	Mated/pregnant at C-section: 20/17 control and 20/17 SO_2 groups

Measured endpoints	Clinical signs, body weight, food and water consumption. At necropsy liver weight, nasal turbinates, trachea and lungs fixed of 5 mice and 6–7 rabbits for microscopical examination. Number of live and dead fetuses, fetal weight and fetal length, sex, external alterations, one-third of the fetuses of each litter was examined immediately for soft tissue alterations and all fetuses for skeletal alterations. Staining of uterus with sodium sulfide of apparently non pregnant females for evidence of early resorptions.
Time of measurement/observation period	Clinical signs daily from GD 6, body weight, food and water consumption at regular intervals. Liver weight, microscopical examination nasal turbinates, trachea and lungs and reproductive data at C-section: GD 29
Methods to measure the endpoints	Light microscopy
Statistical analysis	
Statistical methods	Fetal alterations: Wilcoxon test as modified by Haseman and Hoel (litter as experimental unit) Continuous data: one-way analysis pf variance and Dunnett's test
Results	
Findings reported by the study author/s	Food consumption was decreased during the first few days of exposure (data not shown). In the SO_2 group several skeletal variants (non-ossified area frontal bone, fused occipital and parietal bones and 13 pairs of ribs) were statistically significantly increased.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	An increased number of skeletal variants was observed at 70 ppm SO_2 (calculated to correspond to an internal dose of 57.8 mg/kg bw per day), the only dose tested.
Further information	
Randomisation not described. Number of pregnant animals not clear. The mice data are reported in a separate table (see above)	

Murray et al. (1979) also studied the developmental effects of sulfur dioxide by inhalation (whole body) in a prenatal developmental toxicity study in New Zealand White rabbits. The mated animals were exposed to 0 or 70 ppm SO_2 for 7 h/day (calculated to correspond to an internal dose of 57.8 mg/kg bw per day) from gestation day (GD) 6–18. A C-section was performed on GD 29. During the first days of exposure the food consumption of the dams was statistically significantly decreased. At C-section, several skeletal variants (non-ossified area frontal bone, fused occipital and parietal bones and 13th pair of ribs) were statistically significantly increased in the sulfur dioxide group. No other effects on maternal or developmental toxicity were reported.

Neurotoxicity

Mice

Oral

No studies sufficiently reliable with respect to their internal validity were identified.

Inhalation

No studies sufficiently reliable with respect to their internal validity were identified.

Rats

Oral

Reference	Derin et al., 2006. The effect of sulfite and chronic restraint stress on brain lipid peroxidation and anti-oxidant enzyme activities
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat-dose 21-day oral neurotoxicity study with a single dose-level in adult male rats
Animal model	
Species and strain	Rat, Wistar
Disease models (e.g. diabetes, allergy, obesity)	No
Housing conditions	
Housing condition	Housed in stainless steel cages, in groups of 5/cage; food and water available ad libitum; 12/12 h light/dark cycles and a constant temperature of 23° ± 1°C
Diet name and source (if reported)	Not specified
Treatment	
Test material	Sodium metabisulfite ($Na_2S_2O_5$)
Provider	Merck, Darmstadt, Germany
Compound purity	Not reported. The Panel noted that the current Merck catalogue lists purity > 98%
Vehicle used	Distilled water
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	0; 520 mg/kg bw per day (corresponding to 350 mg SO_2 equivalents/kg bw per day)
Route of administration (diet, drinking water, gavage)	Gavage
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	21 days
Study design	
Sex and age at the start of the treatment	Males, 3 months of age
Number animals/sex/group	10
Measured endpoints	Brain CAT, Cu/Zn SOD and GSH-Px activity; TBARS
Time of measurement/observation period	After 3 weeks of treatment
Methods to measure the endpoints	Antioxidant enzyme activities: following published standard methods; TBARS: using the thiobarbituric acid (TBA) fluorometric assay
Statistical analysis	
Statistical methods	Differences among the groups were analysed via one-way analysis of variance and all pairwise multiple comparisons were performed by Tukey's test
Results	
Findings reported by the study author/s	Significantly increased brain lipid oxidation and decreased GSH-Px activity; no effects on CAT, Cu/Zn SOD
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted that only a single dose level was tested (corresponding to 350 mg SO_2 equivalents/kg bw per day) and that the study did not include apical endpoints. The adversity of the observed effects is not clear.

Further information

–

The study by Derin et al. (2006) examined the effects of oral sodium metabisulfite exposure on brain parameters indicative of lipid peroxidation (TBARS) and antioxidant capacity (CAT, Cu/Zn SOD and GSH-Px activity). In addition, the influence of chronic restraint stress was tested. Groups of 10 male Wistar rats, aged 3 months, were exposed by gavage to sodium metabisulfite (purity not reported) at doses of 0 or 520 mg/kg bw per day (corresponding to 350.5 mg SO_2 equivalents/kg bw per day) for 21 days. TBARS and antioxidant enzyme activity were measured in brain homogenates after the end of exposure. The level of TBARS increased, whereas GSH-Px activity decreased. No effects were seen on CAT and Cu/Zn SOD. The Panel noted that the downstream adversity of the observed effects cannot be predicted with any certainty.

Reference	**Ozturk et al., 2011. Dose-dependent effect of nutritional sulfite intake on visual evoked potentials and lipid peroxidation**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat-dose (35 days) oral (gavage) neurotoxicity study (visual evoked potentials)
Animal model	
Species and strain	Rat, Wistar
Disease models (e.g. diabetes, allergy, obesity)	None; healthy adult
Housing conditions	
Housing condition	Animals were housed in stainless steel cages (4–5/cage) at standard conditions (23 ± 1°C and 50 ± 5% humidity) with a 12 h light–dark cycle and fed ad libitum with standard rat chow and tap water
Diet name and source (if reported)	Not reported
Treatment	
Test material	Sodium metabisulfite ($Na_2S_2O_5$)
Provider	Merck, Darmstadt, Germany
Compound purity	98%
Vehicle used	Distilled water
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	0; 10; 100; 260 mg/kg bw per day; corresponding to 0, 7, 67 and 175 mg SO_2 equivalents/kg bw per day
Route of administration (diet, drinking water, gavage)	Gavage
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	35 days
Study design	
Sex and age at the start of the treatment	Male, 3-month-old, 270 g
Number animals/sex/group	13
Measured endpoints	Body weight; visual evoked potentials (VEP); plasma-S-sulfonate levels; TBARS, 4-hydroxy-2-nonenal (4-HNE), GSH and oxidised glutathione (GSSG) levels in brain and retina
Time of measurement/observation period	After 35 days of treatment
Methods to measure the endpoints	VEP: recorded with stainless steel subdermal electrodes (Nihon Kohden NE 223 S, Nihon Kohden Corporation, Tokyo 161, Japan) under ether anaesthesia after 5 min of dark adaptation from right and left eyes. A photic

	stimulator (Nova-Strobe AB, Biopac System Inc. Santa Barbara, CA 93117, USA) provided flash stimuli at a distance of 15 cm. Plasma-S-sulfonate blood levels: reaction product sulfite–pararosaniline hydrochloride–formaldehyde measured spectrophotometrically at 560 nm. TBARS: by a fluorimetric method after butanol extraction using wavelengths of 525 nm for excitation, and 547 nm for emission. 4-HNE: by immunoblot analysis after SDS-PAGE Tissue GSH and GSSG: by GSH assay kit (Cat. #703002. Cayman Chemical Ann Arbor, MI).
Statistical analysis	
Statistical methods	Analysis of variance (ANOVA) was performed on all parameters of VEPs for the factors of side (right and left) and groups. Differences of other data were also analysed by ANOVA. Post hoc comparisons of the means were carried out using the Tukey's test.
Results	
Findings reported by the study author/s	All components of visual VEP were prolonged at 100 and 260 mg/kg bw per day. Plasma-S-sulfonate levels, thiobarbituric acid reactive substances (TBARS) and 4-hydroxy-2-nonenal (4-HNE) levels were increased in a dose-dependent manner. GSH and GSSG levels were observed to decrease with increasing doses of $Na_2S_2O_5$ (statistically significant at 260 mg/kg bw per day in brain)
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted that 10 mg/kg bw per day (corresponding to 7 mg SO_2 equivalents/kg bw per day) did not affect the latency of visual evoked potentials.
Further information	
This study was selected by the Panel to perform a BMD analysis based on the VEP potential latencies.	

The study by Ozturk et al. (2011) examined the effects of sodium metabisulfite ingestion on retina and brain by measuring VEP, parameters indicative of lipid peroxidation (TBARS, 4-HNE) and antioxidant capacity (GSH, GSSG), and plasma-S-sulfonate levels as an indicator of exposure to the test substance. Groups of 13 male Wistar rats, aged 3 months, were exposed by gavage to sodium metabisulfite (purity 98%) at doses of 0, 10, 100 or 260 mg/kg bw per day for 35 days. VEPs were recorded under anaesthesia stimulating the right and left eyes individually with a flash stimulator. All components of VEP were found to be prolonged in the groups treated with 100 or 260 mg/kg bw per day, but not in the group given 10 mg/kg bw per day. Peak-to-peak amplitudes were not different between the groups. The levels of plasma-S-sulfonate and levels of TBARS and 4-HNE in retina and brain increased in a dose-dependent manner, while GSH and GSSG levels showed a decreasing trend with increasing doses of sodium metabisulfite. The Panel concluded that the dose of sodium metabisulfite at 10 mg/kg bw per day (corresponding to 7 mg SO_2 equivalents/kg bw per day) did not affect the latency of visual evoked potentials.

A BMD analysis of the data was performed and the Panel estimated the BMDL from these data for VEP component N2, i.e. 37.6 mg SO_2 equivalents/kg bw/day (see Section 4.5.4).

Reference	Derin et al., 2009. Effect of alpha-lipoic acid on visual evoked potentials in rats exposed to sulfite
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat-dose 35-day oral neurotoxicity study with a single dose-level in adult male rats
Animal model	
Species and strain	Rat, Wistar
Disease models (e.g. diabetes, allergy, obesity)	No
Housing conditions	
Housing condition	Housed in stainless steel cages in groups of 5/cage; food and water available ad libitum. 12 h light–dark cycles and a constant temperature of 23 ± 1°C
Diet name and source (if reported)	Not specified
Treatment	
Test material	Sodium metabisulfite ($Na_2S_2O_5$)
Provider	Merck, Darmstadt, Germany
Compound purity	Not reported The Panel noted that the current Merck catalogue lists purity >98%
Vehicle used	Distilled water; controls also treated with corn oil, the vehicle for alpha-lipoic acid
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	0; 260 mg/kg bw per day (corresponding to 175 mg SO_2 equivalents/kg bw per day)
Route of administration (diet, drinking water, gavage)	Gavage
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	5 weeks
Study design	
Sex and age at the start of the treatment	Males, 5-month old, 300–350 g
Number animals/sex/group	13
Measured endpoints	Body weight; VEP; TBARS, GSH-Px, GSH, GSSG in brain and retina
Time of measurement/observation period	After 5 weeks of exposure
Methods to measure the endpoints	VEP: recorded with stainless steel subdermal electrodes (Nihon Kohden NE 223 S, Nihon Kohden Corporation, Tokyo 161, Japan) under ether anaesthesia after 5 min of dark adaptation from right and left eyes. A photic stimulator (Nova-Strobe AB, Biopac System Inc. Santa Barbara, CA 93117, USA) provided flash stimuli at a distance of 15 cm. TBARS: measured using the thiobarbituric acid (TBA) fluorometric assay, with 1,1,3,3-teraethoxypropane as a standard Tissue GSH and GSSG: by GSH assay kit (Cat. #703002. Cayman Chemical Ann Arbor, MI). GSH-Px: by a commercially available GSH-Px assay kit (Cat. #354104. Calbiochem, Darmstadt, Germany).

Statistical analysis	
Statistical methods	One-way analysis of variance (ANOVA); pairwise multiple comparisons by Tukey's test. The Pearson Correlation and Linear Regression analysis was performed via SPSS to obtain the given correlation values.
Results	
Findings reported by the study author/s	Latencies of VEP components (P1, N1, P2, N2, P3) were significantly prolonged. TBARS levels were significantly higher than those detected in controls. Sulfite caused a significant decrease in retina and brain GSH-Px activities and non-significant reductions of GSH and GSSG in both tissues. Concomitant exposure to alpha-lipoic acid (100 mg/kg per day) abrogated the effects of sulfite treatment on these parameters.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted an effect on the latency of visual evoked potentials (prolonged) at 175 mg SO_2 equivalents/kg bw per day, the only dose tested.
Further information	
–	

In the study by Derin et al. (2009) the effects of oral sodium metabisulfite treatment on retina and brain were examined by measuring VEP, parameters indicative of lipid peroxidation (TBARS) and antioxidant capacity (GSH, GSSG, GSH-Px). Groups of 13 male Wistar rats, aged 5 months, were exposed by gavage to sodium metabisulfite at doses of 0 or 260 mg/kg bw per day for 35 days. VEPs were recorded under anaesthesia stimulating the right and left eyes individually with a flash stimulator. All components of VEP were found to be prolonged in the treated group. Peak-to-peak amplitudes did not differ from the control group. The levels of TBARS were increased, while GSH-Px was decreased in retina and brain. It is noted that these effects were abrogated by concomitant dosing with an antioxidant, alpha-lipoic acid. The Panel concluded that sodium metabisulfite at 260 mg/kg bw per day (corresponding to 175 mg SO_2 equivalents/kg bw per day), the only dose tested, had an adverse effect on the latency of visual evoked potentials after repeated gavage administration for 35 days.

Reference	**Kencebay et al., 2013. Merit of quinacrine in the decrease of ingested sulfite-induced toxic action in rat brain**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat dose (5-week) rat oral (gavage) neurotoxicity study (effect on somatosensory evoked potentials)
Animal model	
Species and strain	Rat, Wistar
Disease models (e.g. diabetes, allergy, obesity)	None; healthy adult
Housing conditions	
Housing condition	housed in stainless steel cages in groups of 5 rats per cage and given food and water ad libitum. Animals were maintained at 12 h light–dark cycles and a constant temperature of 23 ± 1°C
Diet name and source (if reported)	Not reported
Treatment	
Test material	Sodium metabisulfite ($Na_2O_5S_2$)
Provider	Not reported
Compound purity	Not reported
Vehicle used	Water

Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	0; 100 mg/kg bw per day (corresponding to 67 mg SO_2 equivalents/kg bw per day)
Route of administration (diet, drinking water, gavage)	Gavage
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	5 weeks
Study design	
Sex and age at the start of the treatment	Male, 3 months
Number animals/sex/group	5
Measured endpoints	Plasma-S-sulfonate levels. Somatosensory evoked potentials (right posterior tibial nerve at the ankle to left somatosensory area of cerebral cortex); Brain homogenate TUNEL (TBARS fluorimetric) and brain slice Caspase-3 (immunohistochemistry). Brain homogenate sPLA2 protein level
Time of measurement/observation period	At end of treatment (5 weeks)
Methods to measure the endpoints	Standard electrophysiology and chemical methods
Statistical analysis	
Statistical methods	One-way ANOVA and Tukey test
Results	
Findings reported by the study author/s	Sulfite prolonged SEP latency, increased brain cell apoptosis (caspase-3 and TUNEL positive cells) and inflammation marker PLA2.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted effects on SEP latency (prolonged), brain cell apoptosis (increased caspase-3 and TUNEL positive cells) and the inflammation marker PLA2 at 67 mg SO_2 equivalents/kg bw per day, the only dose tested.
Further information	
Test substance purity and provider were not reported, reducing confidence in the results of this study	

Neurotoxic effects (prolonged SEP latency, increased brain cell apoptosis (caspase-3 and TUNEL positive cells) and inflammation marker PLA2) were seen after oral sodium metabisulfite administration at 100 mg/kg bw per day (corresponding to 67 mg SO_2 equivalents/kg bw per day) for 5 weeks in the study by Kencebay et al. (2013).

Reference	**Küçükatay et al., 2005. Effect of sulfite on cognitive function in normal and sulfite oxidase deficient rats**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat dose (6-week) rat oral (drinking water) neurotoxicity study (Motor function, active avoidance learning and memory, hippocampal lipid oxidative stress marker)
Animal model	
Species and strain	Rat, Wistar
Disease models (e.g. diabetes, allergy, obesity)	None (healthy adults) (but SOX-depleted and oral vitamin E-supplemented rats were also tested)
Housing conditions	
Housing condition	Housed four to five per cage at 22–25°C with a 12-h light/dark cycle.
Diet name and source (if reported)	Not reported

Treatment	
Test material	Sodium metabisulfite ($Na_2O_5S_2$)
Provider	Not reported
Compound purity	Not reported
Vehicle used	Water
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	25 mg/kg bw per day for 6 weeks (nominal dose; drinking water concentration and consumption not reported) Corresponding to 17 mg SO_2 equivalents/kg bw per day
Route of administration (diet, drinking water, gavage)	Drinking water
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	6 weeks
Study design	
Sex and age at the start of the treatment	Male, adult ('weighing 180–200 g')
Number animals/sex/group	10
Measured endpoints	Motor function (Hanging Wire Test), active avoidance (light/dark shuttle box). Hippocampal lipid oxidative stress marker (TBARS, (fluorimetry)). Plasma-S-sulfonate and liver homogenate SOX activity (spectrophotometry)
Time of measurement/observation period	Behaviour/learning memory in the last week of treatment (in the middle of the 6^{th} week), brain measurements after the end of treatment
Methods to measure the endpoints	Standard assays (see Measured endpoints)
Statistical analysis	
Statistical methods	one-way ANOVA followed by TUKEY post hoc test
Results	
Findings reported by the study author/s	Sulfite treatment impaired active avoidance learning and memory and increased hippocampal oxidative stress marker (TBARS) in SOX-deficient rats but not in SOX-normal rats. Concomitant oral vitamin E attenuated the effects on hippocampal TBARS but not on learning and memory in SOX-deficient rats.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted adverse effects on active avoidance learning and memory and an increase of hippocampal oxidative stress at 17 mg SO_2 equivalents/kg bw per day, the only dose tested.
Further information	
Test substance purity and provider were not reported, reducing confidence in the results of this study	

Neurotoxic effects (impaired active avoidance learning and memory and increased hippocampal oxidative stress marker (TBARS) in SOX-deficient rats but not in SOX-normal rats) were seen after oral sodium metabisulfite administration at 25 mg/kg bw per day (corresponding to 17 mg SO_2 equivalents/kg bw per day) for 6 weeks in the study by Küçükatay et al. (2005). It is unclear whether the SOX-deficient rat model (low molybdenum and high tungstate diet) is more relevant for human health assessment than 'SOX-normal' rats.

Reference	**Küçükatay et al., 2006. Visual evoked potentials in normal and sulfite oxidase deficient rats exposed to ingested sulfite**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No

Guideline studies (if yes, specify)	No
Overview of the study	Repeat dose (6-week) rat oral (drinking water) neurotoxicity study (cortical visual evoked potentials and brain and retina lipid peroxidation)
Animal model	
Species and strain	Rat, Wistar
Disease models (e.g. diabetes, allergy, obesity)	None (healthy adults) (but SOX-depleted and oral vitamin E-supplemented rats were also tested)
Housing conditions	
Housing condition	Groups of four to five rats in stainless steel cages at standard conditions (24 ± 2 degC and 50 ± 5% humidity) with a 12-h light–dark cycle and fed ad libitum with standard rat chow and tap water
Diet name and source (if reported)	Not reported
Treatment	
Test material	Sodium metabisulfite ($Na_2O_5S_2$)
Provider	Not reported ('All chemicals used in this experiment were obtained from Merck (Darmstadt, Germany) and Sigma (St. Louis, MO, USA)', but this may refer only to reagents)
Compound purity	Not reported
Vehicle used	Water
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	25 mg/kg bw per day (nominal dose; drinking water concentration and consumption not reported) Corresponding to 17 mg SO_2/kg bw per day
Route of administration (diet, drinking water, gavage)	Drinking water
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	6 weeks
Study design	
Sex and age at the start of the treatment	Male, 3 months
Number animals/sex/group	10
Measured endpoints	VEP (flash stimulus to visual cortex). Brain and retina homogenate TBARS (fluorimetry). Plasma-S-sulfonate and liver homogenate SOX activity (spectrophotometry)
Time of measurement/observation period	At end of treatment (6 weeks)
Methods to measure the endpoints	Standard assays
Statistical analysis	
Statistical methods	Levene homogeneity of variance, ANOVA, post-hoc Tukey test
Results	
Findings reported by the study author/s	Sulfite treatment caused a significant delay in P1, N1, P2 and P3 components of VEPs, increased brain and retina lipid peroxidation (TBARS) in normal and to a greater extent in SOX-deficient rats. The effects were attenuated by concomitant oral vitamin E.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted effects on components of VEPs (delayed) and increased brain and retina lipid peroxidation (TBARS) in normal and to a greater extent in SOX-deficient rats at 17 mg SO_2 equivalents/kg bw per day, the only dose tested.
Further information	
Test substance purity and provider were not reported, reducing confidence in the results of this study	

Neurotoxic effects (significant delay in P1, N1, P2 and P3 components of VEPs, increased brain and retina lipid peroxidation (TBARS) in normal and to a greater extent in SOX-deficient rats) were seen after oral sodium metabisulfite administration at 25 mg/kg bw per day (corresponding to 17 mg SO_2 equivalents/kg bw per day) for 6 weeks in the study by Küçükatay et al. (2006). It is noted that these effects were attenuated by concomitant dosing with an antioxidant, vitamin E. It is unclear whether the SOX-deficient rat model (low molybdenum and high tungstate diet) is more relevant for human health assessment than 'SOX-normal' rats.

Reference	**Öztürk et al., 2006. Expressions of N-methyl-D-aspartate receptors NR2A and NR2B subunit proteins in normal and sulfite-oxidase deficient rat's hippocampus: effect of exogenous sulfite ingestion**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat dose (6-week) rat oral (drinking water) neurotoxicity study (hippocampal expression of NMDA receptors NR2A and NR2B)
Animal model	
Species and strain	Rat, Wistar
Disease models (e.g. diabetes, allergy, obesity)	None (healthy adults) (but groups of SOX-depleted rats were also tested)
Housing conditions	
Housing condition	housed four to five per cage at 22–25°C with a 12-h light/dark cycle, feed and tap water ad libitum
Diet name and source (if reported)	Standard rat chow (not specified) for SOX-competent groups; low molybdenum diet (AIN 76, Research Dyets Inc., USA) and 200 ppm tungsten (NaWO4) in drinking water for SOX deficient-groups
Treatment	
Test material	Sodium metabisulfite ($Na_2O_5S_2$)
Provider	Not reported
Compound purity	Not reported
Vehicle used	Water
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	0; 25 mg/kg bw per day (nominal reported dose, concentration in drinking water not reported) Corresponding to 17 mg SO_2 equivalents/kg bw per day
Route of administration (diet, drinking water, gavage)	Drinking water
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	6 weeks
Study design	
Sex and age at the start of the treatment	Male, adult, body weight 180–200 g
Number animals/sex/group	10
Measured endpoints	Body weight; food and water consumption; hippocampal NR2A and NR2B protein expression; liver SOX activity
Time of measurement/observation period	After 6 weeks of exposure
Methods to measure the endpoints	SOX activity: by measuring enzymatic reduction of cytochrome c at 550 nm NMDAR subunits: by SDS-PAGE and Western blot analysis of hippocampal homogenates

Statistical analysis	
Statistical methods	One-way analysis of variance (ANOVA) procedure, followed by least significant differences post hoc tests, was used to determine the different means among groups
Results	
Findings reported by the study author/s	Both NR2A and NR2B expressions showed approximately 75–90% decrease after sulfite treatment in SOX-competent and SOX-deficient rats compared to the control group. These subunits were also significantly decreased in SOX-deficient rats without sulfite treatment.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted decreased levels of NR2A and NR2B in hippocampal homogenates at 17 mg SO_2 equivalents/kg bw per day, the only dose tested.
Further information	
No apical endpoints were examined Test substance purity and provider were not reported, reducing confidence in the results of this study	

Öztürk et al. (2006) examined effects of sodium metabisulfite exposure as on hippocampal NMDA receptor subunits NR2A and NR2B in rats. Adult male Wistar rats (10/group), weighing 180–200 g, were exposed to a dose of 25 mg/kg bw per day of sodium metabisulfite through their drinking water for 6 weeks. Two groups made SOX-deficient by feeding them a low molybdenum diet (AIN 76a, Research Dyets Inc., Bethlehem, PA) and addition of 200 ppm tungsten to their drinking water in the form of sodium tungstate were included to examine the importance of sulfite detoxification. SOX activity was determined in liver. Administered sulfite both significantly decreased the levels of NR2A and NR2B in hippocampal homogenates. SOX-deficient animals showed a similar decrease without being exposed to sodium metabisulfite, suggesting that endogenous production of sulfite was sufficient to elicit the effect in the absence of the detoxifying enzyme. It is unclear whether this SOX-deficiency rat model (low molybdenum and high tungstate diet) is more relevant for human health assessment than SOX-competent rats. The Panel noted that the change in NMDR subunit protein expression has not been connected to an apical endpoint or to a specific level of sulfite tissue concentration in this study.

Reference	**Küçükatay et al., 2007. Effect of ingested sulfite on hippocampus antioxidant enzyme activities in sulfite oxidase competent and deficient rats**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat dose (6-week) rat oral (drinking water) mechanistic neurotoxicity study (hippocampal antioxidant potential) comparing SOX-competent and SOX-deficient animals
Animal model	
Species and strain	Rat (albino), strain not reported
Disease models (e.g. diabetes, allergy, obesity)	Model for SOX deficiency included (enzyme activity reduced to 1–5% of normal)
Housing conditions	
Housing condition	Housed 5/cage at 22–25°C with a 12-h light/dark cycle, food and water available ad libitum
Diet name and source (if reported)	Standard rat chow (not specified) for SOX-competent groups; low molybdenum diet (AIN 76, Research Dyets Inc., USA) and 200 ppm tungsten in drinking water for SOX deficient-groups

Treatment	
Test material	Sodium metabisulfite ($Na_2O_5S_2$)
Provider	Not reported 'The reagents used during all experimental protocols were obtained from Merck (Darmstadt, Germany) and Sigma (St. Louis, MO, USA)'. but this may refer only to the assay constituents
Compound purity	Not reported
Vehicle used	Water
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	0; 25 mg/kg bw per day (nominal dose; drinking water concentration and consumption not reported) Corresponding to 17 mg SO_2 equivalents/kg bw per day
Route of administration (diet, drinking water, gavage)	Drinking water
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	6 weeks
Study design	
Sex and age at the start of the treatment	Male, adult ('weighing 180–200 g')
Number animals/sex/group	10
Measured endpoints	Activity of antioxidant enzymes in hippocampus (SOD, CAT, GSH-Px); liver SOX activity
Time of measurement/observation period	After 6 weeks of treatment
Methods to measure the endpoints	SOX activity: by measuring enzymatic reduction of cytochrome c at 550 nm SOD, CAT: by standard tests for enzymatic activity GSH-Px: by GSH-Px Cellular Activity Assay Kit CGP-1 (Sigma)
Statistical analysis	
Statistical methods	Differences between mean values in the study groups were evaluated by 1-way ANOVA followed by TUKEY post hoc test.
Results	
Findings reported by the study author/s	In SOX-competent rats, exposure to sulfite resulted in an increase in all antioxidant enzyme activities determined. No significant effect of sulfite was observed on hippocampal SOD, CAT and GSH-Px status in SOX-deficient rats
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted significant increases in antioxidant enzyme activity in SOX-competent but not in SOX-deficient animals at 17 mg SO_2 equivalents/kg bw per day, the only dose tested.
Further information	
No apical endpoints were examined; the adversity of the findings is unclear Test substance purity and provider were not reported, reducing confidence in the results of this study	

The study of Küçükatay et al. (2007) examined effects of sodium metabisulfite exposure on hippocampal antioxidant enzyme activity in rats. Adult male rats (10/group), weighing 180–200 g, were exposed to a dose of 25 mg/kg bw per day of sodium metabisulfite through their drinking water for 6 weeks. Two groups made SOX-deficient by feeding them a low molybdenum diet (AIN 76a, Research Dyets Inc., Bethlehem, PA) and addition of 200 ppm tungsten to their drinking water in the form of sodium tungstate were included to examine the importance of sulfite detoxification. Enzyme activities of SOD, CAT and GSH-Px were measured in hippocampus homogenates. SOX activity was determined in liver. The authors found significant increases in antioxidant enzyme activity in SOX-competent but not in SOX-deficient animals. It is unclear whether this SOX-deficiency rat model (low molybdenum and high tungstate diet) is more relevant for human health assessment than 'SOX-normal' rats. The Panel noted that the downstream adversity of the observed effects cannot be predicted with any certainty.

Reference	Kocamaz et al., 2012. Sulfite leads to neuron loss in the hippocampus of both normal and SOX-deficient rats
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat dose (6-week) rat oral (drinking water) neurotoxicity study (effects on hippocampal CA1-3 pyramidal cell counts)
Animal model	
Species and strain	Rat, Wistar
Disease models (e.g. diabetes, allergy, obesity)	Induced sulfite oxidase deficiency in 2 additional groups
Housing conditions	
Housing condition	Rats were housed 3/cage. No further information
Diet name and source (if reported)	Standard rat chow and tap water, ad libitum; low molybdenum diet (AIN 76, Research Diets Inc., USA) for SOX-deficient groups
Treatment	
Test material	Sulfite (material not further identified)
Provider	Not reported
Compound purity	Not reported
Vehicle used	Water
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	0; 70 mg sulfite/kg bw per day
Route of administration (diet, drinking water, gavage)	Drinking water
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	6 weeks
Study design	
Sex and age at the start of the treatment	Male, 3-month-old, body weight 200–280 g
Number animals/sex/group	6
Measured endpoints	Total number of pyramidal neurons in hippocampus CA1 and CA3–2 subfields
Time of measurement/observation period	At the end of the treatment period
Methods to measure the endpoints	Stereology, optical fractionator method on horizontally cut cryostat brain sections (150 μm) using an unbiased counting frame and unbiased counting rules
Statistical analysis	
Statistical methods	Kolmogorov–Smirnov test for homogeneity of variance, followed by one-way analysis of variance and post hoc least significant difference for multiple comparisons of the means
Results	
Findings reported by the study author/s	All animals had normally organised hippocampal structures. Analysis at higher magnification revealed a loss of pyramidal neurons in the subdivisions of the hippocampus areas (CA1 and CA3–2) of about 15% for the sulfite-treated group (statistically significant for CA3-2 region).
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted that neurotoxic effects were observed at the only dose level tested (70 mg sulfite /kg bw per day)

Further information
Individual data on hippocampal neuron numbers are reported. Full identity regarding counter ion(s) of the tested substance was not indicated by the authors (identified only as 'sulfite'), in addition test substance purity and provider were not reported, reducing confidence in the results of this study

Kocamaz et al. (2012) investigated the effect of ingested sulfite on the number of pyramidal neurons in CA1 and CA3–2 subdivisions of the rat hippocampus. Male, 3-month-old Wistar rats (6/group), weighing 200–280 g, were exposed to 'sulfite' at 70 mg/kg bw per day through their drinking water for 6 weeks. Two additional groups were made SOX-deficient by feeding them a low molybdenum diet (AIN 76a, Research Dyets Inc., Bethlehem, PA) and addition of 200 ppm tungsten to their drinking water in the form of sodium tungstate to examine the importance of sulfite detoxification of endogenous and exogenous sulfite. At the end of the experimental period, the livers were examined for SOX activity assay to confirm enzyme depletion. Neuronal counts in a known fraction of the CA1 and CA3–2 subdivisions of the left hippocampus were obtained using the optical fractionator method. The authors reported a significant decrease in the estimated total numbers of pyramidal neurons in the CA3–2 layers of the hippocampus in the sulfite-treated and the SOX-deficient groups compared with the control group. The loss of pyramidal neurons in the CA1 region reached statistical significance only in SOX-deficient males with exogenous sulfite. Adverse effects (reduced hippocampal CA1 pyramidal cell counts) were seen at 70 mg/kg bw per day (the only dose level tested) only in SOX-deficient but not in 'SOX-normal' rats. It is unclear whether this SOX-deficiency rat model (low molybdenum and high tungstate diet) is more relevant for human health assessment than 'SOX-normal' rats. The Panel noted that the full identity regarding counter ion(s) was not indicated by the authors (identified only as 'sulfite'); the SO_2 equivalent dose, therefore, cannot be calculated.

Reference	**Ozsoy et al., 2016. The effect of ingested sulfite on visual evoked potentials, lipid peroxidation, and antioxidant status of brain in normal and sulfite oxidase-deficient aged rats**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat dose (6-week) rat oral (gavage) neurotoxicity study
Animal model	
Species and strain	Rat, Wistar
Disease models (e.g. diabetes, allergy, obesity)	Induced sulfite oxidase deficiency in 2 additional groups
Housing conditions	
Housing condition	4–5 animals housed in stainless steel cages, 24 ± 2°C, 50 ± 5% humidity, 12 h light–dark cycle
Diet name and source (if reported)	Standard rat chow, source unknown
Treatment	
Test material	Sodium metabisulfite ($Na_2O_5S_2$)
Provider	Not reported
Compound purity	Not reported
Vehicle used	Distilled water
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	0; 25 mg/kg bw per day (corresponding to 17 mg SO_2 equivalents/kg bw per day)
Route of administration (diet, drinking water, gavage)	Gavage
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult

Duration of the exposure	6 weeks
Study design	
Sex and age at the start of the treatment	Male, 24 months, 500–550 g
Number animals/sex/group	10
Measured endpoints	VEP; brain antioxidant status (activity of superoxide dismutase (SOD); catalase (CAT); GSH-Px; brain lipid oxidation status (TBARS); liver SOX activity
Time of measurement/observation period	After 6 weeks of exposure
Methods to measure the endpoints	VEP: recorded with stainless steel subdermal electrodes (Nihon Kohden NE 223 S, Nihon Kohden Corporation, Tokyo 161, Japan) under ether anaesthesia after 5 min of dark adaptation from right and left eyes. A photic stimulator (Nova-Strobe AB, Biopac System Inc. Santa Barbara, CA 93117, USA) provided flash stimuli at a distance of 15 cm. TBARS: by fluometry after butanol extraction using wavelengths of 525 nm for excitation, and 547 nm for emission. SOX: by monitoring the reduction of cytochrome c at 550 nm. SOD activity: by SOD activity assay kit (Cayman Chemical, Ann Arbor, Michigan, USA, no. 706002) CAT: using a commercially available kit (Cayman Chemical, no. 707002) GSH-Px: by GSH-Px assay kit (Sigma–Aldrich Chemie, Steinheim, Germany, no. CGP-1)
Statistical analysis	
Statistical methods	Analysis of variance (ANOVA) for all parameters of VEP with prior calculation of homogeneity of variance by the SPSS Version 20 Statistic software (SPSS Inc., Chicago, Illinois, USA). Post hoc comparisons of the means were carried out using Tukey's test. Differences of other data were also analysed by Kruskal–Wallis test, followed by Dunn's multiple comparison test.
Results	
Findings reported by the study author/s	Sulfite treatment did not affect any of the endpoints examined, regardless of SOX status.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted that sodium metabisulfite at 25 mg/kg bw per day (corresponding to 17 mg SO_2 equivalents/kg bw per day), the only dose tested, did not induce effects on VEP latency and brain/hippocampal oxidative stress markers in aged rats.
Further information	
Test substance purity and provider were not reported, reducing confidence in the results of this study	

The study of Ozsoy et al. (2016) investigated the effect of oral sodium metabisulfite exposure on visual evoked potentials in aged rats and the possible contribution of lipid oxidation and activity of antioxidant enzymes in the brain. Male, 24-month-old Wistar rats (10/group), weighing 500–550 g, were exposed to a dose of 25 mg/kg bw per day of sodium metabisulfite by gavage for 6 weeks. Two groups made SOX-deficient by feeding them a low molybdenum diet (AIN 76a, Research Dyets Inc., Bethlehem, PA) and addition of 200 ppm tungsten to their drinking water in the form of sodium tungstate were included to examine the importance of sulfite detoxification. VEPs were recorded under anaesthesia stimulating the right and left eyes individually with a flash stimulator. Enzyme activities of SOD, CAT, GSH-Px and SOX were measured in brain homogenates. The authors did not see any significant changes in the endpoints examined; a reduced SOX status did not influence the results. In this study sodium metabisulfite at 25 mg/kg bw per day (corresponding to 17 mg SO_2 equivalents/kg bw per day), the only dose tested, did not induce any statistically significant changes in VEP latency or in brain/hippocampal oxidative stress marker levels after repeated gavage administration for 6 weeks

in aged rats. It is unclear whether this SOX-deficiency rat model (low molybdenum and high tungstate diet) is more relevant for human health assessment than 'SOX-normal' rats.

The authors noted that previous studies from their institute had reported delayed visual evoked potentials and increased brain lipid oxidation (TBARS) after sulfite treatment in the same dose range (e.g. Ozturk et al., 2011). They suggested that the lack of effect in the present study was due to the advanced age of the animals (24 months, close to end-of-life expectancy).

Reference	**Ozsoy et al., 2017. The effect of ingested sulfite on active avoidance in normal and sulfite oxidase-deficient aged rats**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat dose (6-week) rat oral (gavage) neurotoxicity study
Animal model	
Species and strain	Rat, Wistar
Disease models (e.g. diabetes, allergy, obesity)	Induced sulfite oxidase deficiency in 2 additional groups
Housing conditions	
Housing condition	4–5 animals housed in stainless steel cages, 24 ± 2°C, 50 ± 5% humidity, 12 h light–dark cycle
Diet name and source (if reported)	Standard rat chow, source unknown
Treatment	
Test material	Sodium metabisulfite ($Na_2O_5S_2$)
Provider	No information
Compound purity	No information
Vehicle used	Distilled H_20
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	0; 25 mg/kg bw per day (corresponding to 17 mg SO_2 equivalents/kg bw per day)
Route of administration (diet, drinking water, gavage)	Gavage
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	6 weeks
Study design	
Sex and age at the start of the treatment	Males, 24 mo of age
Number animals/sex/group	10
Measured endpoints	Active avoidance response; hippocampal cyclooxygenase (COX), caspase-3 enzymes, prostaglandin E2 (PGE2), nitrate/nitrite
Time of measurement/observation period	Active avoidance training for 5 days before termination; all other measurements at the end of the experimental period
Methods to measure the endpoints	Active avoidance response: automated shuttle box (Ugo Basile 7,502) COX activity: COX Activity Assay Kit (Cayman Chemical, Ann Arbor, MI) PGE2: enzyme immunoassay system (Amersham Pharmacia Biotech, Buckinghamshire, UK) Casp-3: Caspase-3 Colorimetric Activity Assay Kit (Chemicon International, Inc., Billerica, MA) Nitrate/Nitrite: Colorimetric Assay Kit (Cayman Chemical, Ann Arbor, MI)

Statistical analysis	
Statistical methods	Analysis of variance (ANOVA) was performed to assess any statistical differences in the number of active avoidance responses. Prior to completion of the ANOVA, homogeneity of variance was calculated by the SPSS 20 statistic (SPSS Inc., Chicago, IL). Post hoc comparisons of the means were carried out using Tukey's test. Differences of other data were also analysed by Kruskal–Wallis followed up Dunn's Multiple Comparison Test. Significance levels were set at $p < 0.05$.
Results	
Findings reported by the study author/s	Sulfite treatment did not affect active avoidance learning, COX activity, caspase-3 or the level of PGE2 and nitrate/nitrite in the hippocampus, regardless of SOX status.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted that sodium metabisulfite at 25 mg/kg bw per day (corresponding to 17 mg SO_2 equivalents/kg bw per day), the only dose tested, did not induce effects on active avoidance learning, COX activity, caspase-3 or the level of PGE2 and nitrate/nitrite in the hippocampus, regardless of SOX status in aged rats.
Further information	
Test substance purity and provider were not reported, reducing confidence in the results of this study	

The study of Ozsoy et al. (2017) investigated the effect of oral sodium metabisulfite exposure on active avoidance learning and on inflammatory processes or apoptosis in rat hippocampus. Male, 24-month-old Wistar rats (10/group), weighing 500–550 g, were exposed to a dose of 25 mg/kg bw per day of sodium metabisulfite by oral gavage for 6 weeks. Two groups made SOX-deficient by feeding them a low molybdenum diet (AIN 76a, Research Dyets Inc., Bethlehem, PA) and addition of 200 ppm tungsten to their drinking water in the form of sodium tungstate were included to examine the importance of sulfite detoxification. Endpoints tested were performance in a shuttle box test during five days of learning and hippocampal COX activity, prostaglandin 2 levels, caspase-3 activity and nitrate/nitrite levels. The authors did not see any significant changes in the endpoints examined; a reduced SOX status did not influence the results. In this study, the NOAEL was 25 mg/kg bw per day (corresponding to 17 mg SO_2 equivalents/kg bw per day), the only dose tested. It is unclear whether this SOX-deficiency rat model (low molybdenum and high tungstate diet) is more relevant for human health assessment than 'SOX-normal' rats.

The authors noted that previous studies reported adverse effects in hippocampus including cell loss and related functional effects (active avoidance). They suggested that the lack of effect in the present study was due to the advanced age of the animals (24 months, close to end-of-life expectancy).

Reference	**Noorafshan et al., 2013. Curcumin, the main part of turmeric, prevents learning and memory changes induced by sodium metabisulfite, a preservative agent, in rats**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat dose (8-week) rat oral (gavage) neurotoxicity study (learning and memory in a partially-baited eight arm radial maze)
Animal model	
Species and strain	Rat, Sprague–Dawley
Disease models (e.g. diabetes, allergy, obesity)	None (healthy adults) (but oral curcumin-supplemented rats were also tested)

Housing conditions	
Housing condition	'housed in plastic cages under standard conditions'
Diet name and source (if reported)	Not reported
Treatment	
Test material	Sodium metabisulfite ($Na_2O_5S_2$)
Provider	Not reported
Compound purity	Not reported
Vehicle used	Water
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	25 mg/kg bw per day (corresponding to 17 mg SO_2 equivalents/kg bw per day)
Route of administration (diet, drinking water, gavage)	Gavage
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	8 weeks
Study design	
Sex and age at the start of the treatment	Male, 'adult'
Number animals/sex/group	10
Measured endpoints	Learning and memory in partially-baited eight arm radial maze.
Time of measurement/observation period	At the end of treatment (8 weeks)
Methods to measure the endpoints	Standard maze endpoints; acquisition/retention working and reference memory
Statistical analysis	
Statistical methods	two-way or one-way ANOVA, post-hoc Tukey test
Results	
Findings reported by the study author/s	Sodium metabisulfite causes learning and memory changes in rats. Sulfite treated animals made more reference and working memory errors during the learning phase, at the end of the learning phase, and during the retention testing. The effects were abrogated by concurrent oral curcumin.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted that changes in learning and memory occurred at 17 mg SO_2 equivalents/kg bw per day, the only dose tested.
Further information	
Test substance source and purity were not reported, reducing confidence in the results of this study	

Neurotoxic effects (learning and memory changes in rats) were seen after oral sodium metabisulfite administration at 25 mg/kg bw per day (corresponding to 17 mg SO_2 equivalents/kg bw per day) for 8 weeks in the study by Noorafshan et al. (2013). The effects were abrogated by concurrent oral curcumin, an antioxidant.

Reference	**Karimfar et al., 2014. Curcumin prevents the structural changes induced in the rats' deep cerebellar nuclei by sodium metabisulfite, a preservative agent**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat dose (56-day) rat oral (gavage) neurotoxicity study (volume of deep cerebellar nuclei and number of neurons)

Animal model	
Species and strain	Rat, Sprague–Dawley
Disease models (e.g. diabetes, allergy, obesity)	None (healthy adults)
Housing conditions	
Housing condition	Not reported
Diet name and source (if reported)	Not reported
Treatment	
Test material	Sodium metabisulfite ($Na_2O_5S_2$)
Provider	Not reported
Compound purity	Not reported
Vehicle used	Water
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	25 mg/kg bw per day (corresponding to 17 mg SO_2 equivalents/kg bw per day)
Route of administration (diet, drinking water, gavage)	Gavage
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	56 days
Study design	
Sex and age at the start of the treatment	Male, adult (weight '250–280 g')
Number animals/sex/group	6
Measured endpoints	Volume of deep cerebellar nuclei and number of neurons
Time of measurement/observation period	At end of treatment (56 days)
Methods to measure the endpoints	Visual point counting, microscopic fields were sampled using a stage micrometre and systematic uniform random sampling.
Statistical analysis	
Statistical methods	Kruskal–Wallis and Mann–Whitney Utest
Results	
Findings reported by the study author/s	Decrease in volume of deep cerebellar nuclei and number of neurons. Effects were abrogated by concomitant curcumin
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted effects (decreases) on volume of deep cerebellar nuclei and the number of neurons at 17 mg SO_2 equivalents/kg bw per day, the only dose tested.
Further information	
Test substance source and purity were not reported, reducing confidence in the results of this study	

Neurotoxic effects (decrease in volume of deep cerebellar nuclei and number of neurons) were seen after oral sodium metabisulfite administration at 25 mg/kg bw per day (corresponding to 17 mg SO_2 equivalents/kg bw per day) for 56 days in the study by Karimfar et al. (2014). It is noted that these effects were abrogated by concomitant dosing with an antioxidant, curcumin.

Reference	**Noorafshan et al., 2015. Protective role of curcumin against sulfite-induced structural changes in rats' medial prefrontal cortex.**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat dose (8-week) rat oral (gavage) neurotoxicity study (median prefrontal cortex mPFC)
Animal model	
Species and strain	Rat, Sprague–Dawley
Disease models (e.g. diabetes, allergy, obesity)	None (healthy adult)

Housing conditions	
Housing condition	Not reported
Diet name and source (if reported)	Not reported
Treatment	
Test material	Sodium metabisulfite ($Na_2O_5S_2$)
Provider	Not reported
Compound purity	Not reported
Vehicle used	Water
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	25 mg/kg bw per day (corresponding to 17 mg SO_2 equivalents/kg bw per day)
Route of administration (diet, drinking water, gavage)	Gavage
Period of exposure (pre-mating, mating, gestation, lactation, adult)	8 weeks
Duration of the exposure	Adult
Study design	
Sex and age at the start of the treatment	Male, adult (weight '250–280 g')
Number animals/sex/group	6
Measured endpoints	Total volume of mPFC and number of neurons and glial cells, neuronal dendritic length, spine density and morphology.
Time of measurement/observation period	At end of treatment (8 weeks)
Methods to measure the endpoints	Standard quantitative microscopical techniques (paraffin-embedded, Cresyl violet-stained sections or Golgi-stained slabs)
Statistical analysis	
Statistical methods	Kruskal–Wallis and Mann–Whitney U test
Results	
Findings reported by the study author/s	Sulfite treatment reduced mPFC volume and number of neurons and glia, and neuronal dendritic length and total number of spines
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted effects (reduced mPFC volume, number of neurons and glia, neuronal dendritic length and total number of spines) at 17 mg SO_2 equivalents/kg bw per day, the only dose tested.
Further information	
Test substance source and purity were not reported, reducing confidence in the results of this study	

Neurotoxic effects (reduced mPFC volume and number of neurons and glia, and neuronal dendritic length and total number of spines) were seen after oral sodium metabisulfite at 25 mg/kg bw per day (corresponding to 17 mg SO_2 equivalents/kg bw per day) for 8 weeks in the study by Noorafshan et al. (2015).

Inhalation

Reference	**Sang et al., 2010. SO_2 inhalation contributes to the development and progression of ischemic stroke in the brain**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Short-term (7-day) repeat dose inhalation neurotoxicity study

Animal model	
Species and strain	Rat, Wistar
Disease models (e.g. diabetes, allergy, obesity)	No
Housing conditions	
Housing condition	Housed in SPF facilities under standard conditions (24 ± 2°C, 50 ± 5% humidity) with a 12-h light/dark cycle, with 6 animals in each stainless steel cage. Food and water were available ad libitum when the rats were not being exposed
Diet name and source (if reported)	Diet type and source not reported
Treatment	
Test material	Sulfur dioxide
Provider	Not reported
Compound purity	Not reported
Vehicle used	Air
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	0; 7.00 ± 0.78; 14.00 ± 0.59; 28.00 ± 3.56 mg/m^3 (0; 2.5; 5; 10 ppm) 6 h per day Calculated to correspond to an internal dose of 1.9 ± 0.21, 3.8 ± 0.16 and 7.6 ± 0.96 mg/kg bw per day
Route of administration (diet, drinking water, gavage)	Inhalation in 1-m^3 exposure chambers
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	7 days
Study design	
Sex and age at the start of the treatment	Male, adult (weighing 270–290 g)
Number animals/sex/group	6
Measured endpoints	mRNA and protein expression for Cox-2, iNOS, ICAM-1, ET-1, beta-actin in cerebral cortex
Time of measurement/observation period	18 h after the last exposure
Methods to measure the endpoints	mRNA: by RT-PCR Proteins: by SDS-PAGE and immunoblot
Statistical analysis	
Statistical methods	ANOVA was applied for between-group statistical comparisons
Results	
Findings reported by the study author/s	SO_2 elevated the levels of ET-1, iNOS, COX-2 and ICAM-1 mRNA and protein in a concentration-dependent manner. A rat model of ischemic stroke after being treated with filtered air also showed elevated expression of ET-1, iNOS, COX-2 and ICAM-1, followed by increased activation of caspase-3 and cerebral infarct volume.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel identified a LOEL of 1.9 mg/kg bw per day based on elevated levels of ET-1, iNOS, COX-2, ICAM-1 mRNA and protein expression in cerebral cortex.
Further information	
Test substance source and purity were not reported, reducing confidence in the results of this study	

Sang et al. (2010) examined whether sulfur dioxide inhalation would have similar effects on the brain as middle cerebral artery occlusion (a model for ischemic stroke). They exposed adult male Wistar rats (n = 6/group) to different concentrations of sulfur dioxide (0, 7, 14 and 28 mg/m^3, calculated to correspond to an internal dose of 1.9, 3.8 and 7.6 mg/kg bw per day) in inhalation chambers for 7 days and measured mRNA and protein expression of endothelin-1 (ET-1), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and intercellular adhesion molecule 1 (ICAM-1) in the cerebral cortex. No apical endpoints for neurotoxicity were included in this study. Sulfur dioxide inhalation elevated the levels of ET-1, iNOS, COX-2 and ICAM-1 mRNA and protein in a concentration-

dependent manner. Effects were observed at all tested doses. The Panel noted that the downstream adversity of the observed effects cannot be predicted with any certainty.

Reference (authors, year, title, other info)	**Yun et al., 2010. SO_2 inhalation modulates the expression of apoptosis-related genes in rat hippocampus via its derivatives in vivo**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Short-term (7-day) repeat dose inhalation neurotoxicity study
Animal model	
Species and strain	Rat, Wistar
Disease models (e.g. diabetes, allergy, obesity)	No
Housing conditions	
Housing condition	Housed in SPF facilities under standard conditions (24 ± 2°C, 50 ± 5% humidity) with a 12-h light/dark cycle, with 6 animals in each stainless steel cage. Food and water were available ad libitum when the rats were not being exposed
Diet name and source (if reported)	Diet type and source not reported
Treatment	
Test material	Sulfur dioxide
Provider	Not reported
Compound purity	Not reported
Vehicle used	Air
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	0; 7.00 ± 0.78; 14.00 ± 0.59; 28.00 ± 3.56 mg/m^3 (0; 2.5; 5; 10 ppm) 6 h per day Calculated to correspond to an internal dose of 1.9 ± 0.21, 3.8 ± 0.16, 7.6 ± 0.96 mg/kg bw per day
Route of administration (diet, drinking water, gavage)	Inhalation in 1-m^3 exposure chambers
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	7 days
Study design	
Sex and age at the start of the treatment	Male, adult (weighing 180–200 g)
Number animals/sex/group	6
Measured endpoints	Hippocampal mRNA and protein expression of p53, bax and bcl-2; c-fos and c-jun
Time of measurement/observation period	18 h after the last exposure
Methods to measure the endpoints	mRNA: by RT-PCR Proteins: by SDS-PAGE and immunoblot
Statistical analysis	
Statistical methods	The data were analysed using one-way analysis of variance (Origin 7.0 software) for significant comparison between groups
Results	
Findings reported by the study author/s	SO_2 statistically increased p53 expression and the ratio of bax to bcl-2 in a concentration-dependent manner. Also, mRNA and protein levels of c-fos and c-jun significantly elevated in proportion to exposure concentration. Then, primary cultured hippocampal neurons treated with SO_2 derivatives (bisulfite and sulfite, 3:1 M/M), show p53, c-fos, c-jun mRNA expression and the ratio of bax to bcl-2

	augmented as functions of SO_2 derivative concentration and exposure time, and confirm the effects observed *in vivo*.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel identified a LOEL of 1.9 mg/kg bw per day based on elevated levels of hippocampal mRNA and protein expression of p53, bax, bcl-2, c-fos and c-jun
Further information	
The study also included in vitro data from primary hippocampal neuron cultures prepared from newborn rat pups. They were incubated with 0, 3, 10, 30, 100 or 300 μM of a mixture of SO_2 derivatives ($NaHSO_3$:Na_2SO_3 3:1 M ratio) for 6, 12 and 24 h and used for real-time RT-PCR analysis of the same genes as in the in vivo study. Test substance source and purity were not reported, reducing confidence in the results of this study	

Yun et al. (2010) examined the effects of sulfur dioxide inhalation on the expression of apoptosis-related genes in rat hippocampus. They exposed adult male Wistar rats (n = 6/group) to different concentrations of sulfur dioxide (0, 7, 14, 28 and 56 mg/m^3, calculated to correspond to an internal dose of 1.9, 3.8 and 7.6 mg/kg bw per day) in inhalation chambers for 7 days. The Panel noted that the highest concentration is not mentioned in the description of the methods. Hippocampal mRNA and protein expression of p53, bax, bcl-2, c-fos and c-jun were measured. No apical endpoints for neurotoxicity were included in this study. Sulfur dioxide inhalation elevated the levels of these mRNAs and proteins in a concentration-dependent manner, indicating that sulfur dioxide exposure activates pro-apoptotic signalling pathways. Effects were observed at all tested doses. The mRNA findings were confirmed in primary hippocampal neurons *in vitro* that were treated with sulfite (a mixture of sodium bisulfite and sodium sulfite). The Panel noted that the downstream adversity of the observed effects cannot be predicted with any certainty.

Reference	**Sang et al., 2011. SO_2-Induced Neurotoxicity Is Mediated by Cyclooxygenases-2-Derived Prostaglandin E2 and its Downstream Signalling Pathway in Rat Hippocampal Neurons.**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Short-term repeat dose (7 day) rat inhalation neurotoxicity
Animal model	
Species and strain	Rat, Wistar
Disease models (e.g. diabetes, allergy, obesity)	
Housing conditions	
Housing condition	Not reported
Diet name and source (if reported)	Not reported
Treatment	
Test material	Sulfur dioxide
Provider	Not reported
Compound purity	Not reported
Vehicle used	Air
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	0, 7.00 ± 0.67, 14.00 ± 0.89 and 28.00 ± 4.12 mg/m^3 SO_2, 6 h/day Calculated to correspond to an internal dose of 1.9 ± 0.19, 3.8 ± 0.24, 7.6 ± 1.1 mg/kg bw per day
Route of administration (diet, drinking water, gavage)	Inhalation in 1-m^3 exposure chambers
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	7 days

Study design	
Sex and age at the start of the treatment	Male, 2-month-old, weighing 180–200 g
Number animals/sex/group	6
Measured endpoints	In hippocampus: expression of COX-2, NF-κB, caspase-3, cleaved caspase-3, EP2 and EP4 receptors, NMDAR2B and beta-actin proteins in hippocampus; expression of mRNA for COX-2, microsomal prostaglandin E synthases (mPGES)-1 and – 2, cytosolic prostaglandin E synthases (cPGES) and beta-actin; prostaglandin E2 and cAMP levels
Time of measurement/observation period	18 h after the last exposure
Methods to measure the endpoints	Immunoblot analysis after SDS-PAGE for protein expression RT-PCR for mRNA
Statistical analysis	
Statistical methods	One-way ANOVA test was used for statistical comparison when appropriate
Results	
Findings reported by the study author/s	SO_2 inhalation resulted in NF-κB and caspase-3 activation, elevated COX-2 expression, increased release of PGE2 and cAMP, upregulated EP2, EP4 and NMDAR2B expression.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel identified a LOEL of 1.9 mg/kg bw per day
Further information	
Test substance purity and provider were not reported, reducing confidence in the results of this study	

Sang et al. (2011) tested the hypothesis that sulfur dioxide might induce neurotoxicity through cyclooxygenase-2-derived prostaglandin E2. They exposed 2 month-old male Wistar rats (n = 6/group) to different concentrations of sulfur dioxide (0, 7, 14 and 28 mg/m^3, calculated to correspond to an internal dose of 1.9, 3.8 and 7.6 mg/kg bw per day) in inhalation chambers for 7 days and measured mRNA and protein expression of relevant enzymes, their products and target receptors in the hippocampi. This included COX-2, NF-κB, caspase-3, EP2 and EP4 receptors, NMDAR2B, microsomal prostaglandin E synthases (mPGES)-1 and -2, cytosolic prostaglandin E synthases (cPGES), prostaglandin E2 and cAMP levels. No apical endpoints for neurotoxicity were examined in this study. Sulfur dioxide inhalation resulted in NF-κB and caspase-3 activation, elevated COX-2 expression, increased release of PGE2 and cAMP, upregulated EP2, EP4 and NMDAR2B expression. Effects were observed at all tested doses.

Reference	**Qin et al., 2012. Sulfur dioxide inhalation stimulated mitochondrial biogenesis in rat brains.**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Long-term repeated dose (30 days) rat inhalation neurotoxicity
Animal model	
Species and strain	Rat, Wistar
Disease models (e.g. diabetes, allergy, obesity)	–
Housing conditions	
Housing condition	Housed in groups of 6 rats in stainless steel cages under standard conditions (24 ± 2°C and 50 ± 5% humidity) with a 12 h light–dark cycle
Diet name and source (if reported)	Not reported
Treatment	
Test material	Sulfur dioxide

Provider	Not reported
Compound purity	Not reported
Vehicle used	Air
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	3.5 ± 0.39, 7.1 ± 1.13 and 14.3 ± 2.07 mg/m^3, 4 h/day Calculated to correspond to an internal dose of 0.63 ± 0.07, 1.3 ± 0.2 and 2.6 ± 0.38 mg/kg bw per day
Route of administration (diet, drinking water, gavage)	Inhalation in 1 m^3 exposure chambers
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Not reported
Duration of the exposure	30 days
Study design	
Sex and age at the start of the treatment	Male, Age not reported
Number animals/sex/group	4 groups, 6 rats/group
Measured endpoints	Effect of SO_2 exposure on the inner mitochondrial membrane potential (MMP), ATP content, MDA content, mtDNA content in rat cortex, mRNA levels of respiratory complex IV and V subunits in rat brain, mRNA and protein levels of nuclear factors associated with mitochondrial biogenesis and function in rat brain.
Time of measurement/observation period	20 h after the last exposure
Methods to measure the endpoints	Lipophilic cationic probe JC-1 for MMP assessment in cerebral mitochondria; luciferin-luciferase method for measurement of the amount of ATP; real time PCR for mitochondrial replication and transcription analysis; Western blotting for protein expression.
Statistical analysis	
Statistical methods	Results were expressed as mean ± SE. The data were analysed using one-way ANOVA for significant differences between the SO_2 groups and the control group. A level of $p < 0.05$ was accepted as statistically significant.
Results	
Findings reported by the study author/s	Cerebral mtDNA content was markedly increased in rats after SO_2 exposure. Paralleling the change in mtDNA content, MMP, ATP content, MDA level, CO1 & 4 and ATP synthase subunits 6 & 8 expression and cytochrome c oxidase activity were increased in rat cortex after SO_2 inhalation. Moreover, mitochondrial biogenesis was accompanied by increased expression of NRF1 and TFAM, whereas PGC-1 was not changed.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel identified a LOEL of 0.63 mg/kg bw per day based on MDA level in rat cortex
Further information	
Test substance purity and provider were not reported, reducing confidence in the results of this study	

Qin et al. (2012) reported that sulfur dioxide inhalation exposure disrupted brain mitochondria and induced oxidative stress (increased inner mitochondrial membrane potential, increased brain malondialdehyde (MDA) and mitochondrial biogenesis) at all doses tested. It is not clear from these data whether oxidative stress precedes or follows mitochondrial disruption. The Panel noted that the downstream adversity of the observed effects cannot be predicted with any certainty.

Reference	Yao et al., 2016. Differential Effects Between One Week and Four Weeks Exposure to Same Mass of SO_2 on Synaptic Plasticity in Rat Hippocampus
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Repeat dose (1 week or 4 week) rat inhalation
Animal model	
Species and strain	Rat, Wistar
Disease models (e.g. diabetes, allergy, obesity)	
Housing conditions	
Housing condition	Not reported; animals had free access to feed and water when not in the inhalation chamber (1 m^3)
Diet name and source (if reported)	Not reported
Treatment	
Test material	Sulfur dioxide
Provider	Not reported
Compound purity	Not reported
Vehicle used	Air
Dose regimen (dose level or concentration per group and frequency) and achieved doses if available	0; 3.5 ± 0.39; 7.1 ± 1.13 mg/m^3, 6 h/day (4 weeks) (Calculated to correspond to an internal dose of 0.95 ± 0.11 and 1.9 ± 0.31 mg/kg bw per day) 0; 14 ± 0.89; 28 ± 4.12 mg/m^3, 6 h/day (1 week) (Calculated to correspond to an internal dose of 3.8 ± 0.24 and 7.6 ± 1.1 mg/kg bw per day)
Route of administration (diet, drinking water, gavage)	Inhalation at a flow rate of 30 L/min
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Adult
Duration of the exposure	4 weeks, 6 h/day 1 week, 6 h/day
Study design	
Sex and age at the start of the treatment	Male, age not reported; body weight 180–200 g
Number animals/sex/group	6
Measured endpoints	mRNA expression for synaptic plasticity marker Arc, glutamate receptors (GRIA1, GRIA2, GRIN1, GRIN2A and GRIN2B); protein expression for memory-related kinase p-CaMKIIα, presynaptic marker synaptophysin, postsynaptic density protein 95 (PSD-95), protein kinase A (PKA) and protein kinase C (PKC) in hippocampus; transmission electron microscopy (TEM) of hippocampal tissue for examination of presynaptic vesicle density and the morphology of the postsynaptic density
Time of measurement/observation period	18 h after the end or exposure
Methods to measure the endpoints	mRNA expression by RT-PCR protein expression by immunoblot analysis after SDS-PAGE
Statistical analysis	
Statistical methods	One-way ANOVA followed by the Fisher's least significant difference test was used for statistical comparison when appropriate
Results	
Findings reported by the study author/s	mRNA expression for Arc and the glutamate receptors as well as protein expression of p-CaMKIIα were decreased SO_2 after exposure for 1 week and 4 weeks. Protein

	expression of synaptophysin, PSD-95, PKA and PKC was increased after 1 week, but decreased after 4 weeks of SO_2 exposure. Opposite results were reported for presynaptic vesicle density (increased after 1 week, decreased after 4 weeks) and morphology of postsynaptic densities (thickened/expanded after 1 week, thinned after 4 weeks).
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel identified a LOEL of 0.95 mg/kg bw per day, based on Arc mRNA expression
Further information	
No toxicological endpoints were examined in this study. TEM data were not quantified Test substance purity and provider were not reported, reducing confidence in the results of this study	

Yao et al. (2016) examined the effect of sulfur dioxide inhalation on the synaptic plasticity markers in the hippocampus of rats. Male Wistar rats (6/group), weighing 180–200 g, were exposed in inhalation chambers for 6 h per day to two concentrations of sulfur dioxide for either 1 week (14 and 28 mg/m^3, calculated to correspond to an internal dose of 3.8 and 7.6 mg/kg bw per day) or 4 weeks (3.5 and 7 mg/m^3, calculated to correspond to an internal dose of 0.95 and 1.9 mg/kg bw per day). In both exposure scenarios, the same total doses were delivered overall. mRNA expression of synaptic plasticity marker Arc, glutamate receptors (GRIA1, GRIA2, GRIN1, GRIN2A and GRIN2B) and protein expression of memory-related kinase p-CaMKIIα, presynaptic marker synaptophysin, postsynaptic density protein 95 (PSD-95), protein kinase A (PKA) and protein kinase C (PKC) were measured. The morphology of synapses in the hippocampus was examined by transmission electron microscopy (TEM). However, no quantitative results were presented for the reported changes in synapse morphology. Sulfur dioxide at both concentrations inhibited the mRNA levels of Arc, the AMPA receptor genes (GRIA1, GRIA2) and the NMDA receptor genes (GRIN1, GRIN2A and GRIN2B) as well as the protein expression of p-CaMKIIα in the group exposed for 1 week. Less severe reductions or no effects were observed when the same doses were spread over 4 weeks, indicating that the daily dose appears to be more important than cumulative dose. The protein expressions of synaptophysin, PSD-95, PKA and PKC were increased in the 1-week exposure scenario, but decreased with the lower daily doses of the 4 weeks exposure schedule. The Panel considered that the LOEL is 3.5 mg/m^3 (calculated to correspond to an internal dose of 0.95 mg/kg bw per day), based on the decreases of Arc and GRIA1 mRNAs. The Panel noted that the downstream adversity of the observed effects cannot be predicted with any certainty.

Reference	**Yargiçoğlu et al. 1999. Age-related alterations in antioxidant enzymes, lipid peroxide levels, and somatosensory-evoked potentials: effect of sulfur dioxide**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Inhalation
Animal model	
Species and strain	Rat, Swiss albino
Disease models (e.g. diabetes, allergy, obesity)	–
Housing conditions	
Housing condition	Housed in groups of four to five rats in stainless steel cages under standard conditions (24 ± 2°C and 50 ± 5% humidity) with a 12-h light–dark cycle.
Diet name and source (if reported)	Not reported
Treatment	
Test material	Sulfur dioxide
Provider	Not reported
Compound purity	Not reported

Vehicle used	Air
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	10 ppm, 7 days/week, 1 h/day Calculated to correspond to an internal dose of 1.2 mg/kg bw per day
Route of administration (diet, drinking water, gavage)	Inhalation 1 m^3 exposure chamber
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Young (3 months), middle-aged (12 months) and old (24 months)
Duration of the exposure	6 weeks
Study design	
Sex and age at the start of the treatment	Male; Young (3 months), middle-aged (12 months) and old (24 months)
Number animals/sex/group	3 age group (each age group was subdivided in two (control and SO_2)): 6 groups in total, 10 animals/group
Measured endpoints	Somatosensory evoked potentials (SEPs); (SEP; tibial nerve to somatosensory cortex), brain homogenate (TBARS; a product of lipid peroxidation) and the activities of Cu/Zn SOD, GSH-Px and CAT.
Time of measurement/observation period	24 h (with food deprivation) after last exposure
Methods to measure the endpoints	SEPs were recorded with stainless steel subdermal electrodes. Enzyme assays: Assay of CAT, GSH-Px and Cu/Zn SOD activity and TBARS assay.
Statistical analysis	
Statistical methods	Differences of SEP parameters were analysed by an analysis of variance (ANOVA). Differences of parameters between groups were tested by student's *t* test.
Results	
Findings reported by the study author/s	SO_2 exposure increased brain TBARS levels and Cu/Zn SOD activity and decreased brain GSH-Px activity in all age groups; brain CAT activities were unaltered. Somatosensory evoked potential (SEP; tibial nerve to somatosensory cortex) latency was prolonged in the young SO_2 group; only the P2 component was prolonged in the middle-aged SO_2 group, and there was no latency change in the older SO_2 group. SO_2 exposure also increased daily food and water consumptions in all age groups. In controls, brain TBARS and SEP latencies were increased age-dependently and Cu/Zn SOD activity decreased.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted effects at 1.2 mg/kg bw per day the only dose tested, consisting of increased brain TBARS and decreased brain GSH-Px activity at all ages, and on increased SEP latency in young animals
Further information	
Test substance purity and provider were not reported, reducing confidence in the results of this study	

Yargiçoğlu et al. (1999) reported that sulfur dioxide inhalation exposure at 10 ppm (calculated to correspond to an internal dose of 1.2 mg/kg bw per day) increased brain oxidative stress (increased lipid peroxidation TBARs, increased Cu/Zn SOD and decreased GSH-Px activity) in young, middle-aged and old rats, and impaired nerve conduction velocity (prolonged SEP latency) in young animals. The lack of effect of sulfur dioxide on SEPs in older animals is possibly a 'floor' effect, since latencies were also significantly prolonged in older controls compared to young controls. Prolonged SEP latency normally suggests defective myelination. The Panel noted that downstream adversity of the brain oxidative stress endpoints cannot be predicted with any certainty.

Reference	Ağar et al., 2000. The effect of sulfur dioxide inhalation on visual evoked potentials, antioxidant status, and lipid peroxidation in alloxan-induced diabetic rats
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Inhalation
Animal model	
Species and strain	Rat, Swiss albino
Disease models (e.g. diabetes, allergy, obesity)	Diabetes mellitus
Housing conditions	
Housing condition	Housed in a group of four or five rats in stainless steel cages at standard conditions (24 ± 2°C and 50 ± 5% humidity) with 12-h light/dark cycle
Diet name and source (if reported)	Not reported
Treatment	
Test material	Sulfur dioxide
Provider	Not reported
Compound purity	Not reported
Vehicle used	Air
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	10 ± 1 ppm, 7 days/week, 1 h/day Calculated to correspond to an internal dose of 1.2 ± 0.12 mg/kg bw per day Model MRU 95/3-CD apparatus was used to monitor the concentration of SO_2 within the chamber. Control groups were exposed to filtered air in the same chamber for the same period of time.
Route of administration (diet, drinking water, gavage)	Inhalation 1 m^3 exposure chamber
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Aged 3 months
Duration of the exposure	6 weeks
Study design	
Sex and age at the start of the treatment	Male, aged 3 months
Number animals/sex/group	40 animals, 4 groups, 10 animals/group: control (C), sulfur dioxide 1 control (CSO_2), diabetic (D) and sulfur dioxide 1 diabetic (DSO_2) groups
Measured endpoints	Effects on visual-evoked potentials (VEPs), TBARS, a product of lipid peroxidation and the activities of Cu/Zn SOD, GSH-Px and CAT in diabetes mellitus
Time of measurement/observation period	24 h (with food deprivation) after last exposure
Methods to measure the endpoints	VEPs were recorded with stainless steel subdermal electrodes. Enzyme assays: Assay of CAT, GSH-Px and Cu/Zn SOD activity and TBARS assay.
Statistical analysis	
Statistical methods	Analysis of variance (ANOVA) was performed on all parameters of VEPs for the factors of side (right and left) and groups. Differences of other data were also analysed by ANOVA. *Post hoc* comparisons of the means carried out using the Tukey's test. Number of rats (n) was equal to 10 for all group. Significance levels were set at p, 0.05.

Results	
Findings reported by the study author/s	SO_2 exposure, though markedly decreasing retina CAT and GSH-Px activities, significantly increased retina Cu/Zn SOD activity in the diabetic and non-diabetic groups. In contrast to SO_2-related increase in the activity of Cu/Zn SOD, decrease in GSH-Px activity was observed in the brain of those groups. Brain CAT activity was unaltered. SO_2 exposure caused the significant elevation in brain TBARS levels of CSO_2 and DSO_2 groups, whereas only in the retina TBARS level of the CSO_2 group. SO_2 exposure caused the significant prolongations of P1, N1, P2 and P3 components of VEPs in the nondiabetic and all components of VEPs in the diabetic groups. SO_2 exposure also resulted in significant amplitude reductions in both experimental groups.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted effects at 1.2 mg/kg bw per day, the only dose tested, consisting of prolonged VEP latency, and increased markers of oxidative stress in brain and retina
Further information	
Test substance purity and provider were not reported, reducing confidence in the results of this study	

Ağar et al. (2000) reported that sulfur dioxide inhalation exposure at 10 ppm (calculated to correspond to an internal dose of 1.2 mg/kg bw per day) prolonged VEP latency, and induced markers of oxidative stress in brain and retina. VEP prolongation is normally a consequence of defective myelination, but the effects of sulfur dioxide on retina may also contribute in this case.

Reference	**Kilic, 2003. The effects of aging and sulfur dioxide inhalation exposure on visual-evoked potentials, antioxidant enzyme systems, and lipid-peroxidation levels of the brain and eye.**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Inhalation
Animal model	
Species and strain	Rat, Swiss albino
Disease models (e.g. diabetes, allergy, obesity)	–
Housing conditions	
Housing condition	Housed in groups of four to five rats in stainless steel cages under standard conditions (24 ± 2°C and 50 ± 5% humidity) with a 12-h light–dark cycle.
Diet name and source (if reported)	Not reported
Treatment	
Test material	Sulfur dioxide
Provider	Not reported
Compound purity	Not reported
Vehicle used	Air
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	10 ppm, 7 days/week, 1 h/day Calculated to correspond to an internal dose of 1.2 mg/kg bw per day
Route of administration (diet, drinking water, gavage)	Inhalation 1 m^3 exposure chamber; constant flow rate of 30 l/min
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Young (3 months), adult (12 months) and mature (24 months)

Duration of the exposure	6 weeks,
Study design	
Sex and age at the start of the treatment	Male; Young (3 months), adult (12 months) and mature (24 months)
Number animals/sex/group	3 age group (each age group was subdivided in two (control; SO_2)): 6 groups in total, 10 animals/group
Measured endpoints	Effects on visual-evoked potentials (VEPs), TBARS, a product of lipid peroxidation and the activities of Cu/Zn SOD, GSH-Px and catalase (CAT) of brain and eye.
Time of measurement/observation period	24 h (with food deprivation) after last exposure
Methods to measure the endpoints	VEPs were recorded with cutaneous needle electrodes over the neocortex (0.5 cm in front of and behind bregma) Enzyme assays: Assay of CAT, GSH-Px and Cu/Zn SOD activity and TBARS assay
Statistical analysis	
Statistical methods	Analysis of variance (one-way repeated measures ANOVA, BMDP7D) was performed on all parameters for the factor of age and groups (i.e. those exposed to SO_2). Amplitude and latency values were subjected to a mixed-design analysis of variance (ANOVA, BMDP2V) using three levels of age (3, 12, 24 months) as the between-subjects variable, and VEPs. I used the Student's test to conduct post hoc comparisons of means. Significance levels were set at $p < 0.05$. ANOVA-BMDP8D was used to calculate the correlations between the antioxidant enzyme and TBARS values in brain and eye with the VEP alterations. The Grapher package programme was used for scatter plots.
Results	
Findings reported by the study author/s	SO_2 inhalation exposure caused increased levels of brain, retina and lens Cu/Zn SOD activity and decreased levels of brain and lens GSHPx activity in all experimental groups with respect to their corresponding control groups, whereas no change was observed in the level of retina GSH-Px activity. No alterations were observed in brain CAT activity. On the other hand, retina CAT activity was slightly decreased in SO_2-exposed rats, but no change was observed in their lens CAT activity. The brain and lens TBARS levels of all SO_2-exposed groups were significantly increased in comparison with their respective control groups. The amount of TBARS was only increased in the retina of the SO_2-exposed 3-month group compared with its control. Of the SO_2-exposed rats, the mean latencies of the P1, N1, P2 and P3 components of the 3-month group, P1, N1 and N2 components of the 12-month group and only P3 of the 24-month group were significantly prolonged in comparison with those of their control groups. The amplitudes of N1P2 and P2N2 in the 12- and 24-month control groups were significantly decreased compared with those of the 3-month group. On the other hand, no differences were observed among those of SO_2-exposed groups.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted effects at 1.2 mg/kg bw per day, the only dose tested, consisting of an age-dependent prolongation of visual evoked potential latency and increased oxidative stress biomarkers in brain, retina and lens.
Further information	
Test substance purity and provider were not reported, reducing confidence in the results of this study	

Kilic (2003) reported that sulfur dioxide inhalation exposure at 10 ppm (calculated to correspond to an internal dose of 1.2 mg/kg bw per day) age-dependently prolonged visual evoked potential latency (most pronounced in young animals) and induced oxidative stress biomarkers in brain, retina and lens. In control animals, there were age-dependent increases in oxidative stress and VEP latency, which possibly explains ('floor effect') why the effects of sulfur dioxide were most pronounced in young animals.

Reference	**Yargicoglu et al., 2007. The effect of sulfur dioxide inhalation on active avoidance learning, antioxidant status and lipid peroxidation during aging**
Source (published/unpublished)	Published
Overview of the study and guideline	
Good laboratory practice (yes/no)	No
Guideline studies (if yes, specify)	No
Overview of the study	Inhalation
Animal model	
Species and strain	Rat, Swiss albino
Disease models (e.g. diabetes, allergy, obesity)	–
Housing conditions	
Housing condition	Housed in groups of four to five rats in stainless steel cages under standard conditions (24 ± 2°C and 50 ± 5% humidity) with a 12-h light–dark cycle.
Diet name and source (if reported)	Not reported
Treatment	
Test material	Sulfur dioxide
Provider	Not reported
Compound purity	Not reported
Vehicle used	Air
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	10 ppm, 7 days/week, 1 h/day Calculated to correspond to an internal dose of 1.2 mg/kg bw per day
Route of administration (diet, drinking water, gavage)	Inhalation 1 m^3 exposure chamber
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Young (3 months), middle-aged (12 months) and old (24 months)
Duration of the exposure	6 weeks
Study design	
Sex and age at the start of the treatment	Male; Young (3 months), middle-aged (12 months) and old (24 months)
Number animals/sex/group	3 age group (each age group was subdivided in two (control and SO_2)): 6 groups in total, 10 animals/group
Measured endpoints	Active avoidance responses Biochemical measurements in hippocampus homogenate: TBARS, a product of lipid peroxidation and the activities of Cu/Zn SOD, GSH-Px and catalase (CAT).
Time of measurement/observation period	24 h (with food deprivation) after last exposure
Methods to measure the endpoints	Active avoidance learning; The animals were trained by using an automated shuttle-box (conditioned stimulus = compartment light), one daily trial for 5 days. Enzyme assays: Assay of CAT, GSH-Px and Cu/Zn SOD activity and TBARS assay.
Statistical analysis	
Statistical methods	Analysis of variance (ANOVA) was performed on all parameters for the factors of age and groups (SO_2 vs. Air). Post hoc comparisons of the means were carried out using the Tukey's test. Significance levels were set at pb0.05. Values were expressed as means±SD.

Results	
Findings reported by the study author/s	The most prominent effect of aging on active performance was also observed in the older group. SO_2 exposure significantly decreased the active avoidance learning in the young group, but it had no effect on this parameter in the middle-aged and the older group compared with their corresponding control groups. SO_2 exposure resulted in increased levels of Cu/Zn SOD activity while decreased level of GSH-Px activity in all experimental groups compared with their corresponding control groups. CAT activities were unaltered. TBARS levels of all SO_2 exposed groups were significantly increased compared with their respective control groups. In conclusion, results from the present research showed that SO_2 exposure resulted in an increase in the lipid peroxidation and caused alterations in antioxidant enzyme activities. Additionally, SO_2 exposure impaired cognitive function only in the young rats during the acquisition phase of active avoidance learning. SO_2 had no effect on body weight and clinical signs. SO_2 exposure increased hippocampal TBARS and Cu/Zn SOD activity and decreased GSH-Px activity; CAT was unaffected by SO_2. SO_2 exposure significantly impaired active avoidance learning in the young group but had no effect in the middle-aged and older group compared with their corresponding control groups. In controls, active avoidance learning was age-dependent (slowest in old animals).
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The Panel noted effects at1.2 mg/kg bw per day, the only dose tested. They consisted of impaired active avoidance learning (light–dark shuttle box) in young animals and increased hippocampal oxidative stress (increased lipid peroxidation and Cu/Zn SOD decreased GSH-Px activity) in young, middle-aged and old rats.
Further information	
Test substance purity and provider were not reported, reducing confidence in the results of this study	

Yargicoglu et al. (2007) reported that sulfur dioxide inhalation exposure to 10 ppm (calculated to correspond to an internal dose of 1.2 mg/kg bw per day) impaired active avoidance learning (light–dark shuttle box) in young animals and increased hippocampal oxidative stress (increased lipid peroxidation and Cu/Zn SOD, decreased GSH-Px activity) in young, middle-age and old rats. The lack of effect of sulfur dioxide on active avoidance learning in older animals is possibly a 'floor' effect, since acquisition was slowest in older controls compared to young controls. Impaired active avoidance learning normally reflects increased anxiety, in which the septo-hippocampal formation plays an important role. The Panel noted that the downstream adversity of the hippocampal oxidative stress endpoints cannot be predicted with any certainty.

Appendix C – Compilation of the results and shortcoming identified in reproductive and developmental toxicity studies

Reference	Zaki et al., 2021	Fathabad et al., 2018	Shekarforoush et al., 2015	Mahmoudi et al., 2017	Li et al., 2018b	Zhang et al., 2016a	Zhang et al., 2016b	Zare et al., 2019	Qureshi et al., 2022
Route	Gavage	Gavage	Gavage	Gavage	Inhalation	Inhalation	Inhalation	Not reported (presumably gavage)	Gavage
Substance	Extract of SO_2[(a)]	$Na_2O_5S_2$	$Na_2O_5S_2$	$Na_2O_5S_2$	SO_2	SO_2	SO_2	$Na_2O_5S_2$	$K_2O_5S_2$
Dose(s) (expressed as mg SO_2 eq/kg bw per day)	Not reported	0; 175.2	0; 6.7; 67.4; 175.2	0; 0.47; 4.7; 47.2	0; 2.1	0; 4.7	0; 5.9	0; 350.5	0; 0.4; 4; 40.3
Vehicle	Distilled water	Distilled water	Not clear (saline or distilled water)	Distilled water	Air	Air	Air	Not clear (saline or distilled water)	Water
Species	Mouse, Swiss Albino	Rat, Wistar	Rat, Wistar	Rat, Sprague Dawley	Mouse, C57BL/6	Rat, Wistar	Mouse, Kunming	Rat, Wistar	Rat, Sprague Dawley
N/group	10	8	8	7	10	24 for body weight and testis weight; 6 for other endpoints	12 for body weight;6 for testes histopathology;10 for sperm quality;5 for blood-testis barrier proteins	10	8
Sex	Male	Male	Male	Male	Male	Male	Male	Male	Male
Age; body weight	6–8 weeks; 22 g	Not provided; 220–250 g	Not provided; 220–250 g	Not provided; 210–270 g	6 weeks; Not provided	12 weeks; Not provided	8 weeks; 20–25 g	Not provided; 220–250 g	Not provided; 115–125 g
Treatment duration	28 days	28 days	28 days	7 weeks	30 days, 6 h/day	14 days, 4 h/day	8 weeks, 3 h/day	7 weeks	28 days
Endpoints	Sperm morphology (100/mouse, 5 males); serum testosterone; testis histology and EM	Serum testosterone; testis histology, lipid oxidation (MDA), antioxidant enzymes; epididymal tubular diameter and epithelial height; cauda epididymis sperm analysis (number, motility, morphology)	Serum testosterone; testis histology; epididymal tubular diameter and epithelial height; cauda epididymis sperm analysis (number, motility, morphology)	Sperm analysis from ductus deferens (counts, morphology, motility); testis histopathology, volumes of components (seminiferous tubules, germinal epithelium, connective tissue); seminiferous tubule	Serum testosterone, enzymes and hormones involved in steroid synthesis; testis weight and histology; tubular diameter and epithelium height, germ cell analysis of stage VII; epididymal sperm number and morphology;	Body weight; testis weight; sperm analysis (number, motility); testis histology; protein expression of P450, CREM and ACT in testes (immunohistochemistry image analysis performed by computer software)	Body weight; sperm analysis (number, morphology); testis histology; TEM for BTB ultrastructure; mRNA and protein expression of BTB proteins	Serum testosterone; testis weight and volume; testis histology, including germ cell and somatic cell counts (image analysis performed by computer	Testis histology, oxidative stress biomarkers (ROS, malondialdehyde/ TBARS, superoxide dismutase, catalase, peroxide dismutase, GSH), tissue total protein concentrations

Reference	Zaki et al., 2021	Fathabad et al., 2018	Shekarforoush et al., 2015	Mahmoudi et al., 2017	Li et al., 2018b	Zhang et al., 2016a	Zhang et al., 2016b	Zare et al., 2019	Qureshi et al., 2022
				length; numbers of different germ cell classes and somatic cells	caspase-3 activity; germ cell apoptosis (TUNEL); testis H2O2, MDA, total superoxide dismutase (T-SOD) activity and total antioxidant capacity (T-AOC).			software); MDA in testis	
Results	Sperm abnormality ↑; testosterone ↓; disorganisation of testicular tubules ↑;various ultrastructural changes.	Testosterone ↓; damage to seminiferous tubules, impaired spermatogenesis; Leydig cells ↓; epididymal parameters ↓; sperm count ↓, normal morphology ↓, motility ↓; glutathione reductase ↓, catalase ↓; MDA ↑	Testosterone ↓; damage to seminiferous tubules, impaired spermatogenesis; Leydig cells ↓; epididymal parameters ↓; sperm count ↓, normal morphology ↓, motility ↓. Effects observed at ≥ 67.4 mg/kg bw/d	Sperm counts ↓, normal morphology ↓; motility ↓; tubule volume ↓, germinal epithelial volume ↓, connective tissue volume ↑, seminiferous tubule length ↓; numbers of germ cells, Sertoli cells and Leydig cells ↓. Effects observed at > 4.7 mg/kg bw/day	Sperm counts ↓; sperm abnormality ↑; testis pathology and apoptosis ↑; impaired spermatogenesis; testicular H2O2 and MDA ↑; T-SOD activity ↓	Body weight ↓ but not testis weight; impaired spermatogenesis, damage of spermatogenic tubules; sperm motility ↓; CREM and ACT↑	Sperm counts ↓; sperm abnormality ↑; testis pathology ↑; desmoglein-2 mRNA and protein ↓, other BTB proteins show lower protein expression	Testis weight and volume ↓; loss of all germ cell types, Leydig cells and Sertoli cells; MDA ↑; testosterone ↓	ROS and TBARS ↑; CAT, SOD, POD, GSH and total protein ↓; testis pathology and germ cell loss ↑
Flaws	Dose not reported; method for allocation of animals to treatment groups not reported; selection of 5/10 mice for sperm analysis not explained or justified; insufficient sperm number/animal examined; results of testis histopathology and TEM not quantified; magnification of testis	Testes fixed in 10% formaldehyde; attrition 2/8 animals in each group; feed not characterised; histomorphometric evaluation and sperm analysis not performed blinded to treatment; morphological abnormality of sperm unusually high in the control group; sperm count and motility measurement not state-of-the-art; time of day of blood sampling for testosterone levels not reported.	Testes fixed in 10% formaldehyde; attrition 2/8 animals in each group; feed not characterised; histomorphometric evaluation and sperm analysis not performed blinded to treatment; morphological abnormality of sperm unusually high in the control group; sperm count and motility measurement not state-of-the-art; time of day of blood sampling for testosterone levels	Source and purity of test substance not reported; housing conditions and feed not reported; sperm analysis by haematocytometer, not blinded; fixation method for testes not reported.	Cages and feed not described; testes fixed in 4% buffered formaldehyde; histomorphometric evaluation and sperm analysis not performed blinded to treatment; morphological abnormality of sperm unusually high in the control group; sperm analysis not state-of-the-art; time of day of blood sampling for testosterone levels not reported; inappropriate statistic procedures.	Attrition 18/24 animals/group; selection of the 6 rats/group for reproductive endpoints was random but has not been explained or justified; absolute testes weight not reported but can be calculated; sperm analysis not state-of-the-art; procedure for the evaluation of testis histopathology not reported and results not quantified; inappropriate statistic procedures.	Not all animals were examined for the various endpoints, and it is unclear how they were selected; results of testis histopathology and TEM not quantified; sperm analysis not state-of-the-art; morphological abnormality of sperm unusually high (18%) in the control group; inappropriate statistic procedures.	Source and purity of test substance not reported; feed not characterised; route of exposure not reported; method for allocation of animals to treatment groups not reported; testis fixation procedure not reported; body weight was measured but not reported; time of day of blood sampling for testosterone	Feed not characterised; method for allocation of animals to treatment groups not reported; testes fixed in 10% formaldehyde; histopathologic evaluation not performed blinded to treatment; sperm and spermatogonia numbers not quantified.

Reference	Zaki et al., 2021	Fathabad et al., 2018	Shekarforoush et al., 2015	Mahmoudi et al., 2017	Li et al., 2018b	Zhang et al., 2016a	Zhang et al., 2016b	Zare et al., 2019	Qureshi et al., 2022
	micrographs incorrectly reported; inappropriate statistic procedure.		not reported; inappropriate statistic procedures.					levels not reported.	

Appendix D – Data extraction from genotoxicity studies

Table D.1: *In vitro* studies

Test system/Test object	Exposure conditions (concentration/duration/ metabolic activation)	Test material	Result	Reliability/ Comments	Relevance of the result	Reference
DNA damage: specific antibody-based detection of phosphorylated H2AX (Ser139) Human hepatoma HepG2 cells	HepG2 cells in the logarithmic growth phase were exposed for 24 h. Five concentrations, selected on the basis of growth inhibition, were assayed in the range of IC5 to IC50. Only the highest concentration tested was specified (0.3 mg/mL).	Sodium sulfite (Na_2SO_3); purity not indicated; prepared in DMSO and diluted in the culture medium	In a multiparameter toxicity assay, treatment with sodium sulfite determined, at the highest concentration tested, a significant increase of signal for phosphorylated H2AX histone, indicating DNA damage. At the same concentration level, and below, altered membrane permeability, mitochondrial membrane potential, intracellular calcium level and increased levels of intracellular ROS, indicative of oxidative stress, were also observed.	2 – Reliable with restrictions There is no specific guideline for this type of study. This study was described and reported with some details. However, limited information on the actual concentrations tested is provided; Only the highest one is disclosed.	Limited As an *in vitro* indicator assay	Qu et al., 2017
Cytogenetic analysis of anaphases in human embryonic lung cells (WI-38)	Cells were treated with 0.0025, 0.025 and 0.25 mg/mL for 24 h. The cytogenetic analysis of anaphases was performed in 263 untreated cells, while lower numbers of score cells were available in treated cultures (171, 96 and 16 at low, intermediate and high dose, respectively). Triethylene melamine (TEM) was used as positive control.	Sodium metabisulphite ($Na_2S_2O_5$); purity not indicated; dissolved in water	Positive Treatments elicited strong toxicity, with few analysable cells. In anaphase cells, increased frequencies of acentric fragments and bridges were observed.	3 – Reliability insufficient The method applied was not adequately validated and it is not considered in the OECD guideline on the conduct of *in vitro* mammalian chromosomal aberration test (TG 473). Moreover, the possible influence of confounders on the results (excessive toxicity, change of pH	Low Because of the lack of validation of the test method and the limited protocol of the study.	Stanford Research Institute, 1972

Test system/Test object	Exposure conditions (concentration/duration/ metabolic activation)	Test material	Result	Reliability/ Comments	Relevance of the result	Reference
				of culture medium) was not considered. The low number of scored cells is also noted.		
Micronucleus test in human lymphocytes	Method: cytokinesis-block protocol with Exp I (3 h exposure, with and without S9): 0.418, 0.731, 1.28 mg/mL Exp II (28 h exposure, without S9): 0.109, 0.19, 0.333 mg/mL For each experimental group 1,000 binucleated cells were analysed from each of two parallel cultures (2,000 cells per concentration, doubled to 4,000 for solvent control and test item in Exp I).	Sodium sulfite (Na_2SO_3); purity 98.5%; dissolved in deionised water	Negative In experiment I, no cytotoxicity and no relevant (i.e. dose related or statistically significant) increase in the number of micronucleated cells was observed up to the highest concentration applied (1.28 mg/mL or 10 mM), either with or without S9. In experiment II, treatments produced concentration-related cytotoxicity, with 55% cytostasis, evaluated with the CBPI, at the highest concentration applied. No relevant increase in the number of micronucleated cells was observed in treated cultures.	1 - Reliable without restriction	High	Documentation provided to EFSA No 11
Micronucleus test in human lymphocytes	Method: cytokinesis-block protocol with cytochalasin-B Exp I (3 h exposure, with and without S9): 0.6, 1.1, 1.9 mg/mL Exp II (28 h, without S9): 0.1, 0.17, 0.29 mg/mL For each experimental group 1,000 binucleated cells were analysed from each of two parallel cultures (2,000 cells	Sodium metabisulphite ($Na_2S_2O_5$); purity 99.0% (w/w); dissolved in deionised water	Negative In experiment I, no cytotoxicity and no increase in the number of micronucleated cells was observed up to the highest concentration applied (1.9 mg/mL or 10 mM), either with or without S9. In experiment II, treatments produced concentration-related cytotoxicity, with 62.9% cytostasis at the highest concentration applied. No relevant (i.e. dose	1 - Reliable without restriction	High	Documentation provided to EFSA No 8

Test system/Test object	Exposure conditions (concentration/duration/ metabolic activation)	Test material	Result	Reliability/ Comments	Relevance of the result	Reference
	per concentration, doubled to 4,000 for solvent control and test item in Exp.I)		related and statistically significant) increase in the number of micronucleated cells was observed cultures treated with the mid and high concentrations. At the lowest concentration, the incidence of micronucleated cells was statistically significantly higher than solvent control (0.75% vs 0.25%), but within the historical control range. Considering the lack of concentration-related response, and the very low value of the concurrent solvent control which likely determined the statistically significance, this finding is considered biologically irrelevant and the overall result of this study as negative.			
Gene mutation assay (at the *Hprt* locus) in Chinese hamster V79 cells	0.04, 0.08, 0.16, 0.32, 0.64, 1.28 mg/mL (4 h exposure, without S9) 0.08, 0.16, 0.32, 0.64, 1.28 mg/mL (4 h exposure, with S9) Maximum concentration 1.28 mg/mL corresponding to 10 mM.	Sodium sulfite (Na_2SO_3); purity 98.5%; dissolved in deionised water	Negative No statistically significant increase of the mutation frequency was observed in any experimental condition. The maximum concentration applied without S9 (1.28 mg/mL) was not analysed for the mutation frequency because of excessive cytotoxicity (cloning efficiency relative to solvent control = 3.3%).	1 - Reliable without restriction	High	Documentation provided to EFSA No 10
Bacterial Reverse Mutation Test: Salmonella Typhimurium strains	Pre-Experiment/Experiment I: 0.003, 0.01, 0.033, 0.1, 0.333, 1, 2.5, 5 mg/plate (plate incorporation test)	Sodium sulfite (Na_2SO_3); purity 98.5%; dissolved	Negative	1 - Reliable without restriction	High	Documentation provided to EFSA No 9

Test system/Test object	Exposure conditions (concentration/duration/ metabolic activation)	Test material	Result	Reliability/ Comments	Relevance of the result	Reference
TA 1535, TA 1537, TA 98, TA 100 and the *Escherichia coli* strain WP2 *uvrA* (pKM101) OECD TG 471 (2020): yes GLP: yes	Experiment II: 0.033, 0.1, 0.333, 1, 2.5, 5 mg/plate (pre-incubation test) With and without S9 (rat) Triplicate plates Negative controls: untreated and deionised water Positive controls: appropriate reference mutagens	in deionised water		Historical control data provided.		
Bacterial Reverse Mutation Test: Salmonella Typhimurium strains TA 1535, TA 1537, TA 98, TA 100 and the *Escherichia coli* strain WP2 *uvrA* (pKM101) OECD TG 471 (2020): yes GLP: yes	Pre-Experiment/Experiment I: 0.003, 0.01, 0.033, 0.1, 0.333, 1, 2.5, 5 mg/plate (plate incorporation test) Experiment II: 0.033, 0.1, 0.333, 1, 2.5, 5 mg/plate (pre-incubation test) With and without S9 (rat) Triplicate plates Negative controls: untreated and deionised water Positive controls: appropriate reference mutagens	Sodium metabisulphite ($Na_2S_2O_5$); purity 99.0% (w/w); dissolved in deionised water	Negative	1 - Reliable without restrictions Historical control data provided	High	Documentation provided to EFSA No 7
Chromosomal aberrations (CA) in human blood lymphocytes	Sodium bisulfite was added to peripheral blood lymphocyte cultures from four donors at the concentrations of 0.005, 0.01, 0.05, 0.1 and 0.2 mg/mL at culture initiation. Treatment time was 48 h (CA) and 72 h (SCE and MN).	Sodium bisulfite ($NaHSO_3$); purity not indicated; dissolved in RPMI 1640 medium	Positive A statistically significant increase in chromatid breaks was observed in lymphocyte cultures of 3 out of 4 donors at 0.01 and 0.05 mg/mL. The incidence of both chromatid and chromosome (isochromatidic) breaks was statistically significantly	2 - Reliable with restrictions The study protocol shows some deviations with respect to the OECD TG473; e.g. from the description provided it is	Limited	Meng and Zhang, 1992

Test system/Test object	Exposure conditions (concentration/duration/ metabolic activation)	Test material	Result	Reliability/ Comments	Relevance of the result	Reference
	Cytochalasin-B was added during the last 24 h in the MN assay. CA and SCE were scored in 200 and at least 25 metaphases per concentration from each donor; MN were scored in 2,000 binucleated cells per donor.		increased in all donors at 0.1 mg/mL, while 0.2 mg/mL was completely toxic in all tests. Treatment determined a concentration-related inhibition of cell proliferation, with a decreased of MI by 40% at 0.1 mg/mL.	concluded that lymphocytes were not cultured in presence of the mitogen prior to the exposure to test chemical, as recommended; moreover, no positive control was performed.		
Sister-chromatid exchanges (SCE) in human blood lymphocytes	The MI and the proportion of first, second and third mitoses were determined in 2,000 lymphocytes and 200 metaphases per experimental point for each donor.		Positive A concentration dependent increase in SCE/cell was observed in all donors, which reached statistical significance at 0.05 mg/mL.	2 - Reliable with restrictions See above	Low Because of reliability restrictions and the lower relevance of SCE in hazard identification	
Micronuclei (MN) in human blood lymphocytes			Positive The number of cells with MN was increased in dose-dependent manner in all donors. The increase reached statistical significance at 1.0 mM in all donors, and at 0.5 mM when individual data were pooled.	2 - Reliable with restrictions See above	Limited Because of reliability restrictions	
in vitro studies of oocyte meiotic maturation (mouse, ewe, and cow oocytes)	Mouse: Metaphase 1: 0, 0.01, 0.025, 0.05, 0.1, 0.15, 0.25, 0.35, 0.5, 0.75, 1, 10 mg/mL Metaphase 2: 0, 0.005, 0.05, 0.1, 0.15, 0.2, 0.25, 0.35, 0.5, 1, 10 mg/mL	Sodium sulfite (Na_2SO_3); purity 98%; vehicle not specified	Inconclusive Inhibition of entry into meiosis of mouse oocytes at all concentrations above 0.01 mg/mL except 0.1 mg/mL. Above 0.5 mg/mL, inhibition was complete. Chromosome 'fuzziness' was observed at 0.025, 0.05, 0.1, 0.35, 0.5 mg/mL but not at 0.15 and	3 – Reliability insufficient Method not validated. Evaluation criteria not standardised. No positive control used. No historical control data reported. The number of oocytes	Low	Jagiello et al., 1975

Test system/Test object	Exposure conditions (concentration/duration/ metabolic activation)	Test material	Result	Reliability/ Comments	Relevance of the result	Reference
	Ewe: 0, 50, 0.1, 0.25, 0.35, 0.5, 1.25 mg/mL Cow: 0, 0.1, 0.25, 0.35, 0.5, 1, 1.25, 1.5 mg/mL		0.25 mg/mL, thus, this effect was not concentration related. Results observed with ewe and cow oocytes were less pronounced. In addition, the authors did not consider the structural changes genetically relevant because they were expected to result in cell death. The authors assigned more relevance to fragmentations with or without rearrangements, however, these effects were observed only sporadically in ewe and cow oocytes.	investigated was not the same for different concentrations and was rather low (6–52 mouse oocytes per concentration). The method does not belong to the methods used for regulatory purposes.		

CA: chromosomal aberrations; CBPI: cytokinesis block proliferation index; DMSO: dimethyl sulfoxide; DNA: deoxyribonucleic acid; GLP: good laboratory practices; IC: inhibitory concentration; LD50: lethal dose, 50%; MI: mitotic index; MN: micronucleus; NCE: normochromatic erythrocytes; OECD: Organisation for Economic Co-operation and Development; PCE: polychromatic erythrocytes; RPMI: Roswell Park Memorial Institute; SCE: Sister Chromatid Exchange; TG: test guideline; TEM: triethylene melamine.

Table D.2: *In vivo* studies

Test system/ Test object	Dose/Route	Test material	Result	Reliability/Comments	Relevance of the result	Reference
Chromosomal aberration in rat bone marrow	Sodium metabisulphite was dissolved in water and administered by oral intubation at three dose levels: 1,200 mg/kg bw, 700 mg/kg bw and 30 mg/kg bw as single and repeated dose (number of dosing not specified). Dose selection was based on oral LD50 determined in the study (2,480 and 1,800 mg/kg bw as single and repeated dose). Animals (3–5 per group, sex not specified) were sacrificed after 6, 24 and 48 h from acute administration. Sacrifice time after repeated dosing not specified. Chromosomal aberrations were scored in 50 metaphases per animal. TEM was used as positive control.	Sodium metabisulphite ($Na_2S_2O_5$); purity not indicated; dissolved in water	Negative There was no increase of cells with chromosomal aberrations in treated rats compared to negative controls. Treatments with sodium metabisulphite did not induce any depression of the mitotic index (evaluated in a number of cells not specified) indicative of cell toxicity.	3 – Reliability insufficient This study is poorly reported, and used a limited protocol with only 50 analysed metaphases per animal (150–250 in total) compared to 200 per animal (1,000 per group) required by OECD test guideline (TG 475). Moreover, no evidence of toxicity to bone marrow was reported, even though the number of cells scored for mitotic index determination was not indicated.	Low For the limitations in study protocol and data reporting.	Stanford Research Institute, 1972
Dominant lethal test in rats	Sodium metabisulphite was dissolved in water and administered by oral intubation to male rats (number not specified) at three dose levels: 1,200 mg/kg bw, 700 mg/kg bw and 30 mg/kg bw as	Sodium metabisulphite ($Na_2S_2O_5$); purity not indicated; dissolved in water	Negative There was no consistent deviation in study parameters which could be attributed to treatment. In particular, the incidence of dead implants/total	2 – Reliable with restrictions The study protocol presents some deviation compared to OECD TG 478 related to the mating schedule (8 weeks instead	Limited. Because of some study limitations.	Stanford Research Institute, 1972

Test system/ Test object	Dose/Route	Test material	Result	Reliability/Comments	Relevance of the result	Reference
	single and repeated dose (number of dosing not specified). Dose selection was based on oral LD50 determined in the study (2,480 and 1,800 mg/kg bw as single and repeated dose). Dominant lethal parameters (average implants, dead implants, corpora lutea and preimplantation losses) were evaluated in females (number not specified) mated with treated males at weeks 1 to 8 after acute treatment (weeks 1 to 7 after subacute-multiple dosing). TEM, administered once, was used as positive control.		implants, representing the dominant lethal factor (% of post-implantation losses), according to OECD TG 478 was not significantly increased at any mating week. A few borderline increases ($P < 0.10$) observed at weeks 7 and 8 after acute treatment were not replicated after repeated administration. TEM produced a distinct positive response (increase of post-implantation losses), with a time trend consistent with the known activity profile of the chemical.	of 10 as recommended for rats) and the lower number of total implants amenable for analysis (approx. 200 instead of 400). However, there are no major scientific issues, and the study results can be considered acceptable even though limited.		
Micronucleus Bone Marrow Mouse (Kunming) 6 M + 6F/group OECD 474: no GLP: no	Inhalation 0, 28 ± 0.89 mg/m^3 6 h/day for 5 days Positive control: no Sampling time: 24 h after last exposure Analysis of 1,000 PCE/mouse	Sulfur dioxide; purity 99.99%	Positive Statistically significant increases of mono-, bi- and polymicronuclei. Statistically significant increase in relative liver and kidney weights and significant decrease in relative lung and spleen weights	3 – Reliability insufficient PCE/NCE ratio not reported Only 1 dose tested No positive control.	Low	Ruan et al., 2003
Chromosomal aberrations	Group 1 (G1): control, Groups 2, 3 and 4 (G2, G3, G4) were given daily oral	Sodium sulfite (Na_2SO_3); purity	A significant and dose-related increase of structural chromosomal	3 – Reliability insufficient	Low Beyond the insufficient reliability, the	Mahmoud et al., 2015

Test system/ Test object	Dose/Route	Test material	Result	Reliability/Comments	Relevance of the result	Reference
8 female albino Wister rats 4 groups (2 rats/group)	doses of Na_2SO_3 dissolved in drinking water at concentrations corresponding to the dose levels of 9.4, 23.6 and 94.5 mg SO_2 equivalents/kg bw per day respectively for 12 weeks	96%; dissolved in drinking water	aberrations in bone marrow cells was reported in animals receiving sodium sulfite. Both chromatid and chromosome-type aberrations were increased. Administration of sodium sulfite also altered haematological parameters (haemoglobin concentration, red and white blood cells count (the latter reduced of 60–80%), haematocrit and platelet count), indicating strong bone marrow toxicity.	The study protocol has major limitations compared to OECD TG 475: only two animals per group analysed, instead of five as a minimum, with less scored metaphases than required (300 instead of 1,000 per group as a minimum); mitotic index not determined; no positive control.	interpretation of the significance of study results is difficult because of the distinct haematotoxicity elicited by the repeated administration of sodium sulfite in all treated groups.	
DNA damage: Comet assay Liver Male Wistar rats (12-week-old) 6/group OECD 489: no GLP: no [The main aim of the study was to investigate the combined effect of NaF and SO_2]	Inhalation 0, 39.3 ± 13.1 mg/m^3 (G3) G1: control. G2: NaF group (100 mg NaF/L in the drinking water). G3: SO_2 group, with SO_2 in ambient air (0.015 mg/mL SO_2, 4 h/day). G4: NaF + SO_2 group. Sampling after 2, 4, 6 and 8 weeks Positive control: No(it was stated that cyclophosphamide was used as positive control but no data were reported). The levels of DNA damage were measured by the BAB Bs Comet assay system.	Sulfur dioxide; purity 99.99%	Positive Statistical increase of ratio tailing and comet tail length (especially at week 4). Grade II at weeks 2, 4 and 6 (general rupture of DNA chain with small comet head) Toxicity: non-significant decrease of the liver organ coefficient. Liver histopathological alterations: liver cords were chaotically arranged, welling of liver cells, granular changes in cytoplasm, degree of dispersion of liver steatosis.	3 - Reliability insufficient Only one dose tested. Only 100 cells scored/group. Unusual parameters used to measure DNA damage (% tail DNA not measured). No positive control data reported.	Low	Liang et al., 2018

Test system/ Test object	Dose/Route	Test material	Result	Reliability/Comments	Relevance of the result	Reference
	Tailing DNA in 100 cells/group. 25 cells chosen randomly and photographed to measure length of DNA migration, grading. Grade I: tailing length/diameter of the nucleus < 1, Grade II: 1 < tailing length/diameter of the nucleus < 2 and grade III: tailing length/diameter of the nucleus ≥ 2. Grades I and II indicate generic rupture of the DNA chain. Grade III indicates severe damage with a small head and a large, bright tail which looks like a broom.					
DNA damage: Comet assay Brain Male Wistar rats 6/group OECD 489: no GLP: no [The main aim of the study was to investigate the combined effect of NaF and SO_2]	Inhalation 0, 39.3 mg/m^3 (G3) 3 h/day G1: control. G2: NaF. G3: SO_2. G4: NaF + SO_2 Sampling: after 8 weeks Positive control: No Counting tailing DNA in 200 cells/sample. 25 cells were chosen randomly and photographed to measure the length of DNA migration and to grade the cells in each sample (see above).	Sulfur dioxide; purity 99.99%	Positive Statistical increase of ratio tailing and comet tail length. Increase grade II and III Histological alterations in brain: shrunken neurons, darkly stained small nucleus and decreased cell numbers. For definition of Grades, see Liang et al., 2018.	3 - Reliability insufficient Only one dose tested The number of cells scored per group is inconsistently reported and lower than recommended. Unusual parameters used to measure DNA damage (% tail DNA not measured). No positive control group.	Low	Wang et al., 2018

Test system/ Test object	Dose/Route	Test material	Result	Reliability/Comments	Relevance of the result	Reference
DNA damage: Comet assay Kidney Male Wistar rats (12-week-old) 6/ group OECD 489: no GLP: no [The main aim of the study was to investigate the combined effect of NaF and SO_2]	Inhalation 0, 39.3 ± 13.1 mg/m^3 (G3) 4 h/day G1: control. G2: NaF. G3: SO_2. G4: NaF + SO_2 Sampling after 2, 4, 6 and 8 weeks Positive control: No(it was stated that cyclophosphamide was used as positive control but no data were reported). Comet length measured by BAB Bs Comet Assay System Evaluation of tailing ratio, comet length and DNA damage grade Tailing DNA in 100 cells/ sample. 25 cells chosen randomly and photographed to measure length of DNA migration, grading (see above).	Sulfur dioxide; purity 99.99%	Positive At 4 and 6 weeks, significant increasing trend of comet length tailing ratio (especially at week 4). Grade II and grade III increased when compared with control group Decrease BW. No effect on kidney weight. Morphological alterations of renal tubules: hyaline cast, interstitial congestion, lymphocytic infiltration in glomeruli, renal tubular epithelial cell exfoliation For definition of Grades see *Liang* et al., 2018.	3 - Reliability insufficient Only one dose tested Only 100 cells scored/group Unusual parameters used to measure DNA damage (% tail DNA not measured). No positive control data reported.	Low	Gao et al., 2018
Damage in meiosis oocytes of female mammalians (Camm mice)	1st series: a single intravenous dose of 1, 2.5 or 5 mg was given to 6 mice (control: another 6 mice)	Sodium sulfite (Na_2SO_3); purity 98%; vehicle not specified	Negative No abnormalities, neither structural changes nor any effects on second metaphase complements were detected	3 – Reliability insufficient Method not validated. Evaluation criteria not standardised. No positive control used. No historical control data reported. The number of oocytes	Low	Jagiello et al., 1975

Test system/ Test object	Dose/Route	Test material	Result	Reliability/Comments	Relevance of the result	Reference
	Exposure: After 96 h, oocytes were removed and cultured *in vitro* for 14 h. 2nd series: a single intravenous dose of 5 mg per mouse was given to 6 mice with appropriate controls. Exposure: After 24, 48, 72 and 96 h, oocytes were removed and cultured *in vitro* for 14 h. 3rd series: a single intravenous dose of 5 mg per mouse was given to 6 mice during the course of induced preovulatory follicular enlargement and meiotic maturation.			investigated was not the same for different doses and was rather low (28–84 mouse oocytes per dose). The method does not belong to the methods used for regulatory purposes.s		

CA: chromosomal aberrations; CBPI: cytokinesis block proliferation index; DMSO: dimethyl sulfoxide; DNA: deoxyribonucleic acid; GLP: good laboratory practices; IC: inhibitory concentration; LD50: lethal dose, 50%; MI: mitotic index; MN: micronucleus; NCE: normochromatic erythrocytes; OECD: Organisation for Economic Co-operation and Development; PCE: polychromatic erythrocytes; RPMI: Roswell Park Memorial Institute; SCE: Sister Chromatid Exchange; TG: test guideline; TEM: triethylene melamine.

Appendix E – Background information on thiamine deficiency

Chauhan et al. (2018) reported that thiamine deficiency in male mice (induced by feeding a thiamine-deficient diet and daily intraperitoneal injection of pyrithiamine for 8–10 days) induced time-related brain oxidative stress (increased LPO TBARS, decreased GSH, CAT, glutathione reductase, GSH-Px and SOD) and moderate to extensive cortical neuron loss. Ikarashi et al. (2009) reported neurotoxicity after feeding rats a thiamine-deficient (TD) diet for 37 days; there was marked vacuolar degeneration, mainly of astrocytes, in medulla, hippocampus and cerebral cortex, also impaired learning and memory and increased anxiety (both hippocampus-related endpoints). The authors proposed astrocyte dysfunction-induced glutamate excitotoxicity as the mechanism for the neurotoxicity, since 'astrocytes are among the first cells to be affected by thiamine deficiency before neuronal cell death'. Inaba et al. (2016) reported that thiamine-deficient mice exhibit a decrease in neurons in the CA1, CA3 and dentate gyrus (DG) regions of the hippocampus and reduced density of wide dendritic spines in the DG, and a corresponding deficit in hippocampus-dependent memory formation; hippocampus-independent memory was not affected.

Inactivation of thiamine by sulfite is frequently discussed in terms of inactivation of dietary thiamine, although EFSA ANS Panel (2016) also referred to inactivation of thiamine in ingested food in the stomach. In principle, sulfite in organs could potentially also inactivate thiamine locally, e.g. in the brain. Experimentally administered sulfite reaches the brain; Wang et al. 2016 reported a peak concentration Cmax in rat prefrontal cortex of 272 uM sulfite, 30 min after intraperitoneal injection of 500 mg/kg bw of Na2SO3 500 mg/kg. Presumably this amount of sulfite will inactivate brain thiamine. There are no direct data showing how quickly brain thiamine depletion could affect physiological responses in nervous tissue, but Doerge et al. (1982) reported that within 1 min of exposure, thiamine 0.1–100 μM dose-dependently inhibited Na^{2+} efflux in Torpedo electroplax membrane fragments. Local depletion of thiamine in the brain will thus likely have relatively rapid effects on neural function.

Thiamine deficiency will impact mitochondrial thiamine, which is present in the brain primarily as thiamine diphosphate (TDP) and thiamine triphosphate (TTP), and which is a co-factor in catalysing the conversion of pyruvate to acetyl-CoA (Hrubsa et al., 2022). Thiamine deficiency thus impairs cerebral energy intermediary metabolism, limits oxidative phosphorylation and efficient ATP generation, leading to mitochondrial dysfunction and oxidative stress and neurotoxicity (Butterworth, 2003). Gangolf et al. (2010) reported that TTP is found mainly in brain mitochondria. TTP could thus be pivotal in thiamine deficiency neurotoxicity, but this has not been investigated (see for example Bettendorff, 2021).

The cellular locus of thiamine deficiency-induced neurotoxicity may be in astrocytes and oligodendrocytes, not primarily in neurons. Hazell et al. (1998), Desjardins and Butterworth (2005) and Afadlal et al. (2014) summarised data that thiamine deficiency-induced oxidative stress and loss of function in astrocytes leading to glutamate-induced NMDA excitotoxicity and neuron loss. Related to this, Zera and Zastre (2018) reported that thiamine deficiency induces pyruvate accumulation in mouse primary astrocytes and increases expression of HIF-1α, a hypoxic/ischemic stress response transcription factor that regulates pro-apoptotic/necrotic responses ('pseudo-hypoxic activation'). In support of the proposed astrocyte glutamate excitotoxicity hypothesis, Makino et al. (2019) reported that memory impairment and anxiogenic effects induced by thiamine deficiency in mice are blocked by memantine, an NMDAR antagonist which suppresses glutamate excitotoxicity. Thiamine deficiency also affects oligodendrocytes (leading to demyelination) (Langlais and Zhang, 1997; Chatterton et al., 2020). Demyelination prolongs VEP latency (You et al., 2011; You et al., 2015), as observed after oral sulfite (e.g. Ozturk et al., 2011).

Annexes A, B, C, D, E, F, G and H

The annexes listed below are available under the Supporting Information Section of the online version of this scientific opinion.

Annex A. Search methodology for the literature search
Annex B. Criteria for assessing toxicity and genotoxicity studies
Annex C. Exposure data and estimate

Table C.1: Summary of reported use levels (mg/kg or mg/L as appropriate) of sulfur dioxide–sulfites (E 220–228)
Table C.2: Summary of occurrence data submitted by Member States on sulfur dioxide–sulfites (E 220–228) (mg/kg)
Table C.3: Number and percentage of food products labelled with food additive sulfur dioxide–sulfites (E 220–228) s out of the total number of food products present in the Mintel GNPD per food subcategory between 2018 and May 2022
Table C.4: Dietary surveys used for the estimation of chronic dietary exposure to sulfur dioxide–sulfites (E 220–228)
Table C.5: Concentration data used in the exposure assessment scenarios (mg/kg or mL/kg as appropriate)
Table C.6: Summary of estimated exposure to sulfur dioxide–sulfites (E 220–228) for the refined maximum level exposure scenario and the refined brand-loyal exposure scenarios per population group and survey: mean and 95th percentile (mg SO2 equivalents/kg bw per day)
Table C.7: Main food categories contributing to the exposure of sulfur dioxide–sulfites (E 220–228) (number of surveys by contribution class) in the MPL scenario
Table C.8: Main food categories contributing to the exposure of sulfur dioxide–sulfites (E 220–228) (number of surveys by contribution class) in the refined brand-loyal scenarios
Table C.9: Main food categories contributing to the exposure of sulfur dioxide–sulfites (E 220–228) (number of surveys by contribution class) in the refined non-brand-loyal scenarios

Annex D. BMD modelling from developmental toxicity studies – rats (study year as a covariate)
Annex E. BMD modelling from developmental toxicity studies – mice (study year as a covariate)
Annex F. BMD modelling from developmental toxicity studies – rats (study year and tested substance as covariates)
Annex G. BMD modelling from developmental toxicity studies – mice (study year and tested substance as covariates)
Annex H. BMD modelling from Ozturk et al. (2011) study