European Food Safety Authority

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SCIENTIFIC OPINION

Scientific Opinion on 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) as food additives[1]

EFSA Panel on Food additives and Nutrient Sources added to Food (ANS)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion re-evaluating 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) when used as food additives. The Panel noted that sulfur dioxide, bisulfite and sulfite ions existed in a series of equilibria and that these would favour bisulfite ions at the pH of the stomach and sulfite ions at physiological pHs. Therefore, it was considered that once ingested, based on their capacity to form sulfite ions, read across between the different sulfite sources is possible; however, the Panel noted the uncertainties about the reactivity of sulfites in different foods and the resulting reaction products. The overall limited database did not indicate any concern for genotoxicity and did not report any effect in the available chronic, carcinogenicity and reprotoxicity studies after oral exposure in the diet, by gavage, or in the drinking water. A no observed adverse effect level (NOAEL) of 70 mg SO_2 equivalent/kg body weight (bw) per day was identified from a long-term toxicity study in rats. However, the 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 equivalent/kg bw per day (derived using a default uncertainty factor) would remain adequate but should be considered temporary while the database was improved. The 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 Panel further concluded that exposure estimates to sulfur dioxide and sulfites were higher than the group ADI of 0.7 mg SO_2 equivalent/kg bw per day for all population groups.

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[1] On request from European Commission, Question No EFSA-Q-2011-00445, EFSA-Q-2011-00446, EFSA-Q-2011-00447, EFSA-Q-2011-00448, EFSA-Q-2011-00449, EFSA-Q-2011-00450, EFSA-Q-2011-00451 and EFSA-Q-2011-00452, adopted on 9 March 2016.

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[3] Acknowledgement: The Panel wishes to thank the members of the Standing Working Group on the re-evaluation of food additives other than gums and colours: Polly Ester Boon, Dimitrios Chrysafidis, Birgit Dusemund, David Gott, Rainer Gürtler, Ursula Gundert-Remy, Claude Lambré, Jean-Charles Leblanc, Daniel Marzin, Peter Moldeus, Pasquale Mosesso, Dominique Parent-Massin, Ivan Stankovic, Paul Tobback, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen and Matthew Wright for the preparatory work on this scientific opinion and EFSA staff members: Davide Arcella, Dario Battacchi, Georges Kass, Francesca Romana Mancini, Ana Rincon and Alexandra Tard for the support provided to this scientific opinion. The ANS Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

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KEY WORDS

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

SUMMARY

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion re-evaluating the safety 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). The term 'sulfites' will be used throughout this document whenever all these substances are referred to as a group.

Sulfur dioxide and sulfites are authorised as food additives in the European Union (EU) in accordance with Annex II and Annex III to Regulation (EC) No 1333/2008. In 1986, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) allocated a group acceptable daily intake (ADI) of 0–0.7 mg SO_2 equivalent/kg body weight (bw) per day for sulfur dioxide and sulfites. In 1994, the Scientific Committee on Food (SCF) similarly allocated a group ADI of 0–0.7 mg SO_2 equivalent/kg bw per day based on pigs and rats studies. The group ADI allocated by JECFA and the SCF has in both cases been determined mainly based on irritating local effects and set under the assumption that results from all sulfiting substances can be compared when taking into consideration the amount of SO_2 being the theoretical result of dosing.

The Panel noted that endogenous sulfites can be generated as a consequence of the body's normal processing of sulfur-containing amino acids and that sulfites may occur as a consequence of fermentation and are naturally present in a number of foods and beverages.

Knowledge on the toxicokinetics of sulfites is primarily based on old data. Sulfites used in foods may be partially liberated as sulfur dioxide both during and after ingestion and the sulfur dioxide inhaled and absorbed through the lungs as sulfite. Sulfite is converted to sulfate, primarily in the liver, by the enzyme sulfite oxidase (SOX). The Panel noted that the activity of this enzyme is lower (10–20 times) in the human liver compared to the rat and that this was the rationale for using rats with a SOX-deficient activity in some toxicity studies. Other studies showed that an alternative pathway of the metabolism of sulfites exists, so that intermediate formation of sulfur trioxide radicals may occur. The Panel noted the absence of specific absorption, distribution, metabolism and excretion (ADME) studies measuring reaction products from the different sulfites. Furthermore, the Panel noted that it was not possible to ascertain the relative contribution of the differing pathways of sulfite metabolism at realistic levels.

Short-term toxicity studies in SOX-competent or -deficient rats indicated a no observed adverse effect level (NOAEL) of 70 mg SO_2 equivalent/kg bw per day. The critical effect was gastric lesions. In subchronic studies in pigs, a NOAEL of 72 mg SO_2 equivalent/kg bw per day was identified, and higher levels caused mucosal lesions in the stomach and the first part of the large intestine.

Based on the available genotoxicity data, the Panel considered 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 did not raise a concern with respect to genotoxicity.

Only old long-term studies, restricted to sodium and potassium bisulfites, were available. No carcinogenic potential was detected in these studies and a NOAEL of 70 mg SO_2 equivalent/kg bw per day was identified. The Panel noted that a possible tumour promoting activity of sulfites in the pylorus of the glandular stomach was reported in two initiation–promotion studies in rats, which may be related to hyperplasia of the fundic glands induced by sodium metabisulfite.

The available two- and four-generation toxicity studies in rats with potassium sulfite were poorly reported, they did not meet the current requirements for end points tested and the doses used were low. Therefore, they were of limited use for this evaluation. Potassium sulfite has also been tested for induction of malformations in offspring in rats and mice with no apparent effects. In addition, sodium metabisulfite and sodium bisulfite have been tested in rats, mice and hamsters with no apparent effects

when dams were dosed during organogenesis with doses up to 262 mg SO_2 equivalent/kg bw per day for 10 days. The Panel noted that studies on reproductive and developmental toxicity were lacking for calcium sulfite, calcium bisulfite and potassium bisulfite.

Sulfite sensitivity occurs mostly in asthmatics and may occur in a small number of non-asthmatic individuals. Numerous studies confirm that sensitivity to sulfites is prevalent and, after oral intake, may present as asthmatic attacks in people suffering from asthma, but also as urticaria and angiooedema in other individuals. Most sulfite sensitivities are not true allergic reactions and the mechanisms of sulfite intolerance are unclear and likely due to various biological reactions, depending on the individual genetic background. The Panel considered that the minimal dose able to elicit a reaction is variable and dependent upon the individual physiological characteristics.

To assess the dietary exposure to sulfur dioxide–sulfites (E 220–228) from their use as food additives, the exposure was calculated based on (1) the maximum permitted levels set out in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*) and (2) usage or analytical data (defined as the *refined exposure assessment scenario*).

Considering all the analytical data received, the Panel decided to assess the refined exposure to sulfur dioxide–sulfites (E 220–228) considering two sets of concentration data: a) reported use levels and analytical results (not exceeding the MPLs) for food categories for which direct addition of sulfur dioxide–sulfites (E 220–228) is authorised, according to Annex II to Regulation (EC) No 1333/2008 and Annex IB to Regulation (EC) No 606/2009; and b) in addition to the previous dataset, the available analytical data for foods categories which may contain sulfur dioxide–sulfites (E 220–228) 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.

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.

However, the Panel noted that the remaining sulfur dioxide, bisulfite and sulfite ions existed in a series of equilibria and that these would favour bisulfite ions at the pH of the stomach and sulfite ions at physiological pHs. Therefore, the Panel considered that once ingested, based on their capacity to form sulfite ions, read across between the different sulfite sources is possible.

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 and 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.

However, the Panel noted that:

- the overall available database was limited;
- this database did not indicate any concern for genotoxicity and did not report effects in chronic, carcinogenicity and reprotoxicity studies after oral exposure in the diet, by

gavage, or in the drinking water. A NOAEL of 70 mg SO2 equivalent/kg bw per day was identified from a long-term toxicity study in rats;

- although the majority of the available toxicological studies were performed using sodium or potassium metabisulfite, because exposure is predominantly to the sulfite ion irrespective of its source, read across of these data to other sulfites and sulfur dioxide is feasible.

In addition, the Panel observed that:

- the exposure to sulfur dioxide–sulfites was:
  - above the group ADI of 0.7 SO_2 equivalent/mg kg per bw in all population groups at both the mean and the high level in the brand-loyal scenario, and at the high level in the non-brand-loyal scenario, when calculated in the refined exposure scenario considering only direct addition of sulfur dioxide–sulfites to food;
  - above the group ADI in all populations at the high level for the non-brand loyal scenario in the refined exposure scenario considering additional exposure taking into account the available analytical data for foods categories which may contain sulfur dioxide–sulfites (E 220–228) 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.
- there are numerous reports of sensitivity/intolerance reactions in humans exposed to sulfited solid foods and beverages.

Overall, considering that**:**

- the group ADI allocated by JECFA and the SCF of 0–0.7 mg SO_2 equivalent/kg bw per day based on a NOAEL in both the pigs and rats studies was on the assumption that they can result from all sulfiting substances;
- the toxicological database on sulfites and their reaction products with food components was limited;
- based on the common exposure to sulfite ions, extrapolation between studies using various sulfite sources was possible;
- there were data suggesting that the critical effects of sulfites (and particularly sulfur dioxide) were site of contact effects, however, it was not possible to ascertain whether there were no systemic effects;
- improving the toxicological database might result in either an increase or a decrease in the group ADI, depending on, for example, the effects detected, the identified point of departure and the use of chemical specific rather than default uncertainty factors.

The Panel concluded that the current group ADI of 0.7 mg SO_2 equivalent/kg bw per day (derived using a default uncertainty factor of 100) would remain adequate but should be considered temporary whilst the database was improved.

The Panel further concluded that exposure estimates to sulfur dioxide–sulfites were higher than the group ADI of 0.7 mg SO_2 equivalent/kg bw per day for all population groups.

The Panel recommended that:

- the database and the temporary group ADI should be re-evaluated. The Panel noted that the studies recommended below could require 5 years for completion;

- additional studies performed according to recent internationally recognised Organisation for Economic Co-operation and Development (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.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The Regulation (EC) No 1333/2008[4] of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union (EU). In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the EU before 20 January 2009 has been set up under the Regulation (EU) No 257/2010[5]. This Regulation also foresees that food additives are re-evaluated whenever necessary in the light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU[6] of 2001. The report 'Food additives in Europe 2000[7]' submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with the highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of the adoption of Regulation (EU) 257/2010, the 2003 Terms of Reference are replaced by those below.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission asks EFSA to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

[4] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.

[5] 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, p. 19–27.

[6] COM(2001) 542 final.

[7] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002, 560.

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety 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). The term 'sulfites' will be used throughout this document whenever all these substances are referred to as a group.

Sulfur dioxide and sulfites are authorised as food additives in the EU in accordance with Annex II and Annex III to Regulation (EC) No 1333/2008. They have been evaluated by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) in 1986 (JECFA, 1987) and in 1998 (JECFA, 1999). Sulfites were also evaluated by the Scientific Committee on Food (SCF) in 1994 (SCF, 1996) and EFSA (EFSA NDA Panel, 2004, 2014).

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following several public calls for data[8,9,10]. To assist in identifying any emerging issue, EFSA has outsourced a contract to deliver an updated literature review on toxicological endpoints, dietary exposure and occurrence levels of sulfur dioxide and sulfites (E 220–228), which covered the period from January 2011 up to the end of 2015.

2. Technical data

2.1. Identity of the substances

2.1.1. Sulfur dioxide (E 220)

Sulfur dioxide (E 220) has a chemical formula SO_2. It has a molecular weight of 64.06 g/mol, Chemical Abstracts Service (CAS) Registry Number 7446-09-5 and the European Inventory of Existing Commercial chemical Substances (EINECS) number is 231-195-2. Its structural formula is given in Figure 1.

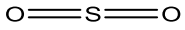

Figure 1: Structural formula of sulfur dioxide

The most commonly used synonyms are sulfurous acid anhydride and sulfurous oxide.

Sulfur dioxide is a colourless, non-flammable gas with a strong pungent suffocating odour (Commission Regulation (EU) No 231/2012[11]). It is soluble in water (110 g/L at 20°C; Ough and Were, 2005) and ethanol (114 v in 1 v) (JECFA, 2006). The pKa values for sulfur dioxide are 1.76 and 7.20 (Ough and Were, 2005).

[8] Call for scientific data on food additives permitted in the EU and belonging to the functional classes of preservatives and antioxidants. Published: 23 November 2009. Available from: http://www.efsa.europa.eu/en/dataclosed/call/ans091123a.htm

[9] Call for food additives usages level and/or concentration data in food and beverages intended to human consumption. Published: 27 March 2013. Available online: http://www.efsa.europa.eu/en/dataclosed/call/130327.htm

[10] Call for scientific data on selected food additives permitted in the EU- Extended deadline: 1 September 2014 (batch A), 1 November 2014 (batch B) Available online: http://www.efsa.europa.eu/en/dataclosed/call/140324.htm

[11] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1–295.

2.1.2. Sodium sulfite (E 221)

Sodium sulfite (E 221) has a chemical formula Na_2SO_3 for the anhydrous and $Na_2SO_3 \cdot 7H_2O$ for the heptahydrate form. The anhydrous form has a molecular weight of 126.04 g/mol, a CAS Registry Number of 7757-83-7 and the EINECS Number is 231-821-4. The heptahydrate form has a molecular weight of 252.16 g/mol and the CAS Registry Number is 10102-15-5 (heptahydrate). The structural formula (anhydrous) is given in Figure 2.

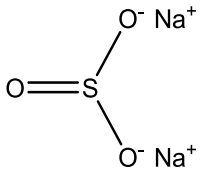

Figure 2: Structural formula of sodium sulfite anhydrous.

Sodium sulfite is a white crystalline powder or colourless crystals (Commission Regulation (EU) No 231/2012). It is freely soluble in water (up to 280 g/L (40°C); Ough and Were, 2005) and sparingly soluble in ethanol (JECFA, 2006). It undergoes oxidation in air. Its solutions are alkaline to litmus and to phenolphthalein (FCC, 2010-2011a).

2.1.3. Sodium bisulfite (E 222)

Sodium bisulfite (E 222) has a chemical formula $NaHSO_3$. It has a molecular weight of 104.06 g/mol, CAS Registry Number 7631-90-5 and EINECS Number 231-548-0. The Panel noted that the EINECS number 231-921-4 indicated in the EU specifications for this food additive is not registered in the EC Inventory[12]. It has the structural formula given in Figure 3.

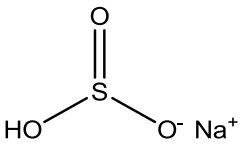

Figure 3: Structural formula of sodium bisulfite.

The most common synonym is sodium hydrogen sulfite.

Commission Regulation (EU) No 231/2012 describes sodium bisulfite as 'a clear, colourless to yellow solution', while JECFA (2006) and the European Pharmacopoeia (European Pharmacopoeia, 2015a) describe it as 'white or almost white, crystalline powder'. It is freely soluble in water (3000 g/L (20°C); Ough and Were, 2005) and slightly soluble in ethanol (JECFA, 2006). It is unstable in air (FCC, 2010-2011b). On exposure to air, it gradually loses some sulfur dioxide and is gradually oxidated to sulfate (European Pharmacopoeia, 2015a).

2.1.4. Sodium metabisulfite (E 223)

Sodium metabisulfite (E 223) has a chemical formula $Na_2S_2O_5$. It has a molecular weight of 190.11 g/mol, CAS Registry Number 7681-57-4 and EINECS Number 231-673-0. It has the structural formula shown in Figure 4.

[12] EC Inventory available online: http://echa.europa.eu/information-on-chemicals/ec-inventory

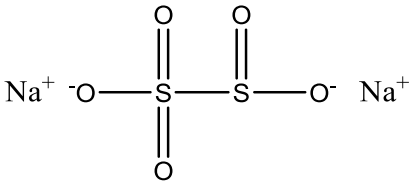

Figure 4: Structural formula of sodium metabisulfite.

The most common synonyms are sodium disulfite, disodium disulfite, disodium pentaoxodisulfate and sodium pyrosulfite.

Sodium metabisulfite occurs in the form white crystals or crystalline powder (Commission Regulation (EU) No 231/2012). It is freely soluble in water (540 g/L (20°C); Ough and Were, 2005) and slightly soluble in ethanol (JECFA, 2006; European Pharmacopoeia, 2015b). Its solutions are acid to litmus (FCC, 2010-2011c).

2.1.5. Potassium metabisulfite (E 224)

Potassium metabisulfite (E 224) has a chemical formula $K_2S_2O_5$, a molecular weight of 222.33 g/mol, CAS Registry Number 16731-55-8 and EINECS Number 240-795-3. It has the structural formula shown in Figure 5.

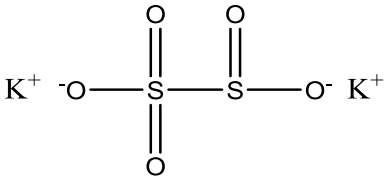

Figure 5: Structural formula of potassium metabisulfite.

Potassium metabisulfite comes in the form of colourless crystals or white crystalline (Commission Regulation (EU) No 231/2012). It is soluble in water (250 g/L (0°C); Ough and Were, 2005) and insoluble in ethanol (JECFA, 2006). It gradually oxidises in air to sulfate and its solutions are acid to litmus (FCC, 2010-2011d).

The most commonly synonyms are potassium disulfite, dipotassium disulfite, potassium pyrosulfite and potassium pentaoxo disulfate.

2.1.6. Calcium sulfite (E 226)

Calcium sulfite (E 226) has a chemical formula $CaSO_3$ and a molecular weight of 120.14 g/mol, CAS Registry Number 10257-55-3 and EINECS Number 233-596-8. The Panel noted that the EINECS number 218-235-4 indicated in the EU specifications for this food additive corresponds to calcium benzoate (EC Inventory[12]). It has the structural formula given in Figure 6.

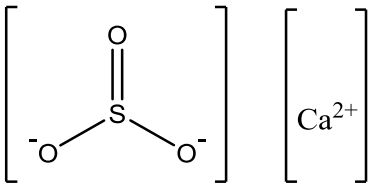

Figure 6: Structural formula of calcium sulfite.

Calcium sulfite occurs as white crystals or white crystalline powder (Commission Regulation (EU) No 231/2012). It slowly oxidises in air to calcium sulfate. It is slightly soluble in water and alcohol, soluble in sulfur dioxide solutions and acids with the liberation of sulfur dioxide (Merck index, 2015).

2.1.7. Calcium bisulfite (E 227)

Calcium bisulfite (E 227) has a chemical formula $Ca(HSO_3)_2$ and a molecular weight of 202.22 g/mol, CAS Registry Number 13780-03-5 and EINECS Number 237-423-7. It has the structural formula shown in Figure 7.

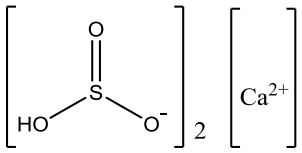

Figure 7: Structural formula of calcium bisulfite

The most commonly used synonym is calcium hydrogen sulfite.

Calcium bisulfite is described as clear greenish-yellow aqueous solution having a distinct odour of sulfur dioxide (Commission Regulation (EU) No 231/2012). On standing in the air, it will form crystals of calcium sulfite dihydrate (Merck index, 2015).

2.1.8. Potassium bisulfite (E 228)

Potassium bisulfite (E 228) has a chemical formula $KHSO_3$, a molecular weight of 120.17 g/mol, CAS Registry Number 7773-03-7 and EINECS Number 231-870-1. It has the structural formula given in Figure 8.

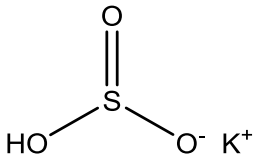

Figure 8: Structural formula of potassium bisulfite

The most commonly used synonym is potassium bisulfite.

Potassium bisulfite occurs in the form of white, crystalline powder with an odour of sulfur dioxide. According to Commission Regulation (EU) No 231/2012, the food additive is an aqueous solution of potassium bisulfite described as clear colourless aqueous solution. Potassium bisulfite is freely soluble in water (1000 g/L (20°C); Ough and Were, 2005).

The theoretical sulfur dioxide yield of the different sulfites is given in Table 1 along with the sulfur dioxide content specified in Commission Regulation (EU) No 231/2012. The Panel noted that the sulfur dioxide yield may vary between different sulfites and the actual specified content may not reach the theoretical yields.

Table 1: Theoretical sulfur dioxide yield (Ough and Were, 2005) and specified content according to Commission Regulation (EU) No 231/2012

Sulfiting agent	**Theoretical yield of SO_2 (%)**	**SO_2 specified content** (Commission Regulation (EU) No 231/2012)
Sulfur dioxide (E 220)	100	Content 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	Content not less than 32% w/w $NaHSO_3$ equal to 19.7%
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	Not less than 39%
Calcium bisulfite (E 227)	None given	6–8% (w/v) (of a solution)
Potassium bisulfite (E 228)	53.5	None specified [150 g SO_2/L] (specified as solution)

2.2. Specifications

Table 2: 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 3: 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 4: 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 5: 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 6: 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	-
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	-

Table 7: 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 8: 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 SO2 content
Selenium	Not more than 5 mg/kg based on the SO2 content
Arsenic	Not more than 3 mg/kg
Lead	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg

Table 9: 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

The Panel noted that, according to the EU specifications, impurities of the toxic elements lead, mercury and arsenic are accepted, respectively, up to concentrations of 5, 1 and 3 mg/kg for sulfur dioxide and 2, 1 and 3 mg/kg for sulfites. The contamination at those levels could have a significant impact on the intake to these metals for which the exposures are already close to the health-based guidance values established by EFSA (EFSA CONTAM Panel, 2009, 2010, 2012).

2.3. Manufacturing process

Sulfur dioxide is produced by burning sulfur in air or oxygen; oxidation of sulfides in the roasting of sulfide minerals; by reduction of sulfuric acid with copper; or by treatment of sulfites or bisulfites with strong acids (Madhavi et al., 1995).

Sodium sulfite (E 221) is commonly produced by reacting sodium carbonate with sulfur dioxide in an aqueous medium. Sodium bisulfite (E 222) is first formed, then neutralised to form sodium sulfite. Sodium bisulfite is neutralised by further addition of sodium carbonate or sodium hydroxide to form sodium sulfite. When sodium carbonate is used for neutralisation, the solution is boiled to expel the carbon dioxide formed during neutralisation. From the neutralised solution, sodium sulfite is obtained by crystallisation. If crystallisation is carried out at temperatures below about 35°C, the crystals formed are sodium sulfite heptahydrate ($Na_2SO_3 \cdot 7H_2O$). When heated at a temperature above 35°C, the heptahydrate melts incongruently resulting in the formation of anhydrous sodium sulfite. In an alternative process, anhydrous sodium sulfite is directly crystallised from the neutralised sodium bisulfite solution by evaporating the water by boiling. Processes for making sodium sulfite involving the above-described reaction, have been described (Butler, 1933; Bowman and Stougaard, 1937; Heinke and Spormann, 1968; Hofmann et al., 1978). These patents generally are concerned with methods for obtaining anhydrous alkali metal sulfite of relatively high degree of purity, hence include certain further purification steps.

Single-step processes for making anhydrous sodium sulfite have also been described. According to Heinke and Spormann (1967), solid alkali metal sulfite salt is obtained by contact of an aqueous solution of sodium hydroxide, sodium carbonate, sodium bicarbonate, with dry sulfur dioxide-containing gas at a temperature sufficiently high that the water introduced with the solution and formed by the reaction of the alkali metal compound with the sulfur dioxide is vapourised.

According to the information provided by industry (Doc. provided to EFSA n. 37), sodium bisulfite (E 222) is produced by chemical reaction of sulfur dioxide gas with aqueous sodium hydroxide solution in usual absorber apparatuses. The concentration of sodium bisulfite solution is controlled by addition of water.

As regards the manufacturing of potassium metabisulfite (E 224), Lüdemann et al. (1968) described a single-step process in which sulfur dioxide, or gases containing sulfur dioxide, reacted with aqueous solutions of potassium hydroxide and/or potassium carbonate. The reaction components are introduced simultaneously into an aqueous solution saturated with potassium sulfite and potassium bisulfite at temperatures between 50°C and 80°C and at a pH in the range between 4 and 7.5. The reaction mixture is then cooled down in order to precipitate the potassium metabisulfite. The potassium metabisulfite is separated by filtration or centrifugation.

2.4. Methods of analysis in food

Many methods exist for the measurement of free, combined (bound) and total sulfites. Most methods are based on removing as much of the free sulfites and the reversibly bound sulfites as possible. Irreversibly bound sulfites cannot be estimated. The determination of free sulfites is important only for industry (wine, beverages, shrimps) to predict the durability of the final product, but there is no maximum authorised amount for free sulfites in EU Legislation.

***Monier–Williams type procedure*:**

According to Fazio and Warner (1990), many available methods for determining sulfites in foods are mostly modifications of the Monier–Williams procedure developed in 1927 and later optimised in 1986 to determine levels down to 10 mg SO_2/kg in foods; meanwhile, methods have been developed with reported limit of detection (LOD) much lower than 10 mg/kg. Many methods used for their determination are based on the Monier–Williams type procedure with volumetric titration, gravimetric, polarographic or via high-performance liquid chromatography (HPLC) quantification. This procedure is based upon distillation of sulfur dioxide from an acidic medium. Sulfur dioxide is then determined either by titration (volumetric method) or by weighting the barium precipitate, having added barium chloride (AOAC 2000; FSA, 2004).

A method employing polarographic detection by differential pulse polarography or squarewave voltammetry also exists (Stonys, 1987).

Pizzoferrato et al. (1990) tested a HPLC method combined with the Monier–Williams procedure and found that it was well suited for analysis in shrimps, mustard and onions where there are otherwise appreciable interference problems. Pizzoferrato et al. (1998) have published the results of the recoveries of sulfites in 10 different food matrices and concluded that the problems of the overestimation of sulfites through the volumetric titration are not relevant when the distillate is consequently analysed via HPLC.

***HPLC after extraction*:**

Chung et al. (2008) presented an analytical method for the determination of free and reversibly bound sulfites in selected foods by using HPLC with fluorometric detection equipped with a pre- and post-column derivatisation system. Sulfites were extracted with a sodium tetrachloromercurate solution, reacted with sodium hydroxide to liberate the reversibly bound sulfites and subsequently separated from other interferences by a size exclusion column and determined by HPLC–fluorescence spectrometry. The method has been applied to a variety of food, with no significant interference encountered in matrixes, such as soy products, cabbage, broccoli, brassica, ginger, fungus, mushroom, mandarin peel, potato chips and biscuits. The LOD was 5 mg/kg.

An analytical method for quantitative detection of sulfites in fresh meat and shrimps has been developed by Iammarino et al. (2010, 2012). The method is based on ion-exchange chromatography with conductivity detection after extraction with a solution of sodium hydroxide, and conformity was demonstrated with Commission Decision 657/2002/EC[13] concerning the performance of analytical methods and the interpretation of results and Regulation 882/2004/EC[14] on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. LODs expressed in sulfur dioxide ranged between 0.34 and 1.03 mg/kg.

Liao et al. (2013) presented a method for the determination of free sulfites in dried fruits by using anion exchange column and conductivity detection after an extraction with a 0.2 N sodium hydroxide aqueous solution.

Robbins et al. (2015) presented a selective method using electrospray ionisation and HPLC–tandem mass spectrometry (HPLC–MS/MS). A total of 12 different types of foods were evaluated. These included dried fruits and vegetables, frozen seafood, molassses and juices. The matrix was extracted with a buffered formaldehyde solution, converting free and reversibly bound sulfite to the stable formaldehyde adduct, hydroxymethylsulfonate. Extracts are prepared for injection using a C18 solid phase extraction (SPE) cartridge and hydroxymethylsulfonate is then separated from other matrix components using hydrophilic interaction chromatography (HILIC) and detected using multiple reaction monitoring (MRM). The limit of quantification (LOQ) expressed in sulfur dioxide varied from 0.12 to 0.75 mg/kg.

Yoshikawa et al. (2015) method using suppressed ion chromatography with the use of a conductivity detector was developed for the determination of free sulfites in wine. The LOD of sulfite expressed in sulfite anion was 0.27 mg/L, calculated by the Panel to be 0.22 mg/L expressed in sulfur dioxide.

For the determination of sulfites in shrimps, Iammarino et al. (2014) applied an ion-exchange chromatographic method with conductivity detection after extraction with the stabilising solution described in the previous publication (Iammarino et al., 2010).

[13]Consolidated version of Commission Decision of 14 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. (2002/657/EC). OJ L 221, 17.8.2002, p. 8.

[14]Consolidated version of Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. OJ L 165, 30.4.2004, p. 1.

***Flow Injection Analysis (FIA)*:**

Numerous flow injection analysis procedures have been described for determining sulfites in food and beverages. Depending on the type of sample, these procedures generally consist of two phases. The first phase is related the extraction process of the sulfating agent, where this must be transferred into the liquid state prior to analysis using appropriate batch pretreatment procedures. The second phase involves injecting the liquid extract into the FIA system where the extracted sulfur dioxide is analysed by a variety of means as described in the review published by Ruiz-Capillas and Jiménez-Colmenero (2009).

Tzanavaras et al. (2009) presented a spectrophotometric method for the determination of total sulfites in white and red wines. The assay is based on the reaction of *o*-phthalaldehyde and ammonium chloride with the analyte in basic medium under sequential injection conditions, where the reaction product passes through a gas diffusion unit followed by alkalisation with NaOH and forms a blue product with an absorption maximum at 630 nm. The reported LOD was 0.3 mg/L expressed in sulfite anion, calculated by the Panel to be 0.24 mg/L expressed in sulfur dioxide.

An automated flow injection analysis system, based on an initial analyte separation by gas-diffusion and subsequent determination by squarewave voltammetry in a flow cell, was developed by Goncalves et al. (2010) for the determination of total and free sulfur dioxide in wine. The proposed method was compared with two iodometric methodologies and demonstrated a LOD of 3 mg/L expressed in sulfur dioxide.

A chemiluminescence method for the determination of sulfite in wine (free and bound) has been developed by combining FIA and its sensitising effect on the known chemiluminescence emission produced by the oxidation of luminol in alkaline medium in the presence of permanganates by Navarro et al. (2010). The LOD was 4.7 µmol of sulfite anion, calculated by the Panel to be 0.3 mg/L expressed in sulfur dioxide.

A compact system encompassing in flow gas diffusion unit and a wall-jet amperometric flow injection analysis detector coated with a supramolecular porphyrin film for the analysis of free sulfites in fruit juices was presented by Martins et al. (2011). The LOD of this method reached the level of 0.043 mg/L expressed in sulfur dioxide.

***Others*:**

Ferrarini et al. (2000) conducted a comparative study to evaluate the total level of sulfites in 12 grape juices containing sulfites at levels around 10 mg/L, determined by three methods involving distillation, one based on aeration–oxidation and one enzymatic method. Analysis of variance disclosed a significant difference among the total SO_2 content in grape juices determined by the five methods. Each analytical method showed limits in relation to their ability to release the combined SO_2. SO_2 bonded to phenolic compounds was better released at low pH in the acidified juice.

A method for the determination of both free and bound sulfites in white wine samples by coulometric titration with electrogenerated iodine was described by Lowinsohn and Bertotti (2001), where the analyte was extracted from samples acidified with hydrochloric acid. Titrations of samples treated with NaOH led to the estimation of the total concentration, the results being in agreement with the ones obtained by the distillation procedure. The LOD was calculated to be 0.6 mg/L expressed in sulfur dioxide.

A reagentless method for sulfites determination is based on the use of an organic conducting polymer, polyaniline, and its absorbance variation at 550 nm, depending on the sulfite concentration. After chemical polymerisation of aniline, a very thin film of polyaniline is obtained. When the change in absorbance at 550 nm was measured for 210 s (stabilisation time), the system showed a linear response, which ranged from 0.025 to 1.50 mg sulfite/L. The method was applied to sulfite

determination in wine samples and the results were in agreement with those obtained by the iodometric titration of free sulfites (de Marcos et al., 2004).

A cyclic voltammetry analysis for the determination of free sulfites in wine was proposed by Makhotkina and Kilmartin (2010). A good correlation was obtained between a cyclic voltammetric measure, based upon the response produced before and after acetaldehyde additions, and the concentration of free sulfur dioxide in eight white wines measured by the Monier–Williams procedure.

Qin et al. (2014) found out that nanoparticles of cobalt oxides have intrinsic oxidase-like activity and can catalytically oxidise peroxidase substrates, such as 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt and 3,3',5,5'-tetramethylbenzidine, to form coloured products (which can be measured via spectrophotometry), a reaction which is inhibited by sulfites. The method was tested in three food matrices and the LOD was 0.053 μmol of sulfite anion, calculated by the Panel to be 0.0034 mg/kg when expressed as sulfur dioxide.

A method for the selective extraction of free and total sulfites from muscle foods (i.e. shrimps) and the following determination by a voltammetric sensor was reported by Schneider et al. (2014). The proposed method was based on the eletrocatalytic oxidation of sulfites at modified glassy carbon electrode fabricated by immobilising 9 μg of acetylferrocene on the surface of the electrode along with 35 μg of carbon black to improve the electron transfer within poly(vinyl butyral) membrane matrix. The LOD was not explicitly given.

A method based on headspace single-drop microextraction in combination with UV–vis microspectrophotometry for the ultrasensitive determination of sulfites in fruits and vegetables was developed by Gómez-Otero et al. (2014). Sample acidification was used for SO_2 generation, which is collected onto a 5,5'-dithiobis-(2-nitrobenzoic acid) microdrop for spectrophotometric measurement. Problems caused by oxidation during the extraction process were addressed. The LOD was 0.06 mg/kg expressed as sulfur dioxide.

Silva et al. (2015) presented a squarewave voltammetric method based on sulfite electrochemical reduction using a carbon-paste electrode chemically modified with multiwalled carbon nanotubes for the quantification of sulfites in commercial beverages. The method is not applicable to red grape juice or red wine samples. The LOD was 1.0 mg/L expressed as sulfur dioxide.

Interference problems from volatile fatty acids in butter flavouring materials were found by Su and Taylor (1995). The authors recommended using alternative methods for the detection of residual sulfites in samples containing significant amounts of volatile fatty acids, such as the sulfite oxidase assay and the colorimetric pararosaniline method.

It is possible to determine the SO_2 content in the headspace of packaged food. The method is based on a gas chromatographic determination, is described by Barnett and Davis (1983) and it has a LOD in the range of ng/ml (μg/L) in the headspace, but there is uncertain how it relates to the content of sulfites in the food as such.

In conclusion, most analytical methods aim to determine the content of free sulfur dioxide/sulfites and the reversibly bound sulfur dioxide/sulfites. Different food matrices may present interference problems with food constituents, and these problems may be overcome by applying the various modified methods according to food type, as described in the literature.

The Panel noted that no analytical methods are available for the determination of irreversibly bound sulfites; therefore, the ingoing amount of sulfites during food production cannot be completely estimated. The Panel also noted that there are methods available which can reach a LOD much lower than 10 mg/kg, which is established by the legislation as a legislative limit for the presence of sulfites.

2.5. Reaction and fate in food

In general, sulfites, when added to foods react with many food components. This has been well described in the review by Taylor et al. (1986). The main reason for the reactivity of sulfites with food is the nucleophilicity of the sulfite ion (SO_3^{2-}) (Wedzicha and Kaputo, 1992).

2.5.1. Reactions of sulfites with reducing sugars

Sulfites have a particular affinity for reactions with aldehydes and ketones. In most foods and beverages, the main reaction products are hydroxysulfonates (Burroughs and Sparks, 1973a,b,c; Adachi et al., 1979). The reaction rates between sulfites and carbonyl groups are fast and in the range of pH 1–8, hydroxysulfonates predominate, while at higher pH values hydroxysulfonates are again dissociated to bisulfite anion and carbonylic substance (Burroughs and Sparks, 1973a,b,c; Adachi et al., 1979). The sulfonated carbonyls formed by reaction of sulfites with α,β-unsaturated carbonyl, intermediates of the Maillard reaction, are stable and their formation is irreversible (McWeeny et al., 1974; Wedzicha and McWeeny, 1974).

Irreversible reactions of sulfites with other intermediates of the browning reactions lead to the formation of stable 3-deoxy-4-sulfo-osuloses. The 3-deoxy-4-sulfo-osuloses can in turn react with other food components to yield other sulfur-containing products. 3-deoxy-4-sulfo-osuloses may account for much of the sulfite originally added to stored dehydrated vegetables (Wedzicha and McWeeny, 1974, 1975) and may be the major end-products of sulfites in jams made from sulfited fruit (McWeeny et al., 1980).

Acetaldehyde is the primary sulfite reactive substance in wines and ciders and acetaldehyde hydroxysulfonate is also considered a stable reaction product (Taylor et al., 1986). D-Glucose may react irreversibly with sulfites to form a stable sulfonic acid derivative (Green, 1976).

2.5.2. Reactions of sulfites with proteins and amino acids

The disulfide bonds of free cystine can be cleaved by sulfites leading to the formation of thiol and *S*-sulfonates. This does not happen with those bonds in proteins, as they are protected. Nevertheless, Gregory and Gunnison (1984) demonstrated sulfitolisis of rabbit plasma albumin. Methionine can be oxidised to methionine sulfoxide via a free radical mechanism and tryptophan can be destroyed by the same mechanism (Gunnison, 1981).

2.5.3. Reactions of sulfites with vitamins

Sulfites can react with a broad range of vitamins including thiamine (vitamins B_1), vitamin C (ascorbic acid), folic acid (vitamin B_9), cobalamin (vitamin B_{12}) and vitamin K. Sulfites can also destroy β-carotene, a precursor of vitamin (Taylor et al., 1986). Sulfur dioxide reacts irreversibly with thiamine to yield 2-methyl-4-amino-5-hydroxymethyl pyrimidine or pyrimidine sulfonic acid and 4-methyl-5-(β-hydroxyethyl)thiazole (Dwivedi and Arnold, 1973; Gunnison et al., 1981b). It has been indicated in the literature that thiamine in foods is cleaved and inactivated by sulfating agents (Davidson, 1992; Studdert and Labuc, 1991). The use of sodium bisulfite during the soaking step in parboiled rice at concentrations above 0.2% severely reduced the thiamine content (Vanier et al., 2015).

2.5.4. Reactions of sulfites with nucleic acids and nucleotides

Significant cleavage of glycosidic linkages of uridine and cytidine nucleosides occurred in a sulfite/free radical environment (Kitamura and Hayatsu, 1974 cited in Gunnison, 1981b). Sulfites can also catalyse the transamination of cytosine with primary and secondary amines (Gunnison, 198b1).

2.5.5. Reactions of sulfites with pigments

Anthocyanins and phenols that are present in wines can react with sulfites forming colourless anthocynin-4-bisulfites. They dissociate easily under acidic conditions at pH 1–2 releasing bisulfite anion and anthocyanins (Burroughs, 1975). Tao et al. (2007) demonstrated that sulfur dioxide is likely

to affect the pathways involving the formation of carbocations at the C4 position of proanthocyanidins and also the way in which these will combine with other polyphenols, including anthocyanins, to generate new tannin and polymeric pigment compounds. The addition of sulfur dioxide concentrations up to 200 mg/L, increased the amount of monomeric anthocyanins and flavan-3-ols, coupled with a decrease in tannin level. Thus, the amount of SO_2 added to a red wine under microoxygenation affects the rate of development of wine polyphenol chemistry, including the stabilisation of colour in polymeric pigment forms and changes in tannin structure affecting wine astringency.

Ojwang and Awika (2010) have investigated the stability of apigenidin and its derivatives in the presence of sulfites. This compound is in the group of 3-deoxyanthocyanin pigments that are more stable than anthocyanins. These pigments were bleached in the presence of sodium metabisulfite at different pHs, mainly at pH 5.0 and 3.0 compared to pH 8. Most of the colour was restored at pH 1.8 in the presence of sulfites. Formation of colourless sulfonates via bisulfite ion addition at C4 was responsible for the bleaching effect.

2.5.6. Reactions of sulfites with fatty acids

Presumably through a free radical mechanism, sulfites can induce oxidation of unsaturated fatty acids (Lamikanra, 1982; Southerland et al., 1982).

2.5.7. Reactions of sulfites with specific foods

The proportion of combined forms of sulfites is variable from one food to another but is usually predominating. An exception is lettuce where almost all sulfites are present under a free form (Taylor et al. 1986). The percentage of total sulfur dioxide existing in the free form was reported to be 2.3% in white wines, 22.3% in concentrated orange juice, 14.8% in molasses and 34.4% in corn starch (Mitsuhashi et al., 1979). In shrimps where most of the sulfites are in the shell, 32.3% were found as a free form in frozen peeled samples.

Vanier et al. (2015) reported that sodium sulfite can act as bleaching agent by demonstrating that 0.2% of sodium bisulfite in the treatment of parboiled rice was able to increase rice whiteness by 21%.

The sulfuring method in dried apricots had significant effects on the colour, as the absorption of sulfur dioxide can depend on many factors as soluble solid content and components, especially sugars, moisture, pH and ambient relative humidity and temperature. The removal of sulfur dioxide during storage fits a first kinetic model, also increases with the temperature (from 39% at 5ºC to 90% at 30ºC for a year) (Coskun et al., 2013). Similar results have been reported for dried apricots containing sulfites at different concentrations and storage temperatures. Also, sulfur dioxide concentrations over 791 mg/kg of dried apricots effectively protected carotenoids during drying, as their colour was lighter as the sulfur dioxide concentration increased, showing its importance in preventing the brown colour formation during drying and storage (Türkilmaz, 2013).

2.5.8. Critical factors in the determination of the fate of sulfites in foods.

The possible reactions with organic ingredients, the equilibrium between the different inorganic forms and the volatilisation of sulfur dioxide have to be considered when studying the fate of sulfites in foods. In addition, processing and storage appear also to be important.

The Panel noted that the measured amounts of free and bound sulfites do not enable to trace back the initially added amount of sulfites. Bound sulfites occur in various forms and percentages of the different reaction products in food are poorly documented. The sulfuring method used for the application of sulfites, the food composition and other conditions together with the time and temperature of storage, could influence the final amount of sulfur dioxide in the food. The Panel considered this information as significant regarding the safety assessment of the actual substances to which consumers are exposed.

The Panel noted that the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) stated in its opinion in 2014 that '*The amounts of sulphites initially used to treat foods do not reflect residue levels after processing. Storage and preparation of food also affects the final amount of sulphites consumed. Mechanisms of loss include volatilisation to SO_2 in acidic conditions, leaching, auto-oxidation, as well as the irreversible reactions with food constituents (Gunnison and Jacobsen, 1987)*'(EFSA NDA Panel, 2014).

2.6. Case of need 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 defined to by the Panel as the 'maximum permitted levels (MPLs)' in this document.

Sulfur dioxide–sulfites (E 220–228) are authorised overall in 40 food categories in the EU according to Annex II to Regulation (EC) No 1333/2008 with MPLs ranging from 20 to 2,000 mg/kg.

Table 10 summarises the food categories that are permitted to contain sulfur dioxide–sulfites (E 220–228) as food additives and the corresponding MPLs as set by Annex II to Regulation (EC) No 1333/2008.

Table 10: MPLs of sulfur dioxide–sulfites (E 220–228) in foods 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	Only white vegetables, including pulses	50[(a)]

Food category number	Food category description	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
	vegetables	Only bottled whiteheart cherries; vacuum packed sweetcorn	100[(a)]
		only bottled, sliced lemon	250[(a)]
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)]

Food category number	Food category description	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
		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)]
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)]

Food category number	Food category description	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
14.2.1	Beer and malt beverages		20[(a)]
		Only beer with a second fermentation in the cask	50[(a)]
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, a 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–sulfites (E 220–228) may also be used in wines and liquors. This use is regulated in Annex IB to Regulation (EC) No 606/2009[15]. In particular, according to this Regulation:

1. The total amount of sulfur dioxide content in wine other than sparkling wines and liqueurs wines, on their release to the market for direct human consumption, may not exceed:

[15] Commission Regulation (EU) No 606/2009 of 10 July 2009 laying down certain detailed rules for implementing Council Regulation (EC) No 479/2008 as regards the categories of grapevine products, oenological practices and the applicable restrictions. OJ L 193, 24.7.2009. p.1–59.

(a) 150 mg/L for red wines;

(b) 200 mg/L for white and rosé wines.

2. Notwithstanding paragraph 1(a) and (b), the maximum sulfur dioxide content shall be raised, as regards wines with sugar content, expressed as the sum of glucose and fructose, of not less than 5 g/L, to:

   (a) 200 mg/L for red wines;

   (b) 250 mg/L for white and rosé wines;

   (c) 300, 350 or 400 mg/L for some wines with high level of residual sugars;

   (d) Where climate conditions make this necessary, the Commission may decide in accordance with the procedure referred to in Article 113(2) of Regulation (EC) No 479/2008 that in certain wine-growing areas of the Community, the Member States concerned may authorise an increase of a maximum of 50 mg/L in the maximum total sulfur dioxide levels of less than 300 mg/L referred to in this point for wines produced within their territory.

3. The total sulfur dioxide content of liqueur wines, on their release to the market for direct human consumption, may not exceed:

   (a) 150 mg/L for wines with sugar content of less than 5 g/L;

   (b) 200 mg/L for wines with sugar content of more than 5 g/L.

4. The total sulfur dioxide content of sparkling wines, on their release to the market for direct human consumption, may not exceed:

   (a) 185 mg/L for all categories of sparkling wine;

   (b) 235 mg/L for other sparkling wines;

   (c) Where climate conditions make this necessary in certain wine-growing areas of the Community, the Member States concerned may authorise an increase of up to 40 mg/L in the maximum total sulfur dioxide content for the sparkling wines referred to in paragraph 1(a) and (b) produced in their territory, provided that the wines covered by this authorisation are not sent outside the Member State in question.

Finally, 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. More in detail, 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) with 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 enzymes preparations in quantities that do not exceed 2 mg/kg in the final food and 2 mg/L in the final beverage. In addition, when the levels of sulfur dioxide or sulfites (E 220–228) are below 10 mg/kg or 10 mg/L, SO_2 is considered to be not present, according to Annex II to Regulation (EC) No 133/2008.

Food categories listed in Annex II to Regulation (EC) No 133/2008 or Annex IB to Regulation (EC) No 606/2009 in relation to sulfur dioxide–sulfites (E 220–228) are referred in the current opinion as food categories for which direct addition of sulfur dioxide–sulfites (E 220–228) is authorised according to Annex II to Regulation (EC) No 1333/2008 or Annex IB to Regulation (EC) No 606/2009.

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

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, information on actual use levels is required for performing a more realistic exposure assessment.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010[16] regarding the re-evaluation of approved food additives, EFSA issued a public call[17,18] for occurrence data (usage level and/or concentration data) on dioxide–sulfites (E 220–228). In response to these calls, both types of data on dioxide–sulfites (E 220–228) were submitted to EFSA by industry and the Member States, respectively.

2.7.1. Summary on reported use levels of sulfur dioxide–sulfites (E 220–228) in foods provided by industry

Information on the actual uses and use levels of sulfur dioxide–sulfites (E 220–228) were made available by FoodDrinkEurope (FDE) (n = 87), the European Starch Industry Association (AAF) (n = 2), the Gelatine Manufacturers of Europe (GME) (n = 8) and the British Meat Processors Association (BMPA) (n = 2) and UNESDA (2010) [Doc. provided to EFSA n..43].

In summary, industry provided EFSA with use levels (n = 101) in foods belonging to 20 out of the 43 food categories in which sulfur dioxide–sulfites (E 220–228) are authorised. Most data were provided for the category '8.2 Meat preparations as defined by Regulation (EC) No 853/2004'.

Usage levels were reported for six food categories for which direct addition of sulfur dioxide–sulfites (E 220–228) is not authorised according to Annex II to Regulation (EC) No 1333/2008 and/or above the MPL. A request for clarification was sent but no feedback was received. Therefore, these data were considered as misclassified and not included in the current assessment.

See Appendix A for an overview of the provided use levels.

2.7.2. Summary of analytical data of sulfur dioxide in foods from the Member States

In total, 27,741 analytical results were available to EFSA but 444 were excluded because no feedbacks were received from the data providers in relation to possible errors identified during the analysis.

The remaining 27,297 analytical results were reported by 14 countries: Austria (n = 1,586), Belgium (n = 160), Cyprus (n = 888), the Czech Republic (n = 792), Germany (n = 18,564), Hungary (n = 579), Spain (n = 994), the United Kingdom (n = 13), Slovakia (n = 865), Ireland (n = 459), Italy (n = 1,217), Luxembourg (n = 138), Malta (n = 20) and Portugal (n = 1,022). Foods were sampled between 2000 and 2014.

In this dataset, 1,410 analytical data were classified at the first level of the FoodEx system (see Section 1.4.1.2). Due to the high number of exceptions and restrictions within the EU legislation concerning the authorisation of sulfur dioxide–sulfites (E 220–228), the first level of the FoodEx system was considered not sufficient to link the analytical results with the food categories listed in Annex II to Regulation (EC) No 1333/2008 and Annex IB to Regulation (EC) No 606/2009. These analytical data were therefore not taken into account in the current assessment.

Of the remaining 25,887 analytical results reported to EFSA, 25,189 concerned food categories listed in Annex II to Regulation (EC) No 1333/2008 and Annex IB to Regulation (EC) No 606/2009 (Appendix B). Among these, 20,576 were above the LOQ, two results were qualitative (binary results) and gave only indication of the absence of sulfur dioxide–sulfites (E 220–228) and 516 samples had analytical values of sulfur dioxide–sulfites (E 220–228) above the relevant MPLs.

[16] 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, p. 19.

[17] Call for scientific data on food additives permitted in the EU and belonging to the functional classes of preservatives and antioxidants. Published: 23 November 2009. Available from: http://www.efsa.europa.eu/en/dataclosed/call/ans091123a.htm

[18] Call for food additives usages level and/or concentration data in food and beverages intended to human consumption. Published: 27 March 2013. Available online: http://www.efsa.europa.eu/en/dataclosed/call/130327.htm

Finally, 706 analytical results related to food categories not listed in Annex II to Regulation (EC) No 1333/2008 or Annex IB to Regulation (EC) No 606/2009 and of which 330 were above the LOQ (Appendix B).

2.8. Information on existing authorisations and evaluations

Sulfur dioxide and sulfites are authorised as food additives in the EU in accordance with Annex II and III to Regulation (EC) No 1333/2008 on food additives, and specific purity criteria have been defined in Commission Regulation (EU) No 231/2012.

Sulfites were evaluated by JECFA in 1986 (JECFA, 1987) and a group acceptable daily intake (ADI) of 0.7 mg SO_2 equivalent/kg body weight (bw) per day was derived. Intake estimates worldwide were gathered and evaluated in 1998 (JECFA, 1999). Data from France and the United Kingdom showed that the intake could exceed the group ADI among high consumers and children. The SCF evaluated sulfites in 1994 and derived a group ADI of 0.7 mg/kg bw based on a no observed adverse effect level (NOAEL) of 70 mg SO_2 equivalent/kg bw per day for gastric irritation in long-term feeding studies in rats and pigs (SCF, 1996).

The Food Standards Australian New Zealand (FSANZ) has also evaluated sulfites as food additives (2005, 2012).

EFSA evaluated sulfites in an opinion on allergenic foods (EFSA NDA Panel, 2004). On that occasion, it was noted that the most sulfite-sensitive individuals can react to ingested metabisulfite in quantities ranging from 20 to 50 mg of sulfites in the food. The smallest concentration of sulfites able to provoke a reaction in sensitive individuals has not been established.

The Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP, 2003) concluded that inorganic sulfites and bisulfites do not pose a health risk when used in cosmetic products at concentrations up to 0.67% in oxidative hair dye products, up to 6.7% in hair waving/straightening products, up to 0.45% in self-tanning products for the face and up to 0.40% in self-tanning products for the body (all expressed as SO_2 equivalent).

The US Food and Drug Administration (FDA) prohibited in 1986 the use of sulfites on fresh fruits and vegetables that were to be served raw or presented as fresh to the public (FDA, 1986).

Sodium sulfite, sodium bisulfite, sodium metabisulfite and potassium metabisulfite are permitted in cosmetic products (European Commission database – CosIng[19]).

Sulfur dioxide, sodium sulfite, sodium bisulfite, sodium metabisulfite, potassium metabisulfite and calcium sulfite have been registered under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation 1907/2006 (ECHA, online).

2.9. Exposure assessment

2.9.1. Food consumption data used for exposure assessment

2.9.1.1. 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 the 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 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure

[19] Available online: http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.simple

Assessment' (EFSA, 2011a)). New consumption surveys added in 2015 in the Comprehensive Database[20] were also taken into account in this assessment.[21]

The food consumption data gathered by EFSA were collected using different methodologies and thus direct country-to-country comparison should be interpreted with caution. Depending on the food category and the level of detail used in the exposure calculations, uncertainties can be introduced owing to possible subjects' underreporting and/or misreporting of consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across the Europe at present.

Food consumption data from the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 11).

Table 11: Population groups considered for the exposure estimates of sulfur dioxide–sulfites (E 220–228)

Population	Age range	Countries with food consumption surveys covering more than one day
Infants	From 4 up to and including 11 months of age	Bulgaria, Denmark, Finland, Germany, Italy, UK
Toddlers	From 12 up to and including 35 months of age	Belgium, Bulgaria, Finland, Germany, Netherlands, Italy, Spain
Children[(a)]	From 36 months up to and including 9 years of age	Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden
Adolescents	From 10 up to and including 17 years of age	Belgium, Cyprus, Czech Republic, Denmark, France, Germany, Italy, Latvia, Spain, Sweden
Adults	From 18 up to and including 64 years of age	Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Spain, Sweden, UK
The elderly[(a)]	From 65 years of age and older	Belgium, Denmark, Finland, France, Germany, Hungary, Italy

(a) The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). The nomenclature from the FoodEx classification system has been linked to the Food Classification System (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D, and in Annex IB to Regulation (EC) No 606/2009, to perform exposure calculations. In practice, FoodEx food codes were matched to the food categories.

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

The 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 IB to Regulation (EC) No 606/2009 were selected from the nomenclature of the EFSA Comprehensive Database at the most detailed level possible of FoodEx (up to FoodEx Level 4) (EFSA, 2011b).

[20] Available online: http://www.efsa.europa.eu/en/press/news/150428.htm
[21] Available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

Some food categories and their relative restrictions/exceptions are not referenced in the EFSA Comprehensive Database and could not be taken into account in the present assessment. This may result in an underestimation of the exposure. The food categories that were not taken into account are described below (in ascending order of the FCS codes):

- 04.1.3 Frozen fruit and vegetables: only white vegetables, including mushrooms and white pulses; only frozen and deep-frozen potatoes;

- 04.2.3 Canned or bottled fruit and vegetables: only white vegetables, including pulses; only bottled white heart cherries; vacuum packed sweetcorn; only bottled, sliced lemon;

- 4.2.5.1 Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EC: only jams, jellies and marmalades made with sulfited fruits;

- 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); only glucose syrup-based confectionery (carry-over from the glucose syrup only); only fruit fillings for pastries;

- 06.1 Whole, broken or flaked grain: only sago and pearl barley;

- 12.2.2 Seasonings and condiments: only citrus juice-based seasonings;

- 14.2.2 Wine and other products defined by Regulation (EC) No 1234/2007, and alcohol-free counterparts, only alcohol-free

- 14.2.4 Fruit wine and made wine;

- 14.2.5 Mead;

- 14.2.6 Spirit drinks as defined in Regulation (EC) No 110/2008: only distilled alcoholic beverages containing whole pears;

- 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 drinks; 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 niskoalkoholowe;

- 15.2 Processed nuts: only marinated nuts.

The following restrictions/exceptions for the respective food categories are not referenced in FoodEx. Therefore, the specific restrictions/exceptions have not been taken into account in the present exposure assessment. This may have resulted in an underestimation of the exposure. The restrictions and exceptions that were not taken into account are described below (in ascending order of the FCS codes):

- ‘only vacuum packed sweetcorn’ and ‘only fresh blueberries’ in 04.1.1 Entire fresh fruit and vegetables;

- ‘only peeled potatoes’ and ‘only horseradish pulp’ in 04.1.2 Peeled, cut and shredded fruit and vegetables;

- 'only dried coconut', 'only white vegetables, processed, including pulses', 'only dried mushrooms', 'only dried ginger' and 'only white vegetables, dried' in 04.2.1 Dried fruit and vegetables;

- 'only golden peppers in brine' in 04.2.2 Fruit and vegetables in vinegar, oil and brine;

- 'only rehydrated dried fruit and lychees, mostarda di frutta' and 'only jellying fruit extract, liquid pectin for sale to the final consumer' in 04.2.4.1 Fruit and vegetable preparations excluding compote;

- 'only glucose syrup, whether or not dehydrated' in 11.1 Sugars and syrups as defined by Directive 2001/111/EC;

- 'only treacle and molasses' in 11.2 Other sugars and syrups;

- 'only dijon mustard' in 12.4 Mustard;

- 'only analogues of meat, fish, crustaceans and cephalopods' in 12.9 Protein products, excluding products covered in category 1.8;

- 'only grape juice, unfermented, for sacramental use' in 14.1.2 Fruit juices as defined by Directive 2001/112/EC and vegetable juices;

- 'only other concentrates based on fruit juice or comminuted fruit; capilé groselha' and 'only concentrates based on fruit juice and containing not less than 2.5% barley (barley water)' in 14.1.4 Flavoured drinks;

- 'only beer with a second fermentation in the cask' in 14.2.1 Beer and malt beverages.

For the following food categories, the restrictions which apply to the use of sulfur dioxide–sulfites (E 220–228) could not be taken into account and the whole food category was considered in the exposure assessment. This may have resulted in an overestimation of the exposure:

- 05.2 Other confectionery, including breath refreshening microsweets: only glucose syrup-based confectionery (carry-over from the glucose syrup only);

- 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; only crustaceans of the Penaeidae, Solenoceridae and Aristaeidae family between 80 and 120 units; only crustaceans of the Penaeidae, Solenoceridae and Aristaeidae family over 120 units;

- 09.2 Processed fish and fishery products, including molluscs and crustaceans: only cooked crustaceans and cephalopods; only cooked crustaceans of the Penaeidae, Solenoceridae and Aristaeidae family up to 80 units; only cooked crustaceans of the Penaeidae, Solenoceridae and Aristaeidae family between 80 and 120 units; only dried salted fish of the 'Gadidae' species; only cooked crustaceans of the Penaeidae, Solenoceridae and Aristaeidae family over 120 units;

- 12.3 Vinegars: only fermentation vinegar.

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 the three food categories according to Annex IB to Regulation (EC) No 606/2009 (see Section 1.1), 12 were not taken into account in the exposure assessment, for 14 food categories only certain restrictions/specifications among those listed

were not included, and four food categories were included in the exposure assessment without considering these restrictions/specifications.

The use of sulfur dioxide–sulfites (E 220–228) in '14.2.7.1 Aromatised wines', '14.2.7.2 Aromatised wine-based drinks' and '14.2.7.3 Aromatised wine-product cocktails' is authorised under Annex II to Regulation (EC) No 1333/2008 (Table 1), whereas maximum levels of sulfur dioxide–sulfites (E 220–228) are defined in Annex IB to Regulation (EC) No 606/2009 for red, white and rosé wine, liqueur wine and sparkling wine (Section 1.1). As no specific food entries are present in FoodEx for aromatised wines, wine-based drinks and wine/product cocktails, the consumption of these products are all coded as wine. Therefore, a unique food category was considered for wine, including also red, white and rosé wine and sparkling wine, when assessing the exposure.

2.9.2. Exposure to sulfur dioxide–sulfites (E 220–228) as food additives

The Panel estimated chronic exposure to sulfur dioxide–sulfites (E 220–228) for the following population groups: infants, toddlers, children, adolescents, adults and the elderly. Dietary exposure to sulfur dioxide–sulfites (E 220–228) from their use as food additives was calculated by multiplying concentration levels (Appendix C and D) for each food category with their respective consumption amount per kilogram body weight for each individual in the Comprehensive database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of surveys days, resulting in an individual average exposure per day for the survey period. Dietary surveys with only one day per subject were excluded as they are considered as not adequate to assess repeated exposure.

The dietary exposure was assessed per survey and per population group, resulting in distributions of individual average exposure per survey and population group (Table 2). Based on these distributions, the mean and 95th percentile exposures were calculated per survey and per population group. High percentile exposure was only calculated for those population groups where the sample size was sufficiently large (> 60 subjects) to allow calculation of the 95th percentile of exposure (EFSA, 2011a). Therefore, in the present assessment, high levels of exposure for infants from Italy and for toddlers from Belgium, Italy and Spain were not included.

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

1. The MPLs set down in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*). The possible presence of sulfur dioxide–sulfites (E 220–228) due to carry-over was not considered in this assessment.

2. Reported use levels and analytical results (not exceeding the MPLs) for food categories for which direct addition of sulfur dioxide–sulfites (E 220–228) is authorised, according to Annex II to Regulation (EC) No 1333/2008 and Annex IB to Regulation (EC) No 606/2009 (**dataset 1**). Overall, a total of 24,436 analytical results reported for sulfur dioxide in foods were considered by the Panel for the exposure calculations, after discarding the analytical results: 1) classified at the first level of the FoodEx system (n = 1,403); 2) expressed as qualitative results (n = 2); 3) exceeding the MPL (n = 516); 4) of foods categories not listed in Annex II to Regulation (EC) No 1333/2008 and Annex IB to Regulation (EC) No 606/2009 (n = 706); and 5) of food categories not referenced in FoodEx (n = 235). Eventually, in this dataset, 27 food categories for which direct addition of sulfur dioxide–sulfites (E 220–228) is authorised according to Annex II to Regulation (EC) No 1333/2008 were included and three food categories according to Annex IB to Regulation (EC) No 606/200 (Appendix C).

3. Reported use levels and analytical data (levels not exceeding the MPLs) for food categories for which direct addition of (E 220–228) is authorised and, in addition, the available analytical data for foods categories which may contain sulfur dioxide–sulfites (E 220–228) 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 (**dataset 2**). This dataset consisted of a total of 24,956 analytical values, after excluding the analytical results expressed as qualitative results (n = 2), analytical results of food categories not listed in Annex II to Regulation (EC) No 1333/2008 and Annex IB to Regulation (EC) No 606/2009, where all analytical results were below the LOQ and of food categories not listed in Annex II to Regulation (EC) No 1333/2008 nor in Annex IB to Regulation (EC) No 606/2009 composed of only one analytical sample (n = 84) and analytical results of food categories not referenced in FoodEx (n = 337). Overall, 43 food categories were considered for the exposure assessment (Appendix C and D).

In order to evaluate the impact of the relatively high number of analytical results found to exceed the MPL (n = 516), the exposure to sulfur dioxide–sulfites (E 220–228) was as well assessed under a scenario including use levels and analytical data for food categories for which direct addition of sulfur dioxide–sulfites (E 220–228) is authorised and, in addition, the available analytical data for foods categories which may contain sulfur dioxide–sulfites (E 220–228) 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.

2.9.2.1. Regulatory maximum level exposure assessment scenario

The *regulatory maximum level exposure assessment scenario* is based on the MPLs as set in the Annex II to Regulation (EC) No 1333/2008 and Annex IB to Regulation (EC) No 606/2009 and listed in Section 2.6.

A MPL of 250 mg/L as established for white and rosé wines with more than 5 g of glucose/L by Annex IB to Regulation (EC) No 606/2009 (Section 2.6) was assigned to the food category 'Wine' (Appendix C).

The exposure estimates derived following this scenario should be considered as the most conservative as it is assumed that the consumer will be continuously (over a lifetime) exposed to sulfur dioxide–sulfites (E 220–228) present in the food at a MPL.

2.9.2.2. Refined exposure assessment scenario

The refined exposure assessment scenarios are based on reported use levels from industry and analytical results submitted to EFSA by the Member States. The refined exposure assessment scenarios were carried out twice, based on the dataset 1 and dataset 2 (Section 2.9.2). Appendix C and D summarise the concentration levels of sulfur dioxide–sulfites (E 220–228) used in the refined exposure assessment scenarios per dataset.

Per dataset, the Panel calculated two estimates based on different model populations:

1. The brand-loyal consumer scenario: It was assumed that a consumer is exposed long term to sulfur dioxide–sulfites (E 220–228) at the maximum reported use/analytical level for one food category. This exposure estimate is calculated as follows:

    a. Food consumption is combined with the maximum of the reported use levels or the maximum of the analytical results, whichever was highest or available, for the main contributing food category at the individual level.

    b. Food consumption is combined with the mean of the typical reported use levels or the mean of analytical results, whichever was highest or available, for the remaining food categories.

2. The non-brand-loyal consumer scenario: It was assumed that a consumer is exposed long term to sulfur dioxide–sulfites (E 220–228) present at the mean reported use/analytical results in

food, whichever was highest or available. This exposure estimate is calculated using the mean of the typical reported use levels or the mean of analytical levels for all food categories.

In the brand-loyal consumer scenario, including values above the MPL, the 95th percentile level of a food category was used instead of the maximum value in order to minimise the impact of possible outliers. However, for food categories listed in Annex II to Regulation (EC) No 1333/2008, in case the 95th percentile level was below the MPL, the maximum value below the MPL as reported in dataset 1, was used also in dataset 2.

To consider left-censored analytical data (i.e. analytical results < LOD or < LOQ) in both refined exposure assessment scenarios, 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.

For all food categories, except 06.2.2 'Starches', analytical data were used to estimate the exposure according to the refined exposure scenarios for both datasets. For 06.2.2, use levels were used.

2.9.2.3. Estimated exposure to sulfur dioxide–sulfites (E 220–228)

Table 12 summarises the estimated exposure to sulfur dioxide–sulfites (E 220–228) from their use as food additives in six population groups. Detailed results per population group and survey are presented in Appendix F and summary results related to the exposure to sulfur dioxide–sulfites (E 220–228) under the scenario including also concentration levels above the MPLs are reported in Appendix E.

Table 12: Summary of estimated exposure to sulfur dioxide–sulfites (E 220–228) from their use as food additives in the *regulatory maximum level exposure assessment scenario* and in the *refined exposure scenarios* in six population groups (min–max across the dietary surveys in mg/kg bw per day).

	Infants (4–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (> 65 years)
Regulatory maximum level exposure assessment scenario						
Mean	0.23–1.10	0.75–2.21	0.63–1.86	0.35–1.02	0.42–0.85	0.37–0.97
High level	1.33–3.95	2.34–6.92	1.55–5.11	0.85–2.31	1.11–2.02	1.03–2.01
Refined exposure scenario considering concentration levels not exceeding the MPLs for food categories listed in Annex II to Regulation (EC) No 1333/2008 and Annex IB to Regulation (EC) No 606/2009 (dataset 1)						
Brand-loyal scenario						
Mean	0.13–0.91	0.41–1.22	0.25–1.16	0.16–0.63	0.3–0.67	0.28–0.89
High level	0.68–3.48	1.55–4.5	0.70–3.63	0.42–1.63	0.97–1.97	0.78–2.41
Non-brand-loyal scenario						
Mean	0.03–0.23	0.14–0.56	0.10–0.53	0.06–0.31	0.12–0.26	0.11–0.30
High level	0.16–0.70	0.61–2.26	0.34–1.65	0.15–0.79	0.42–0.76	0.39–0.69
Refined exposure scenario considering in addition to dataset 1, the available analytical data for foods categories which may contain sulfur dioxide–sulfites (E 220–228) 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 (dataset 2)						
Brand-loyal scenario						
Mean	0.25–0.99	0.74–1.6	0.57–1.45	0.37–0.88	0.48–0.75	0.45–0.95
High level	1.4–3.61	1.98–4.64	1.18–3.78	0.74–2.09	1.16–2.06	0.94–2.46
Non-brand-loyal scenario						
Mean	0.08–0.31	0.26–0.74	0.25–0.69	0.14–0.4	0.19–0.34	0.2–0.34
High level	0.45–0.85	0.79–2.4	0.55–1.83	0.3–0.9	0.51–0.87	0.48–0.74

MPL: maximum permitted level.

Using the *regulatory maximum level exposure assessment scenario*, the anticipated mean exposure to sulfur dioxide–sulfites (E 220–228) from their use as food additives ranged from 0.23 to 2.21 mg/kg bw per day across all population groups. The high exposure to sulfur dioxide–sulfites (E 220–228) under this scenario could be as high as 6.92 mg/kg bw per day in toddlers.

The refined mean exposure to sulfur dioxide and sulfites (E 220–228), considering concentration levels not exceeding the MPLs for food categories listed under Annex II to Regulation No 1333/2008 and Annex IB to Regulation (EC) No 606/2009, ranged from 0.13 to 1.22 mg/kg bw per day and 0.68 to 4.5 mg/kg bw per day at the high level (95th percentile) in the *brand-loyal scenario*. The corresponding estimates for the *non-brand-loyal scenario* were 0.03–0.56 and 0.16–2.26 mg/kg bw per day, respectively.

The refined exposure estimates of sulfur dioxide and sulfites (E 220–228), considering concentration levels not exceeding the MPLs for food categories for which direct addition of sulfur dioxide–sulfites is authorised and, in addition, the available analytical data for foods categories which may contain sulfur dioxide–sulfites (E 220–228) 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 were slightly higher compared to those previous refined exposure scenario considering only direct addition to food. In the *brand-loyal scenario*, the mean exposure ranged from 0.25 to 1.6 mg/kg bw per day and the high level ranged from 0.74 to 4.64 mg/kg bw per day. The corresponding figures for the *non-brand-loyal scenario* were 0.08–0.74 and 0.3–2.4 mg/kg bw per day.

The inclusion of analytical results above the MPLs further increased the exposure estimates up to 6.11 mg/kg bw per day for the high level under the *brand-loyal scenario* (Appendix E).

2.9.3. Main food categories contributing to exposure to sulfur dioxide–sulfites (E 220–228) using the regulatory maximum level exposure assessment scenario

The main food categories contributing more than 5% to the exposure to sulfur dioxide–sulfites (E 220–228) in the regulatory maximum level exposure assessment scenario are presented in Appendix G. For infants and toddlers, the FCS 04.2.6 ‘Processed potato products, not dehydrated’ and the FCS 04.2.1 ‘Dried fruit and vegetables’ were the main contributors to the total mean exposure to sulfur dioxide–sulfites (E 220–228), while for other children and adolescents, the FCS 04.2.1 ‘Dried fruit and vegetables’ and the FCS 14.1.2 ‘Fruit juices as defined by Directive 2001/112/EC and vegetable juices’ contributed most. Finally in adults and elderly, the FCS 08.2 ‘Meat preparations as defined by Regulation (EC) No 853/2004 (M42)’ and ‘Wine’ represented the main food contributors.

2.9.4. Main food categories contributing to exposure to sulfur dioxide–sulfites (E 220–228) considering only direct addition to food

The main food categories contributing more than 5% to the exposure to sulfur dioxide–sulfites (E 220–228) in the refined exposure assessment scenarios including use levels and analytical results (not exceeding the MPLs) for food categories for which direct addition to food is authorised are presented in Appendix H and I. The FCS 08.2 ‘Meat preparations as defined by Regulation (EC) No 853/2004’ was one of the main contributors to the exposure to sulfur dioxide–sulfites (E 220–228) in all population groups in both the scenarios. For infants and toddlers, the FCS 04.2.6 ‘Processed potato products, except dehydrated potatoes’ contributed most to the total exposure to sulfur dioxide–sulfites (E 220–228) in both scenarios. For other children, the highest contribution was ascribable to the FCS 14.1.2 ‘Fruit juices as defined by Directive 2001/112/EC and vegetable juices’, and for adolescents, the FCS 14.1.4 ‘Flavoured drinks’. Finally, ‘Wine’ was the main contributor to the exposure in adults and elderly in both the scenarios.

2.9.5. Main food categories contributing to the exposure to sulfur dioxide–sulfites (E 220–228) considering additional exposure taking into account the available analytical data for foods categories which may contain sulfur dioxide–sulfites (E 220–228) 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

The main food categories contributing more than 5% to the exposure to sulfur dioxide and sulfites (E 220–228) in the refined exposure assessment scenarios considering additional exposure taking into account the available analytical data for foods categories which may contain sulfur dioxide–sulfites (E 220–228) 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 are presented in Appendix J and K. In both the scenarios, the FCS 04.2.6 'Processed potato products, except dehydrated potatoes', the FCS 04.2.1 'Dried fruit and vegetables' and the FCS 08 'Meat, only chicken meat' were the food categories that contributed most to the exposure in infants and children. The FCS 08.2 'Meat preparations as defined by Regulation (EC) No 853/2004' was the largest contributor in most of the other population groups, whereas together with the FCS 08 'Meat, only chicken meat' and 'Wine' in adults and elderly only.

2.10. Uncertainty analysis

Uncertainties in the exposure assessment of sulfur dioxide–sulfites (E 220–228) have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and summarised in Table 13:

Table 13: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction [(a)]
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Correspondence of reported use levels and analytical data to the food items in the EFSA Comprehensive Food Consumption Database: uncertainties to which types of food the levels refer to	+/–
Food categories selected for the exposure assessment: exclusion of 12 food categories and 20 restrictions due to missing FoodEx linkage	–
Food categories selected for the exposure assessment: inclusion of food categories without considering the restriction/exception (n = 4 food categories)	+
Food categories included in the exposure assessment: data not available for certain restrictions which were excluded from the exposure estimates (n = 5 for the refined scenarios)	–
Concentration data: levels considered applicable for all items within the entire food category, exposure calculations based on the maximum or mean levels (reported use from industry or analytical data from the Member States)	+
Regulatory maximum level exposure assessment scenario: - food categories which may contain sulfur dioxide–sulfite due to carry-over not considered - food categories authorised at MPL according to Annex II to Regulation (EC) No 1333/2008	– +
Refined exposure assessment considering only direct addition to food: - food categories which may contain sulfur dioxide–sulfite due to carry-over not considered - concentration data: levels considered applicable for all items within the entire food category, exposure calculations based on the maximum	–

Sources of uncertainties	Direction [(a)]
reported use levels/p95 of analytical data or mean levels	+/–
- analytical data above the MPLs were excluded	–
Refined exposure assessment considering additional exposure taking into account the available analytical data for foods categories which may contain sulfur dioxide–sulfites (E 220–228) 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:	
- food categories which may contain sulfur dioxide–sulfite due to carry-over considered	+/–
- concentration data: levels considered applicable for all items within the entire food category, exposure calculations based on the maximum reported use levels/p95 of analytical data or mean levels	+/–
- analytical data above the MPLs were excluded	–
Uncertainty in possible national differences in use levels of food categories, concentration data not fully representative of foods on the EU market	+/–

MPL: maximum permitted level; EU: European Union.
(a): +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

Overall, the Panel considered that the uncertainties identified could, in general, not exclude an underestimation of exposure to sulfur dioxide–sulfites (E 220–228) as a food additive in European countries for the regulatory maximum level exposure scenario when considering only direct authorised addition to foods (i.e. according to Annex II to Regulation No 1333/2008 and Annex IB to Regulation (EC) No 606/2009).

The refined estimated exposure scenario using the dataset 1 results in an adequate estimation of sulfur dioxide–sulfites (E 220–228) used as food additives in European countries, however, it considers only concentration levels not exceeding the MPLs for only direct addition to foods (i.e. according to Annex II to Regulation No 1333/2008 and Annex IB to Regulation (EC) No 606/2009).

2.10.1. Exposure via other sources

Some sulfites are permitted in cosmetic products and are used in drugs. Sulfur dioxide is a chemical compound, which is present in the environment as a consequence of the industrial activity that process sulfur-containing materials, and it is also emitted by present in motor vehicles. Quantification of exposure via all these sources is not precisely known and could therefore not be taken into account in this opinion.

3. Biological and toxicological data

Given that the exposure to gaseous SO_2 resulting from the use of sulfites as food additives is likely to be extremely low and taking into account differences in the environmental conditions in the lung and gastrointestinal tract, the Panel did not consider all the inhalation toxicity studies available for SO_2.

3.1. Physiological occurrence of sulfite

The Panel noted that endogenous sulfite is generated as a consequence of the body's normal processing of sulfur-containing amino acids. JECFA (1987) reviewed the metabolism of sulfite and concluded that small amounts of sulfite are regularly formed in the intermediary metabolism of the body in the catabolism of cysteine by the non-enzymatic decomposition of 8-sulfinyl pyruvic acid to pyruvic acid and SO_2. However, data on the intra- and extracellular concentrations of sulfites and their variability *in vivo* arising from endogenous sulfite production were limited.

Gardiner et al. (1992) reported that rabbit polymorphonuclear neutrophils can produce sulfites when incubated *in vitro* in the presence of sulfate. The authors concluded that their results suggest that

sulfite is not only an exogenous toxic substance and an endogenous metabolite, but also acts as a mediator of neutrophil function, with antimicrobial and proinflammatory activities.

Mitsuhashi et al. (1998) reported that *in vitro* human neutrophils released significant amounts of sulfite (1.0 nmol/h/10^7 cells) in response to lipopolysaccharide (LPS), a major component of bacterial endotoxin. A putative role of sulfite as an endogenous biological mediator was further underscored by the observation that *in vivo* administration of LPS was associated with a marked increase in the serum concentration of sulfite in Wistar rats.

Togawa et al. (1992) described a high-performance liquid chromatographic method for the determination of sulfide, sulfite and thiosulfate, which was applied to the determination of bound sulfide and sulfite, and thiosulfate in normal human serum. Thiosulfate could be determined directly by the use of an ultrafiltered sample. The LOQs were 0.05 μM for thiosulfate, 0.5 μM for bound sulfide and 0.2 μM for bound sulfite. Sera from healthy control subjects contained sulfite at a concentration of 0.1–2.2 μM. The mean concentration of serum sulfite in healthy Japanese subjects was 0.47 ± 0.25 μM.

Ji et al. (1995) developed an assay for the determination of total serum sulfite by utilising: (a) reductive release of serum protein-bound sulfite; (b) derivatisation of free sulfite with monobromobimane; (c) separation of sulfite-bimane from thiol-bimanes by reversed-phase HPLC; and (d) quantitation of sulfite-bimane by fluorescence detection. The detection limit of this assay was 0.44 μmol/L serum sulfite. The intra- and interassay CVs for total serum sulfite at 5.4 μmol/L were 8.1% and 22.0%, respectively. The standard addition method was used to determine total serum sulfite in normal subjects. More than 70 samples were prepared in 2–3 h followed by automated overnight analysis. The mean concentrations (+/- SD) of total serum sulfite in female (n = 41) and male (n = 35) donors were 4.63 +/- 2.33 and 5.16 +/- 2.68 μmol/L, respectively (not statistically significant: p = 0.368). The combined mean concentration of total sulfite in both sexes was 4.87 +/- 2.49 μmol/L. There was no correlation between total serum sulfite and total serum cysteine, cysteinylglycine, homocysteine, subject age, serum cobalamin or serum folic acid. The reference range (mean +/- 2 SD) for total serum sulfite in normal subjects was 0–9.85 μmol/L.

Kajiyama et al. (2000) reported that mean ± SD of serum sulfite in healthy subjects (n = 20) was 1.55 ± 0.54 μM.

Mitsuhashi et al (2004) reported that the serum sulfite concentration in pneumonia patients (n = 25) was significantly higher than that in control subjects (3.75 +/- 0.88 vs. 1.23 +/- 0.48 μM, respectively, $p < 0.05$). Among 20 patients, serum sulfite was serially determined before and after antibiotic therapy. The levels of serum sulfite were significantly reduced during the recovery phase compared with those during the acute phase (1.34 +/- 0.56 vs. 3.65 +/- 0.92 μM, respectively, $p < 0.05$). Moreover, neutrophils obtained from three patients during the acute phase of pneumonia spontaneously produced higher amounts of sulfite *in vitro* than those obtained after recovery. The authors suggested that sulfite may act as a mediator in inflammation and activated neutrophils might be responsible, at least in part, for the upregulation of sulfites during systemic inflammation.

3.2. Absorption, distribution, metabolism and excretion (ADME)

The toxicokinetics of sulfur dioxide and a number of sulfites were reviewed by the Agency for Toxic Substances and Disease Registry in USA (ATSDR, 1998) and JECFA (1987).

The sodium and potassium salts of sulfite and bisulfite exist in aqueous solutions as a mixture of sulfite (SO_3^{2-}) and bisulfite (HSO_3^-) ions.

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

pKa = 1.8 pKa = 7.2

Metabisulfite is the anhydride of the acid sulfite and will lead to the production of bisulfite ions in aqueous solution:

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

For all the above starting substances, the relative levels of sulfur dioxide, sulfite and bisulfite in aqueous solutions are dictated by the pH-dependent equilibria depicted above.

Therefore, the Panel considered that the equilibrium between bisulfite and sulfur dioxide would vary dependent on the pH of the stomach, with bisulfite predominating during fasting phases and in achlorhydria, and sulfur dioxide in acidic conditions. However, sulfite and bisulfite will exist as near equimolar mixtures in the intestine and upon absorption. The liberation of sulfur dioxide gas from ingested sulfited foods has been reported (Lester, 1995). However, the Panel noted that sulfur dioxide gas was highly soluble in water (up to 110 g/L at 20°C) (Ough and Were, 2005) allowing this species to interconvert to bisulfite in the stomach and after passing the pylorus to sulfite as the prevailing pH increases in the intestinal tract.

After absorption, sulfite is converted to sulfate (SO_4^{2-}), a reaction catalysed by sulfite oxidase (SOX). In mammalian tissues, high SOX activity has been measured in the liver, kidney and heart, the highest enzyme expression being in the liver, but the brain, spleen, lungs and testis have been found to have low SOX activity (Gunnison, 1981). Johnson et al (1974) examined SOX activity in the intestine of rats and reported that, in the terminal ileum, the activity was approximately 20% of the activity per gram tissue found in the liver. The levels of SOX mRNA expression in small intestine and colon in humans were reported to be very low compared to liver and kidney (Woo et al., 2003). The presence of SOX in terminal ileum and its activity in the liver suggested that sulfite undergoes significant first pass metabolism, although it is not known to what extent.

While the metabolism of sulfite to sulfate by SOX is well characterised (see below), additional pathways of sulfite metabolism occurring in the gastrointestinal tract have been described. Bisulfite radicals can be formed in the stomach through the reaction between nitrite and sulfite with the formation of NO (Takahama and Hirota, 2012). Another route of sulfite metabolism is by the gut microflora. An early study by Pfleiderer et al. (1968), although very limited due to the low number of animals (n = 2), indicated a metabolism of radiolabelled sulfite by the microflora in the rat intestine. This has subsequently been confirmed in humans with the demonstration of the metabolism of a range of oxidised sulfur compounds, including sulfate and sulfite, by sulfate-reducing bacteria (SRB) to form hydrogen sulfide (H_2S) (Rey et al., 2013). However, the contribution of these gastrointestinal metabolic pathways to the overall metabolism of sulfite is not known.

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, rapidly metabolised by SOX and eliminated in the urine within 24 h, essentially all as sulfate. Studies in monkeys and human volunteers have shown that the half-lives of intravenous (i.v.) administered sulfite were 10 and 15 min, respectively. Elevated levels of thiosulfate in body fluids have been found in cases of SOX deficiency (JECFA, 1987).

JECFA furthermore described non-enzymatic reactions of sulfite with tissue components including lysis of disulfide bonds, with the formation of S-sulfonates and thiols, especially under conditions of sulfite loading. Cysteine-S-sulfonate has been found in urine, while glutathione-S-sulfonate has been detected in bovine ocular lenses. The protein S-sulfonates formed slowly breakdowns and release sulfite ions in the presence of sulfhydryl compounds. S-sulfonates are strongly bound by plasma proteins and are gradually cleared from the blood by mechanisms, which are not yet clearly characterised. Plasma S-sulfonate fractions have been reported to have half-lives of 4 and 8 days in the rat and rhesus monkey, respectively (JECFA, 1987).

In conclusion, the ingested sulfites may be absorbed from the intestine and rapidly metabolised by oxidation to sulfate. However, under high load or when SOX activity is low, the formation of S-sulfonates, including protein S-sulfonates occurs.

SOX activity was found to be lower in the liver of young versus mature rats (Cohen et al., 1974). In 1-day-old rats, SOX activity was approximately 1/10 the level of adult rats. The activity increased as the rats matured and at 32 days of age, the liver SOX activity was approximately half the levels found in adult rats. The efficiency of the sulfite oxidation reaction depends primarily on the activity of SOX (Gunnison and Palmes, 1976). Significant species differences were detected in SOX activity in the liver and kidney of five laboratory animal species (Tejnorová, 1978). The highest activity values were determined in rats, and in the remaining species, the values decreased in the following order: mice, guinea pigs, hamsters and rabbits. Human liver homogenates have 20 times lower SOX activity than rat liver homogenates (Johnson and Rajagopalan, 1976).

Sulfur dioxide is excreted primarily in the urine as sulfate (Yokoyama et al., 1971). Increased levels of sulfates have been detected in the urine of dogs (Yokoyama et al., 1971) and humans (Savic et al., 1987) after exposure to sulfur dioxide.

Data obtained in human polymorphonuclear leucocytes have shown that the addition of sulfite significantly stimulated oxygen (O_2) uptake (Constantin et al., 1994, 1996). Electron spin resonance spectra were consistent with the presence of sulfur trioxide radicals formed during autooxidation of sulfite. The authors concluded that two different routes exist for human polymorphonuclear leucocytes to oxidise sulfite to sulfate: one involving SOX and the other involving the intermediate formation of sulfur trioxide radicals. The rate of sulfur trioxide radical formation was higher in leucocytes with low SOX activity, such as in cells isolated from very old persons and patients with Down's syndrome (Constantin et al., 1996).

Conclusions

In summary, once ingested, sulfites 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 different stomach phases in the pH neutral environment of the intestine and these will be sulfite and hydrogen sulfite. Sulfur dioxide gas is highly soluble in aqueous media but some may be inhaled and absorbed in the lungs as either sulfur dioxide and/or sulfite during and after oral ingestion. A portion of the ingested sulfites may undergo reductive metabolism by the gut microflora to form hydrogen sulfide (H_2S). Of the ingested sulfites, 70–97% may be absorbed from the intestine. Once absorbed, sulfite is converted to sulfate, primarily in the liver, by the enzyme sulfite oxidase. Sulfate, is excreted in the urine along with endogenously formed sulfate. The half-life of sulfites in humans is estimated to be 15 min, but this can vary as studies have shown, particularly in very old people and patients with Down's syndrome can have a lower activity of sulfite oxidase. Polymorphonuclear leucocytes can metabolise sulfites to sulfate via a sulfur trioxide radical intermediate. In addition, under high load, the formation of S-sulfonates, including protein S-sulfonates may occur.

Based on chemical considerations, for all the sulfite-based food additives, the predominant species in aqueous fluids will be bisulfite and sulfite ions. The Panel considered that once ingested, based on their capacity to form sulfite ions, read across between the different sulfite sources is possible.

However, the Panel also acknowledged the existence of significant data gaps on the identity of the reaction products between sulfites and food components and their metabolic fates. The only exception is the stable reaction product 3-deoxy-4-sulfohexosulose, where evidence has shown that it is eliminated predominantly in the faeces and to a limited extent in the urine, unchanged as 3-deoxy-4-sulfohexosulose.

3.3. Toxicological data

3.3.1. Acute oral toxicity

A vomiting reflex in man was reported with doses of sulfite equivalent to less than 3.5 mg SO_2/kg bw (Lafontaine and Goblet, 1955, as referred to by JECFA 1987).

The rabbit oral median lethal dose (LD_{50}) of **sodium sulfite** was determined to be 600–700 mg SO_2 equivalent/kg bw. In man, a single oral dose of 4 g of sodium sulfite caused toxic symptoms in 6 out of 7 persons. In another subject, 5.8 g caused severe irritation of the stomach and intestine (Rost and Franz, 1913, as referred to by JECFA 1987).

A report on **sodium sulfite** (BASF, 1981 [Doc. provided to EFSA n. 5]) in Sprague–Dawley rats (5 males, 5 females) was available and indicated a LD_{50} of 3,160 mg/kg bw. The animals showed similar symptoms as described for bisulfite.

Two reports on acute toxicity studies, one in male and one in female rats were available for **sodium bisulfite** (BASF, 1982b,c [Doc. provided to EFSA n. 7 and 8]). The LD_{50} in female rats was estimated to be 3.85 mL/kg bw and 2.9 mL/kg bw in male rats. The reports indicated that a saturated aqueous solution was administered which is a 38–40% solution. The Panel calculated[22] that the LD_{50} value of 3.85 mL/kg correspond to 1.54 g/kg bw (female rats) and that of 2.9 mL/kg bw to 1.16 g/kg bw (male rats). The difference between the LD_{50} values could be due to the fact that the male rats were 9 weeks at the onset of the study and the female rats were 14 weeks old. The symptoms in both studies were cyanosis, shabby fur, sedation, narcosis, reduced health condition and prone position.

Sodium metabisulfite was tested in mice by intraperitoneal (i.p.) administration and in rats by per orally (p.o.) administration (BASF, 1973a [Doc. provided to EFSA n. 3]). The LD_{50} in mice was 560 mg/kg bw and 3,200 mg/kg bw in rats.

The LD_{50} **of potassium metabisulfite** was tested in mice (i.p.) and in rats (p.o.) (BASF, 1973b [Doc. provided to EFSA n. 4]). The LD_{50} in mice was 640 mg/kg bw and 2,300 mg/kg bw in rats.

For those salts where data were available, acute toxicity of sulfites (sulfite, bisulfite and metabisulfite) was low. Where comparable data were available, these acute toxicity values were similar irrespective of the cation. Although data were not available on all salts permitted as food additives currently, acute toxicity of these salts would also be expected to be low.

3.3.2. Short-term and subchronic toxicity

From JECFA's latest assessment (1987), short-term studies in rats, rabbits and pigs were available. The most important ones are referred to in the following section. Additionally, more recent studies on sodium metabisulfite in rats were identified in the literature.

Rats

Groups of weanling female rats (5 per group) were fed 0.6% **sodium metabisulfite** (not less than 0.34% as equivalent SO_2) for 6 weeks (Bhagat and Lockett, 1964). The test diets were either freshly prepared or stored at room temperature before use. A reduction in growth occurred in rats receiving the fresh diet, which was attributed to lack of thiamine. Rats fed the diet, which had been stored for 75 days developed signs of thiamine deficiency and additional toxic effects, including diarrhoea and stunting of growth, which could not be reversed by the administration of thiamine. The Panel noted that owing to the confounding adverse effects resulting from thiamine deficiency, this study cannot be used to identify a NOAEL; but the authors calculated that 6,000 mg metabisulfite/kg food (~3400 mg

SO_2/kg food), destroyed the thiamine content in the diet to the extent that the diet cannot support any longer the thiamine nutrition of the test animals.

A subchronic oral toxicity study of **sodium metabisulfite** was conducted in groups of eight normal or SOX-deficient female Sprague–Dawley rats (Hui et al., 1989). The authors calculated that the rats were given doses of 0, 7 or 70 mg SO_2 equivalent/kg bw per day; or a higher dose with the drinking water, which was available ad libitum for 8 weeks. In all groups, drinking water was fortified with 50 mg/L thiamine. At the highest dose level (350 mg SO_2 equivalent/kg bw per day for 3 weeks, followed by 175 mg SO_2 equivalent/kg bw per day for 5 weeks of either compound), histopathological examination demonstrated gastric lesions in both normal and sulfite oxidase-deficient rats (hyperkeratosis of the forestomach and dilatation of the deep fundic glands; hypertrophy of chief cells was only seen in sulfite oxidase-deficient rats). The lesions were more pronounced and more frequently encountered in the sulfite oxidase-deficient rats. A NOAEL for sodium metabisulfite was 70 mg SO_2 equivalent/kg bw per day in both normal and sulfite oxidase-deficient rats. A increased urinary excretion of sulfite was noted in sulfite oxidase-deficient rats whether or not they were given exogenous sulfites.

Changes in mucosal disaccharidases and alkaline phosphatase after **sodium metabisulfite** administration were investigated in the small intestine of rats (Rodriguez et al., 1994). Female Wistar rats (10 per group) were given a diet supplemented with 0.25 or 2.5% sodium metabisulfite (corresponding to 125 and 1250 mg metabisulfite/kg bw per day as reported by the authors) for 5 weeks. Sucrase, maltase, lactase and alkaline phosphatase were assayed in intestinal homogenates and in brush border membrane fractions. The intake of 2.5% sulfite induced an increase in the specific activities of sucrase, maltase and alkaline phosphatase compared to control levels ($p < 0.05$). No effect except apart from a significant decrease in lactase activities were reported in animals exposed to 0.25% sodium metabisulfite.

A study was conducted by Ribera et al. (2001) to determine the subacute and subchronic toxicity of sulfite-bound compounds in manufactured biscuits. Groups of 10 male and 10 female rats Sprague–Dawley rats were fed diets prepared with biscuits containing **sodium metabisulfite** or untreated (controls) biscuits enriched with nutrients appropriate for rats for 28 and 85 days. The diets contained sulfited biscuits at levels of 0, 10, 35 or 75%, corresponding to 10–15, 35–45, 150–170 and 310–340 mg SO_2/kg diet as reported by the authors. In both studies, no death or clinical abnormalities were reported. The growth rate, food consumption and food conversion efficiency were not affected by the treatment. No dose-related changes were observed for haematology, clinical chemistry, ocular examination, renal function, urinalysis, organ weights or gross and microscopic examinations. The liver concentrations of vitamins A, B_1, C and E were not significantly changed except for an increase in vitamin E in high-dose males after 28 days exposure. Based on these data, the NOAEL of sulfites in baked biscuits was judged to be 310 mg SO_2/kg diet or 25 mg SO_2/kg bw per day, the highest dose tested. The authors considered the absence of changes in the digestive tract and in the liver as showing that the irritant power and the toxicity of sulfites were modified when submitted to food processing.

In the study of Öztürk et al. (2010), adult male Wistar albino rats (40 animals per group) were divided into two groups: SOX-competent and SOX-deficient rats. The latter group was made deficient by administration of a low-molybdenum diet with concurrent addition of 200 mg tungsten/L to the drinking water for at least 3 weeks in advance of sulfite dosing. Within each of the two groups, a further two groups of 10 animals each were formed: control and **sodium metabisulfite**-treated with 25 mg/kg bw per day in drinking water for 6 weeks. Sulfite treatment induced a low (< 10%) but statistically significant increase in erythrocyte lipid peroxidation in SOX-competent animals and a 40% increase in SOX-deficient animals as measured by thiobarbituric acid reactive substances (TBARS) level. Statistically significant increases in glucose-6-phosphate dehydrogenase, Cu, Zn superoxide dismutase and glutathione peroxidase activities following a 6-week treatment with sulfite were recorded in erythrocytes from SOX-competent and SOX-deficient animals treated with 25 mg sulfite/kg bw per day in drinking water.

In another study (Öztür et al., 2011), male Wistar rats (13 animals per group) were given **sodium metabisulfite** by gavage at doses of 0, 10, 100 or 260 mg/kg bw per day for 35 days. The authors of that study reported a dose-dependent and significant increase in plasma S-sulfonate levels and brain and retina lipid peroxidation levels (measured as TBARS). The levels of 4-hydroxy-nonenal-modified proteins in brain and retina homogenates were also increased in a dose-dependent way. At the highest dose of 260 mg/kg bw per day, the levels were approximately sevenfold higher than the corresponding control levels. No effect on reduced glutathione (GSH)/oxidised glutathione (GSSG) ratio was observed. The authors reported that the general health and body weights of the animals were not affected by the treatment and they suggested that the observed oxidative effect of sulfite correlates with prolonged visual evoked potential latencies suggesting that the increased ingestion of sulfite may cause damage to the visual system. The Panel noted that the effects were observed after treatment for only 35 days, that they were dose dependent and significance ($p < 0.05$ and $p < 0.001$) was frequently achieved at the mid-dose (100 mg sodium metabisulfite/kg bw per day approximately to 50 mg SO_2 equivalent/kg bw per day), no effects were reported at the dose of 10 mg sodium metabisulfite/kg bw per day (approximately 5 mg SO_2 equivalent/kg bw per day). The Panel considered that the study was suggestive of a potential toxic effect for the eyes but needs further supporting data before being possibly used for identifying a NOAEL.

Pigs

In a study of Til et al. (1972a), groups of 20 castrated male and 20 female weanling Dutch landrace pigs were placed on diets supplemented with 50 mg/kg thiamine containing 0, 0.06, 0.16, 0.35, 0.83 or 1.72% **sodium metabisulfite**. A total of 14 males and 14 females per group were sacrificed at 15–19 weeks and the remainder were killed at 48–51 weeks. In addition, a paired-feeding study on 15 male and 15 female weanling pigs/group was performed for 18 weeks at 0 and 1.72% sodium metabisulfite. Food intake and weight gain were reduced at the 1.72% level; however, in the paired-feeding study, growth and food conversion were not affected. Mortality was not related to metabisulfite ingestion. Urinary and liver thiamine levels decreased with increasing dose, but they were reduced below the levels found in pigs on basal diet alone only at 1.72%. Haematology and faecal occult blood determinations were comparable in all groups. Organ/body-weight ratios were elevated at 0.83 and 1.72% for the heart, kidneys and spleen, and at 1.72% for the liver. The paired-feeding study showed liver- and kidney-weight ratios to be increased at 1.72% metabisulfite. Mucosal folds in the stomach and black colouration of the caecal mucosa at the top two dose levels were observed on gross pathological examination. At 0.83 and 1.72% metabisulfite, histopathological examination showed hyperplasia of mucosal glands and surface epithelium in the pyloric and cardiac regions. Intraepithelial microabscesses, epithelial hyperplasia and accumulations of neutrophilic leucocytes in papillae tips were observed in the pars oesophagea. In the caecal mucosa, macrophages laden with pigment granules (PAS-positive containing Cu and Fe) were observed at all dose levels, including controls. The incidence was markedly increased at 0.83% and above. At 1.72% metabisulfite, fat-containing Kupffer cells were present in unusually high numbers in the liver. The authors identified a NOAEL of 0.35% sodium metabisulfite from this pig study. JECFA considered a yield of 67.39% SO_2 from sodium metabisulfite to recalculate to 0.24% SO_2 equivalent from a NOAEL of 0.35% sodium metabisulfite. Assuming a body weight of 100 kg and a feed consumption of 3 kg/day, a final NOAEL of 72 mg SO_2 equivalent/kg bw per day was identified by JECFA (1987).

Overall, the Panel noted that short-term studies with sodium metabisulfite in both SOX-competent or -deficient rats indicated a NOAEL of 70 mg SO_2 equivalent/kg bw per day; the critical effect being gastric lesions. In a subchronic study in pigs, a NOAEL of 72 mg SO_2 equivalent/kg bw per day was identified, higher levels caused mucosal lesions in the stomach and the first part of the large intestine. At 6,000 mg sodium metabisulfite/kg food (~ 3,400 mg SO_2 equivalent/kg food), the thiamine content in the diet was destroyed at such an extent that the diet cannot support the thiamine nutrition of the test animals.

3.3.3. Genotoxicity

More than 60 studies are available in which the genotoxicity of sulfur dioxide, sodium sulfite, sodium bisulfite, sodium metabisulfite and potassium metabisulfite was investigated *in vitro* and/or *in vivo*. No genotoxicity studies are available on potassium bisulfite, calcium sulfite and calcium bisulfite. However, the Panel considered that a read-across approach can be applied for the assessment of genotoxicity.

The Panel evaluated the reliability of the studies and the relevance of their results. The latter is based on the relevance of the genetic endpoint investigated and the reliability of the study. The relevances of the study results were classified into *high*, *limited* and *low*. The study results of only low relevance were not further considered for the assessment of the genotoxicity.

A table on the available *in vitro* and *in vivo* genotoxicity studies with indication of their reliability and relevance is presented in Appendix L and the studies are summarised below.

Sodium sulfite

In vitro *studies*

In a Conference proceeding abstract, Valencia et al., (1973) reported negative results on sex-linked recessive lethal mutation by sodium sulfite in adult males of *Drosophila melanogaster* following administration of a feeding solution at 0.04 and 0.08 M of sodium sulfite.

In the study by Ishidate et al. (1984), **sodium sulfite** was assessed for its mutagenicity in the Ames test with the *Salmonella* Typhimurium tester strains TA1535, TA1537, TA92, TA94, TA98 and TA100 using the preincubation method both in the absence and presence of rat liver S9 metabolic activation up to a concentration of 5 mg/plate. A total of six concentrations were employed. No increases of revertant colonies were observed at any experimental test point.

In a chromosome aberration assay on 242 food additives, **sodium sulfite** was assayed for its clastogenic properties in a Chinese hamster lung (CHL) cell line using three concentrations in the absence of S9 metabolic activation only (Ishidate et al., 1984). The highest concentration employed (0.5 mg/ml) was selected from a cytotoxicity test (based on estimation of the 50% growth inhibition). Negative results were obtained.

In an unpublished report (BASF, 1989a [Doc. provided to EFSA n. 11]), the mutagenicity of **sodium sulfite** (degree of purity 96–98%) in the Ames test with *Salmonella* Typhimurium tester strains TA1535, TA1537, TA98 and TA100 using the standard plate and preincubation assays both in the absence and presence of rat liver S9 metabolism was investigated. Dose levels of 20, 100, 500, 2,500 and 5,000 µg/plate were used. No bacteriotoxic effect was observed. The results obtained for mutagenicity indicated that the test compound did not increase the number of his^+ revertants both in the standard plate test and in the preincubation test either without S9 mix or after the addition of an exogenous S9 metabolising system.

In the study by Meng and Zhang (1999), **sodium sulfite** (in a mixture of 75 mM sodium sulfite and 25 mM sodium bisulfite) was assayed for the induction of gene mutations at the *gpt* locus in AS52 cells. The test was performed in the absence of S9 metabolic activation at dose levels of 5 and 10 mM. The results obtained indicated dose-related and statistically significant increases in mutant frequency. A fourfold increase over the concurrent negative control was observed at the highest concentration which was associated with a marked cytotoxicity (34.5% relative survival). At this dose level, a high percentage of mutants was characterised by deletions within the *gpt* locus as detected by a nested PCR procedure. The authors suggested that the increased mutation frequency by deletions might be related to DNA damage caused by the high cytotoxicity induced at the highest concentration assayed. The Panel agrees with this conclusion.

In a conventional and spore rec-assay employing *Bacillus subtilis* strains M45 rec^-, unable to repair DNA damage and the wild-type strain H17 rec^+ as control, **sodium sulfite** was investigated for its mutagenicity at single dose level of 5 mg/plate. The results reported indicated sodium sulfite as a mutagenic compound. It should be noted that this mutagenicity assays (conventional and spore rec-assay) are not frequently used and are not validated (Ueno et al., 2002; article in Japanese).

Summary of in vitro *data*

Sodium sulfite yielded no evidence for mutagenicity in the bacterial reverse mutation assay (Ishidate et al., 1984; BASF, 1989a [Doc. provided to EFSA n. 11]) and a positive finding in the spore rec-assay using the *Bacillus subtilis* strain M45 (Ueno et al., 2002). In the latter case, the Panel noted that the test is not frequently used and did not receive sufficient validation and considered the study of limited value. In mammalian cells *in vitro*, sodium sulfite did not show clastogenic activity in a limited *in vitro* chromosome aberration assay (Ishidate et al., 1984) and a positive outcome for the induction of gene mutation at the *gpt* locus in AS52 cells (Meng and Zhang, 1999), which was attributed by the authors to the marked cytotoxicity at the highest concentration assayed and the Panel agreed with this conclusion.

In vivo *studies*

In an unpublished report (BASF, 2008 [Doc. provided to EFSA n. 15]) **sodium sulfite** (purity 98.1%) was assessed for its potential clastogenicity and aneugenicity in a mouse bone marrow micronucleus test. Dose levels of 250, 500 and 1,000 mg/kg bw of test compound dissolved in purified water were administered once, subcutaneously, to groups of five male NMRI mice. The treatment schedule included vehicle control and two positive control treated groups, cyclophosphamide for clastogenicity and vincristine sulfate for aneugenicity. The highest dose level employed was selected from dose-range finding experiments as maximum tolerated dose (MTD) as deaths were observed at 1,500 mg/kg. The animals were sacrificed 24 h (all test substance concentrations, vehicle control and both positive controls) and 48 h (highest test substance concentration, vehicle control) after the treatment, respectively. Cytogenetic analyses revealed no significant increase of micronucleated polychromatic erythrocytes (PCEs) in any of test compound treatment groups. A marked reduction of the ratio between PCE and normochromatic erythrocytes (NCE) observed at the high-dose level. A 48-h sampling time indicate that the test compound reached the bone marrow. The study was performed in compliance with the OECD Mammalian Erythrocyte Micronucleus Test Guideline 474, with the exception that the subcutaneous route of treatment employed was not scientifically justified.

In the study by Meng et al. (2004), **sodium sulfite** (in a mixture of 75 mM sodium sulfite and 25 mM sodium bisulfite) was assayed for its capability to induce DNA damage *in vivo* in different organs of mice by means of the alkaline Comet assay. The organs used included brain, lung, heart, liver, stomach, spleen, thymus, bone marrow and kidney. Three groups of six mice each received an i.p. dose of the mixture of 125, 250 or 500 mg/kg bw daily for 7 days. The highest dose used was selected from previous dose-range finding experiments and represents the MTD. A control group of six mice received 200 µl of normal saline i.p. daily for 7 days. The animals were sacrificed at 24 h after the final injection and Comet slides prepared according to standard procedures. A minimum number of 50 cell per animal (300 per group) were analysed for DNA damage using an adequate comet image processing and analysis system and the olive tail moment (OTM) was used to measure the degree of DNA damage. The results obtained indicated dose-related and statistically significant increases in the OTM in cells from all organs tested. However, the Panel noted that no historical control data for the OTM in the different organs employed were presented to assess the biological significance of the results obtained and no concurrent positive control treatment group was available. Furthermore in the absence of an earlier sampling time (2–6 h) following a single administration of the test compound, it is difficult to justify the persistence of elevated levels of DNA damage 24 h from the last treatment which could likely be mediated by cytotoxic effects. On these bases, the positive findings obtained in this study should be critically considered in.

Summary of in vivo *data*

Sodium sulfite yielded no evidence for clastogenicity and aneugenicity in a micronucleus test in the mouse bone marrow, compliant with the OECD Mammalian Erythrocyte Micronucleus Test Guideline 474 (BASF, 2008 [Doc. provided to EFSA n. 15]), but proved to induce DNA damage *in vivo* in different organs of mice in the alkaline comet assay (Meng et al., 2004). In the latter case, the Panel noted major shortcomings in the study, which consequently was considered of limited validity.

Sodium bisulfite

In vitro *studies*

In a Conference Proceeding Abstract, Khoudokormoff (1978) reported negative results on mutagenicity of **sodium bisulfite** at dose levels of 100, 200 and 400 ppm in a *Bacillus subtilis* mutant strain M45 rec^-, unable to repair DNA damage, and the wild-type strain H17 rec^+ as control.

MacRae and Stich (1979) assessed **sodium bisulfite** for its capability to induce sister chromatid exchanges (SCE's) in a Chinese hamster ovary (CHO) cell line in the absence of an exogenous S9 metabolism. A number of six dose levels ranging from 0.03 mM to 7.3 mM were selected. Treatment time was conducted for 2 and 24 h. The results obtained showed dose-related and statistically significant increase of SCE's, both in the short and long treatments. However, it should be noted here that sodium bisulfite has acidic capabilities (pH 5 in 25% water solution) because it has been shown that factors like pH and osmotic pressure might cause genetic damage (Brusick, 1987; Scott et al., 1991; Seeberg et al., 1988). Although authors report a generic neutralisation of induced acidity by 0.1 M NaOH, it is believed that, in the absence of accurate measurements of pH and osmolality values throughout the study, the induction of SCE's might be related to unphysiological culture treatment conditions.

In the study by Mallon and Rossman (1981), **sodium bisulfite** was assessed for its mutagenicity in a Chinese hamster V79 cell line and in the *Escherichia coli* WP2 (wild-type for DNA repair) and $WP2_s$ (uvrA), WP6 (polA), WP5 (lexA) and WP10 (recA) which differ from the wild-type only for DNA repair markers. For mutagenicity in Chinese hamster V79 cell line (mutation to ouabain resistance), dose levels of 10 and 20 mM were employed for 15 min and dose levels of 1 and 5 mM for 48 h. For mutation to 6-thioguanine resistance, a dose level of 10 mM for 15 min was employed. Assay for Trp^+ revertants in *E. coli* was performed at dose levels up to 0.1 M for 15 min with no effects on cell survival. The results for mutagenicity indicate that sodium bisulfite was not mutagenic to either Chinese hamster V79 cells or *E. coli* prokaryotic cells.

In a study by De Giovanni-Donnelly (1985), the mutagenicity of **sodium bisulfite** (mixture of sodium bisulfite and sodium metabisulfite, purity not reported) was investigated in *Salmonella* Typhimurium strains *hisG46* and TA92 with wild-type DNA repair and in several strains with DNA repair deficiencies (TA1950, TA2410, TS24 and GW19) in the absence of metabolic activation, respectively. The bacteria were incubated in 0.2 M acetate buffer at pH 5.2 (negative control) or in the presence of sodium bisulfite at a single concentration of 1 mol/L in 0.2 M acetate buffer at pH 5.2. Samples were plated at zero time and after 30, 60 and 90 min of preincubation. The results were expressed as mutants per plate and as mutants per 10^8 survivors. Sodium azide was used as positive control (but the data were not reported). The treatment with sodium bisulfite resulted in all strains in increased revertant frequencies compared to the negative control. The effect was larger in strain *hisG46* than in the strains with reduced repair capacity. The mutation frequency was increased in strain *hisG46* already at zero time (2–3 min of exposure before plating) and it further increased with time of preincubation, while strain *hisG46* did not respond when the standard plate incorporation assay was used (data on standard plate incorporation assay were not reported in detail). When strain *hisG46* was tested with 30 min preincubation at concentrations of 0, 0.1, 0.5, 1.0, 1.5 and 2.0 mol/L (the highest concentration was equal to 0.2 mmol/plate), a concentration-related increase in the frequency of mutants per plate was observed (up to 19-fold compared to control at the highest non-toxic concentration of 1.0 mol/L, however, the frequency of 0.3 mutants per plate in the negative control

was very low). The Panel noted that the study deviated from the OECD guideline 471 (1983) which would have been applicable at the time when this study was performed (e.g. with respect to the bacterial strains and reporting details). Accordingly, its reliability is limited.

In a study by Pagano and Zeiger (1987), the mutagenicity of **sodium bisulfite** (purity not reported) was investigated in *Salmonella* Typhimurium strains carrying the *his*G46 mutation (TA92, TA1535, TA100, SB2802, SB2061), the *his*D6610 mutation (TA88, TA110, TA90, TA97), the *his*D3052 mutation (TA1538, TA98) and the *his*C3076 mutation (TA1537, TA1977) in the absence of metabolic activation, respectively. The test substance was dissolved in 0.1 M sodium phosphate buffer (pH 5.0, 6.0, 7.0 or 8.0) and preincubated with bacteria for 30 min at eight concentrations up to 0.3 mol/L. It produced a weak concentration-related mutagenic response in strains carrying the *his*D6610 or the *his*G46 mutations (about twofold in TA88, TA110, TA97 and TA92 compared to the negative control, respectively), however, strains TA1535, TA100 and TA90 (carrying also the *uvr*B *rfa* mutations) did not respond. A fivefold increase in the mean frequency of revertants per plate was observed with strain SB2802, however, the Panel noted that the frequency of 3 ± 1 revertants per plate in the negative control was low. In strains TA1538, TA98, TA1537 and TA1977 (carrying the *uvr*B and/or *rfa* mutations), sodium bisulfite was not mutagenic when tested up to a concentration of 0.64 mol/L in the preincubation solution. The mutagenic response was highest when 0.1 M sodium phosphate buffers at pH 5.0–6.0 were used as solvent (after sodium bisulfite was added, the resulting solutions had a pH ranging from 4.1 to 5.9). The mutagenic response observed with strain TA97 decreased with increasing pH, while this range of pH did not have any effect upon the levels of spontaneous revertants. When sodium bisulfite was preincubated for 60, 90 or 120 min, the mutagenic response increased with time but remained to be weak (up to about twofold compared to the negative control) in strain TA97. The Panel noted that the protocol of this study entailed the incubation of bacteria in buffers of different pH in the presence of high concentrations of bisulfite (from 10 mM up to 300 mM), which is different from the direct plate incorporation method recommended by the OECD guideline 471 (1983) applicable at the time when the study was performed. In addition, most of the bacterial strains used have been rarely used and are not recommended for routinely testing. The Panel also noted that the identity of the test substance is not fully clear (the results were described for sodium bisulfate while a supplier was reported for sodium metabisulfite), the purity of the test substance was not reported and no positive control substance was used. Thus, the reliability of the study is limited.

In the study by Popescu and DiPaolo (1988), **sodium bisulfite** was shown to induce *in vitro* cell transformation and SCEs in hamster fetal cells (HFC) but not chromosomal aberrations. However, the Panel noted that the observed effects were obtained at dose levels of 20 mM a concentration far exceeding the physiological limits of 10 mM for *in vitro* treatments with mammalian cells.

Tsutsui and Barrett (1990) investigated the induction of gene mutation, structural and numerical chromosome aberration, SCE's and DNA strand breakage by **sodium bisulfite** in Syrian hamster embryo (SHE) cells. For induction of gene mutation and chromosomal aberration, five dose levels ranging from 0.2 to 5 mM were selected. Cells were exposed to the test compound for 24 h for induction of gene mutation and for 24 or 48 h for chromosomal aberrations. For induction of SCE's, cells were exposed to dose levels of 5, 10 and 20 mM in the short treatment (15 min) and 0.5, 2 and 5 mM in the long treatment (24 h). The results obtained indicate that sodium bisulfite did not prove to induce under the reported experimental conditions in either gene mutation or chromosomal aberrations (structural and numerical). In contrast, dose-related and statistically significant increases were reported for induction of SCE's in the long treatment (24 h). However, it should be noted here that sodium bisulfite has acidic capabilities (pH 5 in 25% water solution) because it has been shown that factors like pH and osmotic pressure might cause genetic damage (Brusick, 1987; Scott et al., 1991; Seeberg et al., 1988). It is believed that in the absence of accurate measurements of pH and osmolality values throughout the study, the induction of gene mutation, structural and numerical chromosome aberration, SCE's and DNA strand breakage might be related to unphysiological culture treatment conditions.

In an unpublished report (Bayer, 1988) **sodium bisulfite** was assessed for its mutagenicity in the *Salmonella*/microsome assay both in the absence and presence of rat S9 metabolism using five dose levels ranging from 0.001 to 10 µl/plate. The following *Salmonella* Typhimurium tester strains were employed: TA1535, TA1537, TA98 and TA100. The results obtained showed negative results. In the repeat study, due to the very low cytotoxicity achieved in the first trial seven dose levels were used ranging from 1.5 to 96 µl/plate. The results obtained confirmed previous negative findings.

In the study by Meng and Zhang (1999), **sodium bisulfite** (in a mixture of 75 mM sodium sulfite and 25 mM sodium bisulfite) was assayed for induction of mutation at the *gpt* locus in AS52 cells. The test was performed in the absence of S9 metabolic activation at dose levels of 5 and 10 mM. The results obtained indicated dose-related and statistically significant increases in mutant frequency. A fourfold increase over the concurrent negative control was observed at the highest concentration which was associated with a marked cytotoxicity (34.5% relative survival). At this dose level, a high percentage of mutants was characterised by deletions within *gpt* locus, as detected by a nested polymerase chain reaction (PCR) procedure. The authors suggest that the increased mutation frequency by deletions may be related to DNA damage caused by the high cytotoxicity induced at the highest concentration assayed. The Panel agrees with this conclusion.

Summary of in vitro *data*

Inconsistent results were reported for the mutagenicity of **sodium bisulfite** in bacterial reverse mutation assays. While mutagenic effects were reported by De Giovanni-Donnelly (1985) and Pagano and Zeiger (1987), sodium bisulfite proved to be not mutagenic in other *Salmonella*/microsome assays both in the absence and presence of S9 metabolic activation (Bayer 1988) and in the tryptophan-requiring *E. coli* strains $WP2_s$ (uvrA), WP6 (polA), WP5 (lexA) and WP10 (recA) (Mallon and Rossman 1981). In mammalian cells, sodium bisulfite did not induce gene mutation in a limited study in V79 cells (Mallon and Rossman), chromosome aberrations in HFC (Popescu and DiPaolo, 1988), gene mutation or structural and numerical chromosomal aberrations (Tsutsui and Barrett, 1990). In contrast, it induced SCE's in different mammalian cell lines (MacRae and Stich, 1979; Popescu and DiPaolo, 1988; Tsutsui and Barrett, 1990) and gene mutation at the *gpt* locus in AS52 cells (Meng and Zhang, 1999). However, these positive findings were attributed by the Panel to non-physiological treatment conditions in the case of SCE's or high cytotoxicity levels in the case of gene mutation.

In vivo *studies*

In the study by Generoso et al. (1978), **sodium bisulfite** was assessed for its capability to induce chromosomal damage in mice germ cells using dominant lethal and heritable translocations assays in male animals and dominant lethal assay in female animals. Dose levels were 400 and 300 mg/kg bw per day for males given by i.p. injection for 20 times over a period of 26 days at the high dose level and for 38 times over a period of 54 days at the lower dose level. For female animals, a dose level of 550 mg/kg was delivered as single i.p. injection. The results obtained showed negative findings for both dominant lethal and heritable translocations.

In the study by Meng et al., (2004), **sodium bisulfite** (in a mixture of 75 mM sodium sulfite and 25 mM sodium bisulfite) was assayed for its capability to induce DNA damage *in vivo* in different organs of mice by means of the alkaline Comet assay. The organs used included brain, lung, heart, liver, stomach, spleen, thymus, bone marrow and kidney. Three groups of six mice each received an i.p. dose of the mixture of 125, 250 or 500 mg/kg bw daily for 7 days. The highest dose used was selected from previous dose-range finding experiments and represents the MTD. A control group of six mice received 200 µl of normal saline i.p. daily for 7 days. The animals were sacrificed 24 h after the final injection and Comet slides prepared according to standard procedures. A minimum number of 50 cell per animal (300 per group) were analysed for DNA damage using an adequate comet image processing and analysis system and the OTM was used to measure the degree of DNA damage. The results obtained indicated dose-related and statistically significant increases in the OTM in cells from all organs tested. However, the Panel noted that no historical control data for the OTM in the different

organs employed were presented to assess the validity of the study and the biological significance of the results obtained, no concurrent positive control treatment group was available and a limited number of cells scored. Furthermore, in the absence of an earlier sampling time (2–6 h) following a single administration of the test compound, it is difficult justify the persistence of elevated levels of DNA damage 24 h from the last treatment which could likely be mediated by cytotoxic effects. On these bases, the positive findings obtained in this study should be critically considered.

Summary of in vivo *data*

Sodium bisulfite did not induce both dominant lethal and heritable translocations in male and female rats (Generoso et al., 1978) and chromosome aberrations and micronuclei in normal and sulfite oxidase-deficient Chinese hamster and NMR1 mice (Renner and Wever, 1983). These studies were conducted according to the acknowledged international protocols at the time. In contrast sodium bisulfite induced DNA damage in different organs of mice by the alkaline comet assay (Meng et al., 2004), in a study judged by the Panel of limited validity due to the presence of major shortcomings which included the absence of concurrent positive control treatment group, the absence of historical control data to assess the validity of the study and the biological significance of the results, the absence of an earlier (2–6 h) sampling time and a limited number of cells scored.

Sodium and potassium metabisulfite

In vitro *studies*

In the study of Abe and Sasaki (1977), **potassium metabisulfite** was assessed for its genotoxicity evaluating the induction of chromosomal aberration and SCE's in a pseudodiploid Chinese hamster (Don) cell line at dose levels of 0.1, 0.5 and 1 mM for 26 h in order to cover two rounds of replication. Results obtained were judged negative for induction of chromosomal aberrations and equivocal for induction of SCE's.

In a chromosome aberration assay on 134 compounds, **potassium metabisulfite** was assayed for its clastogenic properties in a CHL cell line at a maximum dose level of 0.6 mM in the absence of exogenous S9 metabolism. The high dose level was selected from preliminary toxicity test based on estimation of the 50% growth inhibition. Treatment was performed for 48 h. The results obtained were negative (Ishidate and Odashima, 1977).

In the study by Ishidate et al. (1984), **potassium metabisulfite** was assessed for its mutagenicity in the Ames test with the *Salmonella* Typhimurium tester strains TA1535, TA1537, TA92, TA94, TA98 and TA100 using the preincubation method both in the absence and presence of rat liver with or without S9 metabolism activation up to a concentration of 3 mg/plate. A total of six concentrations were employed. No increases of revertant colonies were observed at any potassium metabisulfite treatment experimental test point.

In a chromosome aberration assay on 242 food additives, **potassium metabisulfite** was assayed for its clastogenic properties in a CHL cell line using three concentrations in the absence of S9 metabolic activation only (Ishidate et al., 1984). The highest concentration employed (0.06 mg/ml) was selected from a cytotoxicity test (based on estimation of the 50% growth inhibition). Negative results were obtained. The Panel noted that the experimental designed followed was limited.

In an unpublished study report (BASF, 1989b [Doc. provided to EFSA n. 14]), negative results were also reported for **sodium metabisulfite** (approximately purity, 98%) in the Ames test with *Salmonella* Typhimurium tester strains TA1535, TA1537, TA98 and TA100 using the standard plate and preincubation assays both in the absence and presence of rat liver S9 metabolism. Dose levels of 20, 100, 500, 2,500 or 5,000 µg/plate were used. No cytotoxicity was also recorded.

Potassium metabisulfite (purity 97–98%) was reported to be negative for its mutagenicity in the Ames test with *Salmonella* Typhimurium tester strains TA1535, TA1537, TA98 and TA100 using the

standard plate and preincubation assays both in the absence and presence of rat liver S9 metabolism (BASF, 1989c [Doc. provided to EFSA n. 13]). Dose levels of 20, 100, 500, 2,500 and 5,000 µg/plate were used. No cytotoxicity was also recorded. A weakly bacteriotoxic effect was observed only using TA 100 at doses > 2,500 µg/plate.

In the study by Prival et al. (1991), **sodium metabisulfite** was assessed for its mutagenicity in the Ames test with the *Salmonella* Typhimurium tester strains TA1535, TA1537, TA1538, TA98 TA100 and the tryptophan-requiring *E. coli* strain WP2, using the standard plate-incorporation method both in the absence and presence of rat liver S9 metabolism. Dose levels of 0.033, 0.10, 0.33, 1.0, 3.3 or 10 mg/plate were used and no mutagenic effects were observed. The Panel noted that the study was performed with a complete set of bacterial tester strains as recommended by the relevant OECD Guideline TG 471.

Rencüzoğullari et al. (2001) investigated the induction of chromosomal aberration and SCE's by **sodium metabisulfite** in human lymphocytes. Cells were treated with dose levels of 75, 150 and 300 µg/ml for 24 and 48 h. The results obtained showed dose-related and statistically significant increases for both SCE's and structural chromosomal aberrations. However, the Panel noted major shortcomings, which include the presence of elevated values of chromosome aberrations in the untreated control (14 chromatid breaks and 8 chromosome breaks out of 400 cells scored). Furthermore, two chromatid exchanges and one polyploid cell out of 400 cells scored were also found, which are not normally observed in healthy donors. This is probably due to inadequate culture conditions, which include the use of high concentration of 5-bromo-2'-deoxyuridine (10 µg/ml), which is not requested in the chromosome aberration assay and needs to be protected from light to avoid spontaneous generation of DNA single strand breaks in the halogen substituted DNA.

Yavuz-Kocaman et al. (2008) investigated the induction of chromosomal aberrations, SCE's and micronuclei by **potassium metabisulfite** in human lymphocytes. Cells were treated with dose levels of 25, 50, 100 and 200 µg/ml for 24 and 48 h. The results obtained showed dose-related and statistically significant increases for induction of SCE's, structural chromosomal aberrations and micronuclei following 24 and 48 h treatment. However, the Panel noted major shortcomings which include for chromosomal aberrations the use of high concentrations of 5-bromo-2'-deoxyuridine (10 µg/ml), which is not requested in the chromosome aberration assay and needs to be protected from light to avoid spontaneous generation of DNA single strand breaks in the halogen substituted DNA. Furthermore in the micronucleus assay, the stimulation of cells with phytohaemoagglutinin and treatment schedule with cytochalasin B and test compound appear to be unusual.

In an unpublished report (Covance, 2010 [Doc. provided to EFSA n. 21]), **sodium metabisulfite** was assayed for the ability to induce mutation at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus (6-thioguanine [6TG] resistance) in mouse lymphoma cells using the fluctuation method. A 3-h treatment incubation period was used and dose levels employed ranged from 100 up to 1,902 µg/mL (equivalent to 10 mM) in three independent experiments; each conducted in the absence and/or presence of metabolic activation by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S9). The results obtained indicate that sodium metabisulfite did not induce mutation at the hprt locus of L5178Y mouse lymphoma cells. The Panel noted that the study was performed in compliance with the methods indicated in the OECD TG 476 guideline.

Summary of in vitro *data*

Potassium metabisulfite did not show mutagenic activity in adequately performed bacterial reverse mutation assays (Ames test) (Ishidate et al., 1984; BASF, 1989c [Doc. provided to EFSA n. 13]; Prival et al., 1991) and did not prove to induce chromosomal aberrations in mammalian cells (Abe and Sasaki, 1977; Ishidate and Odashima, 1977; Ishidate et al., 1984 and SCE's (Abe and Sasaki 1977). In contrast, it induced chromosome aberration, SCE's and micronuclei in a study (Yavuz-Kocaman et al., 2008) judged by the Panel to be of limited validity due to methodological shortcomings.

Sodium metabisulfite did not show mutagenic activity in an adequately performed bacterial reverse mutation assay (BASF, 1989b [Doc. provided to EFSA n. 12]) and in a gene mutation assay at the HPRT locus of L5178Y mouse lymphoma cells performed in compliance with the methods indicated in the OECD TG 476 guideline (Covance, 2010 [Doc. provided to EFSA n. 21]). However, it induced chromosomal aberrations and SCE's in a study (Rencuzogullari et al., 2001) judged by the Panel to be of limited validity due to methodological shortcomings.

In vivo *studies*

Renner and Wever (1983), investigated the induction of SCE's, chromosomal aberrations and micronuclei by **sodium metabisulfite** in normal and sulfite oxidase-deficient Chinese hamsters and NMR1 mice. To induce sulfite-oxidase deficiency, animals were maintained on a low-molybdenum diet in combination with distilled water supplemented with 200 ppm of sodium tungstate. For induction of SCE's, normal animals were administered with the test compound in water or fruit juice solution by oral gavage at a dose level of 660 mg/kg bw. In addition, in order to maintain exposure to test compound at levels sufficiently high, animals were subsequently injected subcutaneously at 20 min intervals with 12 dose levels of 50 mg/kg bw. The sulfite oxidase-deficient Chinese hamsters and NMR1 mice received one single dose level by oral gavage at 330 and 165 mg/kg bw in water, respectively, and 12 dose levels of 50 mg/kg bw by subcutaneous injections. For chromosomal aberrations and micronuclei induction, normal animals were administered twice with test compound in water or fruit juice solution by oral gavage at a dose level of 660 mg/kg bw. The sulfite oxidase-deficient Chinese hamsters and NMR1 mice received by oral gavage, two dose level each in water or fruit juice at 330 and 165 mg/kg bw, respectively. Cytogenetic end points were analysed and results obtained showed negative results even when SOX activity reached extremely low values. Studies were performed according to the acknowledged international protocols at the time.

In the study by Pal and Bhunya (1992), **sodium metabisulfite** was assayed for its capability to induce chromosome aberrations and micronuclei in Swiss mice bone marrow cells. Sperm shape abnormality was also investigated but this end-point is not considered relevant for genotoxicity. For chromosome aberrations, the authors used several experimental designs: animals were injected i.p. with the highest does (400 mg/kg bw) and sacrificed after 6, 24 and 48 h; animals were injected i.p. with three different doses (200, 300 and 400 mg/kg bw) and sacrificed after 24 h ; animals dosed with 400 mg/kg bw i.p., p.o. and subcutaneous injection were sacrificed after 24 h. The highest dose (400 mg/kg bw) was divided into 5 equal parts and each part was successively administered i.p. with a gap of 24 h in-between and the animals were sacrificed 120 h after the first injection.

For the micronucleus test, dose levels of 200, 300 and 400 mg **sodium metabisulfite**/kg bw were administered twice by i.p. injection 24 h apart. The results reported indicated slight but significant increases of chromosomal aberrations for sodium metabisulfite administered by i.p. and subcutaneously at different dose levels and sampling times. Negative results were observed in the animals administered by oral gavage at any dose-level tested. Similarly, for micronucleus test, slight but significant increases in the incidence of micronucleated PCE were only observed at the intermediate (300 mg/kg bw) dose level. However, for chromosomal aberrations, the results included chromatid and isochromatid gaps which are mandatorily excluded from the evaluation, and for the micronucleus test, the significant increase observed was very small and it was often found in untreated control animals. Furthermore, the study bears a number of shortcomings which include an inadequate selection of treatment regimens and sampling times which markedly deviates from the current OECD Guidelines 473 and 474 for the *in vivo* chromosomal aberrations and micronucleus tests, respectively, and the absence of concurrent positive control treatment groups. In addition, the number of cells scored and the number of animals used were insufficient. On these bases, the Panel considered this study unreliable for risk assessment.

In an *in vivo* rat bone-marrow chromosomal aberration assay, Yavuz-Kocaman et al. (2008) investigated the clastogenicity of **potassium metabisulfite**. Four albino rats (two males and two females) were used for each test substance treatment and control groups. The rats were administered once by i.p. injection with dose levels of 150, 300 and 600 mg/kg bw 12 and 24 h before sacrifice.

One-hundred metaphases per animal (400 metaphases per group) were analysed. The results obtained indicate dose related and statistically significant increases in the chromosomal damage both at the 12- and 24-h sampling times which consisted uniquely of chromatid and chromosome breaks. It should be noted here that the data obtained for structural and numerical chromosome aberrations from individual animals were pooled and reported accordingly. However, since in *in vivo* studies, the statistical unit is the individual animal, the procedure followed appears inadequate for correct evaluation of results. Furthermore, the simultaneous coexistence of chromatid and chromosome breaks in the same treatment group which are not compatible with the genotoxic mechanism of chemical compounds. Furthermore, the criteria followed for scoring of aberrations refer to a human cytogenetic biomonitoring study not usually used (Paz-y-Miño et al., 2002). Taking into account the above mentioned observations, the study appears to be not useful for evaluation.

Carvalho et al. (2011a) evaluated the *in vivo* genotoxic effects of **sodium metabisulfite** on different tissues of the mouse, by use of the comet assay (liver, blood and bone marrow cells) and the micronucleus test (blood and bone marrow cells). Ten mice per group (five females and five males) received once by oral gavage dose levels of 0.5, 1, or 2 g/kg bw of sodium metabisulfite and were sacrificed 24 h later. In the comet assay, genetic damage was based on the calculation of a 'damage index' obtained by manual allocation of cells into five classes according to tail size (0 = no tails and 4 = maximum-length tails), which resulted in a single DNA damage score for each sample analysed. In the micronucleus test, genetic damage was based on the evaluation of the number of micronuclei observed out of 2,000 immature bone marrow erythrocytes (PCE) and 2,000 peripheral blood reticulocytes (RET) per animal. Cell proliferation status was evaluated by measuring in the bone marrow, the ratio of immature (PCE) to mature erythrocytes (NCE) based on the scoring of 1,000 PCE. The results obtained showed significant increases of DNA breakage in the comet assay at 1 and 2 g/kg in blood, liver and bone marrow cells. Additionally, in the micronucleus test, the authors reported significant increases in micronucleus frequencies both in blood and bone marrow cells at 2 g/kg bw only. It should be noted here that for comet assay, positive results were based on the calculation of a 'damage index' which is seldomly used and not validated. Furthermore, conclusions raised are not fully supported by data presented since DNA breakage observed at 24 h after treatment appear to be not coherent with induction of micronuclei at the same sampling time following acute exposure to the test compound. The absence of an earlier sampling time in the comet assay (3–6 h) further weakens the raised conclusions. For micronucleus test, positive effects were not dose-related and were only observed at the highest dose, level used (2 g/kg bw), both in the blood and bone marrow. Although the results were based on the scoring of a sufficient number of PCE and RET, some concerns arise from the staining procedures based solely on the use of 5% Giemsa solution which does not a correct discrimination of mature and immature erythrocyte. This is also supported by the unusual reported high level of PCE/NCE ratio in the untreated negative control in the bone marrow (1.67 $\pm$ 0.67) which is usually close to 1 in different mouse strains. Historical control data for both micronucleus and comet assays have not been reported. On the basis of the observations presented, the study appears to be not suitable for evaluation.

In a following study, Carvalho et al., (2011b) investigated the clastogenic potential of **sodium metabisulfite** in sea waters and sediments collected in a shrimp farm in Cajueiro da Praia (Brazil) by analysis of induction of chromosomal aberrations and micronuclei in the *Allium cepa* (onion). Water and sediment samples were collected in the dry and in the rainy seasons, in three sites: upstream the shrimp farm, at a point sodium where metabisulfite is discharged and 100 m downstream the farm. Three sample dilutions were used (50%, 25% and 10%) for all samples. A negative control (well water) and a positive control (copper sulfate) were used in each experiment. Following 72-h exposure, onion roots were measured and removed and cytogenetic slides prepared according to standard procedures. Cytogenetic analyses did not reveal any significant induction of both chromosomal aberrations and micronuclei. However, the Panel noted that the tests used are rarely employed and did not receive adequate validation.

Summary of in vivo *data*

Potassium metabisulfite was reported to induce chromosomal aberrations in rat bone marrow cells in a study (Yavuz-Kocaman et al., 2008) judged by the Panel to be not useful for risk assessment due to the presence of major shortcomings which included an inadequate statistical evaluation of data and the simultaneous coexistence of chromatid and chromosome breaks in the same treatment group which are not compatible with the genotoxic mechanism of chemical compounds.

Sodium metabisulfite proved to induce chromosomal aberrations and micronuclei in mouse bone marrow cells (Pal and Bhunya, 1992), and in *Allium cepa* (Carvalho et al., 2011b), DNA damage in mouse blood, liver and bone marrow cells by the use of alkaline comet assay and micronuclei in blood and bone marrow cells. However, these studies were judged by the Panel to be unsuitable for a correct risk assessment for major shortcomings or the absence of validation.

Sulfur dioxide

In vitro *studies*

In the study by Uren et al. (2014), **sulfur dioxide** was investigated for its capability to induce SCEs and micronuclei in human lymphocytes treated *in vitro* in the absence of S9 metabolic activation system only. Dose levels of 0, 10, 0.50 and 1.00 ppm of SO_2 were employed. The reported results indicate significant increases of SCE and micronuclei at dose levels of 0.50 and 1.00 ppm. However, the maximum increases observed for both SCE and micronuclei (4.62 and 5.50, respectively) are small in absolute terms and are normally observed in negative control human lymphocyte cultures. In addition, in the SCE assay, the positive control cyclophosphamide did not show positive results. It should be noted that cyclophosphamide, a positive control substance which needs metabolic activation to be active and routinely used in these *in vitro* assays in the presence of S9 metabolic activation, was employed in this case in the absence of S9 metabolic activation with unexpected positive results in the micronucleus test. In addition, the negative historical control values to check for biological significance were not presented. On these bases, the Panel considered this study not adequate for risk assessment.

In vivo *studies*

Meng et al. (2002) investigated the induction of micronuclei in bone marrow cells of male and female Kunming mice following inhalation exposure to **sulfur dioxide**. Concentrations of 0, 14, 28, 56 and 84 mg SO_2/m^3 were administered to groups of five male and five female mice for 4h/day for 7 days. The animals were sacrificed 24 h after the last SO_2 inhalation and bone marrow smear slides were prepared for induction of micronuclei. For each animal a number of 1000 immature PCE were scored for induction of micronuclei. The results obtained indicated dose-related and statistically significant increases of micronucleated PCE compared to the concurrent negative control. The authors concluded that sulfur dioxide (SO_2) is a genotoxic compound. However, the Panel noted that the study bears major shortcomings, which include a limited number of immature PCE scored (1,000 PCE/animal), no historical control data were used to assess the validity of the study and the biological significance of the results obtained and no scoring of mature NCE which is important to assess toxicity of the target organ and to evaluate the health status of animals for the presence of micronuclei before treatment. Furthermore, no concurrent positive-control treatment group was available to assess correct functioning of the experimental system. On these basis, the Panel considered that the conclusions raised by the authors were not supported by experimental data.

In the study by Meng and Zhang (2002), **sulfur dioxide** was investigated for its capability to induce, by inhalation, chromosomal aberrations in bone marrow cells of male and female Kunming mice. Concentrations of 0, 7, 14, 28 and 56 mg SO_2/m^3 were administered in one case to groups of four male and four female mice for 4 h/day for 7 days and at 14 mg SO2/ m^3 for 1, 3, 5 and 7 days to groups of 10 male mice in the other case. Twenty-two hours after the last SO_2 inhalation, mice were injected with colchicine to accumulate cells in metaphase and sacrificed 2 h later. Bone marrow metaphase spreads were prepared and cytogenetic slides were stained with Giemsa and analysed for the presence of chromosomal aberrations out of 100 metaphase per animal. In both cases, statistically significant increases in chromosomal aberrations which were also dose-related in one case and exposure related in

the other case. Furthermore, dose-related reduction in mitotic indices indicate, according to the authors, that SO_2 reached the bone marrow cells. On this basis, the authors concluded that SO_2 is a clastogenic compound *in vivo*. However, the Panel noted that the highest increase observed in the frequency of aberrant cells was not marked (4.86% aberrant cells at 56 mg SO_2/m^3 vs 1.81% in the untreated control) and no historical control data to check for biological relevance were available. In addition, it is difficult to support time-related increases in the frequency of aberrant cells for a clastogenic compound, up to 7 days, since damaged cells are selectively eliminated during cell divisions and therefore cannot accumulate with the time. Furthermore, the statistical method applied to assess the statistical significance of aberrant cells does not appear to be appropriate and no positive-control treatment groups were available. On this basis, positive findings obtained in this study should be critically considered.

In the study by Meng et al. (2005), **sulfur dioxide** was assayed for its capability to induce DNA damage *in vivo* in different organs of mice by means of the alkaline Comet assay following inhalation exposure. The organs used included brain, lung, heart, liver, stomach, spleen, thymus, bone marrow and kidney. The animals were randomly allocated to five groups, each containing six male and six female mice. Four groups were exposed to 14, 28, 56 and 112 mg/m^3 SO_2 in 1 m^3 exposure chamber for 6 h/day for 7 days. The fifth group was used as negative control group and was exposed to filtered air in a separate 1 m^3 exposure chamber using the same schedule. The animals were sacrificed immediately after last exposure and Comet slides prepared according to standard procedures. A minimum number of 50 cell per animal (300 per group) were analysed for DNA damage using an adequate comet image processing and analysis system, and the OTM was used to measure the degree of DNA damage. The results obtained indicated dose-related and statistically significant increases in the OTM in cells from all organs tested. However, the Panel noted that no historical control data for the OTM in the different organs employed were presented to assess the validity of the study and the biological significance of the results obtained. Furthermore, no concurrent positive control treatment group was available to assess correct functioning of the experimental system. On these bases, the significant increases of DNA breakage obtained in the different organs of the SO_2-treated rats obtained in this study should be critically considered.

In the study by Ziemann et al. (Fraunhofer, 2008a,b [Docs. provided to EFSA n. 35 and 36]; Ziemann et al., 2010), the induction of micronuclei in the bone marrow PCE of male outbred NMRI mice was investigated following treatment with **sulfur dioxide** by inhalation at dose levels of 0 (clean air), 2.7, 8, 27, or 80 mg/m^3 (0, 1, 3, 10, or 30 ppm) SO_2 for 4 h/day on seven consecutive days. Animals were sacrificed 24 h after administration of the last exposure, and blood samples (for clinical analyses) and bone marrow smears for micronuclei analysis were prepared. The results obtained indicated that SO_2 did not induce micronuclei in PCE of the bone marrow at any dose level tested and that haematological parameters, such as haematocrit, haemoglobin, erythrocyte/platelet/total leucocyte counts, differential white blood cell counts and indicators of blood formation (reticulocyte counts, ratio of PCE/NCE in the bone marrow) remained unchanged by SO_2 treatments compared with the clean-air controls. The positive control cyclophosphamide (60 mg/kg bw) induced as expected significant increases over the concurrent untreated control of micronucleated PCEs. In addition, SO_2 treatments significantly enhanced the levels malondialdehyde in erythrocyte lysates by the thiobarbituric acid-reactive substances method indicating potential SO_2-mediated oxidative stress, but also demonstrating systemic availability of the inhaled SO_2. The Panel noted that this study was fully compliant with the methods and recommendations indicated in the relevant OECD Guideline 474.

Summary of in vitro *and* in vivo *data*

Sulfur dioxide was reported to induce SCE's and micronuclei in human lymphocytes *in vitro* in a study (Uren et al., 2014) judged by the Panel to be inadequate for risk assessment due to the presence of major shortcomings. *In vivo*, it was also reported to induce micronuclei (Meng et al., 2002) chromosome aberrations (Meng and Zhang, 2002) and DNA damage by means of the alkaline comet assay (Meng et al., 2005) following inhalation exposure. However, the above mentioned studies are judged by the Panel to be inadequate for risk assessment for the presence of major shortcomings which

include the absence of concurrent positive control treatment groups, the absence of historical control values to assess the validity of the assays and the biological significance of the results obtained.

In contrast, negative results were obtained in a bone marrow micronucleus test in mice following inhalation exposure (Ziemann et al., 2010), which was mainly compliant with the methods and recommendations indicated in the relevant OECD Guideline 474.

Conclusion

Inconsistent results from studies of limited relevance were observed with bacterial gene mutation assays. Some positive results were obtained with sodium bisulfite (De Giovanni-Donelli 1985; Pagano and Zeiger 1987), however, the results were of limited relevance and there are other studies of limited relevance in which an induction of gene mutations in bacteria was not observed with sodium bisulfite (Mallon and Rossman, 1981; Bayer 1988 [Doc. provided to EFSA n. 18]), sodium and potassium metabisulfite and sodium sulfite (BASF, 1989b,c [Doc. provided to EFSA n. 12 and 13]; Ishidate et al 1984), respectively. Negative results of limited relevance were also observed with sodium bisulfite in gene mutation assays in mammalian cells *in vitro* (Mallon and Rossmann 1981; Tsutsui and Barrett 1990). In addition, there is a negative result of high relevance obtained with sodium metabisulfite *in vitro* in a gene mutation assay in mouse lymphoma cells (Covance, 2010 [Doc. provided to EFSA n. 21]). Thus, based on the *in vitro* data available, there is no concern with respect to the potential induction of gene mutations and, accordingly, no need for an *in vivo* follow-up on this genetic endpoint.

Negative results were observed in some *in vitro* chromosomal aberration assays and *in vitro* micronucleus assays with sodium sulfite (Ishidate et al. 1984), sodium bisulfite (Tsutsui and Barrett 1990) and potassium metabisulfite (Ishidate et al. 1984). However, the results were of limited relevance and chromosomal aberrations and micronuclei were observed with sodium and potassium metabisulfite in human lymphocytes *in vitro* (Rencuzogullari et al. 2001; Yavuz-Kocaman et al. 2008) in studies of high relevance. In addition, positive results were observed *in vivo* with sodium metabisulfite in a chromosomal aberration assay in SOX-deficient Chinese hamsters after subcutaneous exposure (Renner and Wever, 1983), with potassium metabisulfite in a chromosomal aberration assay in rat bone marrow after single i.p. administration and with sulfur dioxide in a micronucleus assay and a chromosomal aberration assay in mouse bone marrow after inhalation, respectively (Meng et al. 2002; Meng and Zhang 2002). A positive result was also observed in several organs of mice in a Comet assay after inhalation (Meng et al. 2005). However, all these *in vivo* results had certain shortcomings and were accordingly of limited relevance. Moreover, these positive results were not confirmed in a reliable micronucleus assay performed with sulfur dioxide by Ziemann et al. (2010). This study has only minor shortcomings (there are only indirect indications of target tissue exposure and the maximum dose was chosen in order to investigate the reproducibility of other studies and was not strictly based on the maximum tolerated dose). It is therefore considered to be of high to limited relevance. A negative result was also observed in a reliable *in vivo* micronucleus assay in mouse bone marrow after subcutaneous administration of sodium sulfite with demonstration of target tissue exposure (BASF, 2008 [Doc. provided to EFSA n. 15]). This study is considered to be of high relevance, too. Further negative results were observed in studies of limited relevance with sodium metabisulfite in *in vivo* chromosomal aberration assays in mice and hamsters after oral exposure (Renner and Wever, 1983).

Overall, based on these data the Panel concluded that the use of sulfur dioxide and sulfites (sodium sulfite, sodium bisulfite, sodium metabisulfite, potassium metabisulfite, potassium bisulfite, calcium sulfite and calcium bisulfite) as food additives does not raise a concern with respect to genotoxicity.

3.3.4. Long-term toxicity and carcinogenicity

JECFA (1987) and the SCF (1996) evaluated several studies in mice and rats. In the lack of any newer long-term studies, these studies are reviewed below.

3.3.4.1. Animal studies

Mice

Groups of 50 male and 50 female ICR/JCL mice received **potassium metabisulfite** in drinking water ad libitum at concentrations of 0, 0.1 or 2% for 24 months. No difference in tumour incidence between groups was found (JECFA, 1987; Tanaka et al., 1979). Under the assumption of a mouse body weight of 25 g and a daily water intake of 5 g/day, the authors calculated the daily doses to be 0, 200 or 4,000 mg/kg bw per day. The NOAEL in this study was the highest dose given, 4,000 mg potassium metabisulfite/kg bw per day corresponding to 2,300 mg SO_2 equivalents/kg bw per day, however, the reliability of this NOAEL is questionable due to the uncertainty in the assumptions made for its calculation.

Rats

Groups of rats (18 to 24 per group) were fed **sodium bisulfite** at dosages of 125, 250, 500, 1,000, 2,500, 5,000, 10,000 or 20,000 mg/kg diet for periods ranging from 1 to 2 years (Fitzhugh et al., 1946). The rats fed a diet containing 500 mg sodium bisulfite (equivalent to 307 mg SO_2/kg diet) for 2 years showed no toxic symptoms. Sodium bisulfite at concentrations of 1,000 mg/kg (615 mg SO_2 equivalent/kg diet, equivalent to 31 mg SO2 equivalent mg/kg bw per day) or more in the diet inhibited the growth of the rats and induced deleterious effects over and above those produced by the removal of thiamine because, according to the authors, the effects were only partly prevented by injection of vitamin B_1.

Lockett and Natoff (1960) conducted a three-generation study for 3 years in rats treated with **sodium metabisulfite**, The animals received a dose of 37 mg SO_2 equivalent/kg bw per day in the drinking water (750 mg/L) and no effects were reported. The authors concluded that the higher toxicity reported in the Fitzhugh et al. (1946) study at a comparable dose could be due to products of the interaction of sulfites with constituents of the solid food.

In a study by Cluzan et al. (1965), a solution containing 1.2 g of **potassium metabisulfite** per litre of water (700 mg SO_2 equivalent/L) was administered to 160 Wistar rats aged 28–32 days (80 of each sex and 40 per dose group) over a period of 20 months. Control rats were given distilled water. The intake of fluid by the test group was the same as that of the controls (but no measurements of SO_2 loss from the metabisulfite solution appear to have been made). The intake of SO_2 was calculated by the authors of the study to range from 190 mg/kg bw per day (days 1–15) to 58 mg/kg bw per day (month 2), 35 mg/kg bw per day (month 9) and 29 mg/kg bw per day (month 20) for males and 190 mg/kg bw per day (days 1–15) to 78 mg/kg bw per day (month 2), 54 mg/kg bw per day (month 9) and 40 mg/kg bw per day (month 20) for females. The following observations provided no evidence of toxic effects, growth rate, food intake, clinical condition, haematological indices of blood and bone marrow (except peripheral leucocyte count, which was increased by 27% in males), relative organ weights (except spleen weight, which was higher in females by 41%), histopathological examination of a large number of tissues and mortality rate. Fatty change in the liver was mostly slight or absent, with a similar incidence and severity in test and control groups. According to the authors, the NOAEL of this study was the highest dose tested, 50 mg potassium metabisulfite/kg bw per day (expressed as a mean intake) corresponding to 29 mg SO_2 equivalent/kg bw per day in males.

Four groups of 20 Wistar rats (10 of each sex on a standard diet) were given daily doses (30 mL/kg bw) of red wine containing 100 or 450 mg SO_2/L, an aqueous solution of **potassium metabisulfite** (containing 450 mg SO_2/L), or pure water, by oral intubation 6 days each week for four successive generations (Lanteaume et al., 1965). The females were treated for 4 months, the males for 6 months and the second generation was treated for 1 year. The only effect reported was a slight reduction in hepatic cellular respiration. All other parameters examined, which comprised weight gain, weight and macroscopic or histological appearance of various organs, appearance and behaviour, proportion of parturient females, litter size and weight and biological value of a protein sample, showed no changes attributable to SO_2.

In the Til et al. (1972b) study, the toxicity of sulfites was studied by feeding rats (20 per sex and per group) on diets containing 0, 0.125, 0.25, 0.50, 1 or 2% **sodium metabisulfite** (calculated by the authors as corresponding to about 0, 37, 75, 150, 300 and 600 mg SO_2 equivalent/kg bw per day) for periods up to 2 years and over three generations. Undue losses of sulfite were reduced by storing the diets at -18°C prior to feeding. Thiamine deficiency due to destruction of this vitamin by sulfite was prevented by enrichment of the diets with thiamine. During storage up to the time of consumption, the losses of sulfite from the diets containing sodium metabisulfite at levels of 0.125, 0.25, 0.50, 1 and 2% averaged 22, 14, 12.8 and 4.5%, respectively, while the decrease in thiamine was 2.7, 1.7, 8.3, 14.5 and 15.4%, respectively. A level of 2% sulfite in the diet caused slight growth retardation in F_1- and F_2-generation rats both before and after weaning. Occult blood was present in the faeces in groups given 1% sulfite or more. The relative kidney weights were increased in animals receiving a diet with 2% sulfite in the F_2 generation females only, but this increase was accompanied by neither functional nor histological changes. Pathological examination revealed hyperplastic changes in both the fore- and glandular stomach at dietary levels of 1 and 2% sulfite in each of the three generations. Some slight alterations (mainly hyperplasia or inflammation) were also found in the F_2 generation rats receiving 150 mg SO_2 equivalent/kg bw per day. There was no indication that sodium metabisulfite had any carcinogenic effect. According to the authors, the dietary level of sodium metabisulfite showing no untoward effect in rats was 0.25 recalculated to 0.215% (equivalent to 72 mg SO_2/kg bw per day) when taking the loss of sulfite from the diet into account. The Panel agreed with this NOAEL.

In another study (Feron and Wensvoort, 1972), male and female Wistar rats were fed a diet containing **sodium metabisulfite** at levels of 0, 0.125, 0.25, 0.5, 1 and 2% for up to 24 months. Thiamine deficiency was prevented by addition of 50 mg thiamine/kg food. No information was provided on the number of animals per sex per dose. The microscopic examinations gave no evidence for the formation of tumours in the stomach but feeding with metabisulfite induced hyperplastic and inflammatory changes in the forestomach at dietary levels of 0.5% and higher. The metabisulfite lesions induced in the glandular stomach consisted of haemorrhagic microerosions, necrosis of epithelial cells, cellular infiltrations and an atypical glandular hyperplasia.

Beems et al. (1982) studied the hyperplastic glands observed by Feron and Wensvoort (1972) in the fundic mucosa of rats treated with **sodium metabisulfite** for 24 months in more detail by light and electron microscopy, as well as enzyme histochemistry. They concluded that sulfite may induce both hyperplastic fundic glands, exclusively lined by hyperactive chief cells and dilated glands lined by dedifferentiated chief cells. The actual mechanism by which sodium metabisulfite induced these alterations remains, however, unclear.

3.3.4.2. Other studies

In a study not designed as a regular carcinogenicity bioassay, 4 out of 149 female rats with low SOX activity induced by low-molybdenum diets in association with tungstate treatment displayed mammary adenocarcinomas after 9 weeks of treatment with **sodium metabisulfite** in their drinking water (Gunnison et al., 1981b). No tumours were seen when the animals were not exposed to exogenous sulfite and in control animals with normal levels of sulfite oxidase.

Takahashi et al. (1986) reported an initiation-promotion study in male Wistar rats given *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) in the drinking water and a diet supplemented with 1% NaCl for 8 weeks. Thereafter, the animals were maintained on drinking water containing 1% **potassium metabisulfite** for 32 weeks. Thirty animals were treated with the initiation procedure alone. Ten rats serving as negative controls without MNNG treatment were given drinking water with 1% potassium metabisulfite during the promotion stage. At the end of the 40th experimental week, all surviving animals were sacrificed for necropsy. Potassium metabisulfite significantly increased the incidence of adenocarcinoma in the pylorus of the glandular stomach (26.3% vs 3.9% in the controls) after initiation with MNNG and sodium chloride. In addition, 10.5% of the rats developed papillomas in the forestomach. No carcinomas developed in rats given potassium metabisulfite without MNNG or sodium chloride. Duodenal adenocarcinomas were induced by the initiation alone (10%) and the

incidence was not affected by metabisulfite. The authors of that study concluded that potassium metabisulfite exerts a tumour-promoting activity in the rat glandular stomach.

Furihata et al. (1989) studied the possible tumour-promoting activities of various salts including potassium metabisulfite in the glandular stomach mucosa of F344 male rats after single administration by gastric intubation. Up to 100-fold increases in ornithine decarboxylase activity in the pyloric mucosa of the stomach with maxima after 8 h were observed after a single administration of **potassium metabisulfite** at doses of 500–1,400 mg/kg bw. Doses of 400 and up to 1,200 mg/kg bw potassium metabisulfite induced up to 10-fold increase in DNA synthesis in the pyloric mucosa of the stomach with maxima after 16–24 h ($p < 0.05$ and $p < 0.01$, respectively). The authors suggested that this food additive may have tumour-promoting activities in the pyloric mucosa of F344 male rat stomach.

Overall, only very old long-term studies exist, and they are restricted to sodium and potassium metabisulfites. No carcinogenic potential was detected in these studies but a possible tumour promoting activity of sulfites in the glandular stomach mucosa was reported in rats which may be related to hyperplasia of the fundic glands induced by sodium metabisulfite.

No long-term studies have been identified with sodium sulfite, calcium sulfite, calcium bisulfite or potassium bisulfite.

3.3.4.3. Other studies related to carcinogenicity

In vitro

Qin and Meng (2009) investigated whether sulfur dioxide derivatives (bisulfite and sulfite) had effects on the expression of several proto-oncogenes and tumour suppressor genes in cultured human bronchial epithelial (BEP2D) cells. The mRNA and protein levels were measured following exposure to differing SO_2-derivative concentrations and exposure times. SO_2 derivatives caused mRNA and protein overexpression of c-fos, c-jun and c-myc at all tested doses (0.001–2 mM). According to the authors, the data supported the hypothesis that SO_2 derivatives could cause the activation of proto-oncogenes and inactivation of tumour suppressor genes and SO_2 derivatives may play a role in the pathogenesis of SO_2-associated lung cancer.

Human studies

In an epidemiological study conducted on a cohort of 57,613 workers from 12 countries, the mortality of workers exposed to sulfur dioxide in the pulp and paper industry was investigated (Lee et al., 2002). The authors of the study reported that lung cancer mortality was marginally increased among exposed workers (SMR = 1.08; 95% CI, 0.98–1.18). Mortality from non-Hodgkin lymphoma and from leukaemia was increased among workers with high sulfur dioxide exposure, and a dose–response relationship with cumulative sulfur dioxide exposure was suggested for non-Hodgkin lymphoma. The authors of the study concluded that occupational exposure to sulfur dioxide in the pulp and paper industry may be associated with an increased risk of lung cancer.

3.3.5. Reproductive and developmental toxicity

Sodium sulfite

In a study by Itami et al. (1989), pregnant Wistar rats were fed diets containing 0, 0.32, 0.63, 1.25, 2.5 and 5% **sodium sulfite** (heptahydrate) from gestation day (GD) 8 to 20. On GD 20, a Caesarean section was performed on 10–12 females/group. The other four or five dams of the 0, 0.32 and 5% groups were allowed to deliver and rear their litters until weaning. Effects on body weight gain and food consumption were reported at the concentration of 5%, but no clinical signs of toxicity were recorded. The corresponding daily intakes of sodium sulfite (heptahydrate) were reported to be approximately 0, 300, 1,100, 2,100 and 3,300 mg/kg bw per day. Some evidence of growth retardation was observed in all treatment groups, but there was no dose-relationship and these effects were not

observed in the live-birth part of the study, which was indicated by a lack of changes in male and female pups 3 weeks after birth. These effects were considered as being possibly related to maternal malnutrition and/or disturbance in metabolism by liberated sulfur dioxide. No other developmental toxicity was seen in the study. According to the authors, the NOAEL for maternal toxicity was 2.5% in the diet (approx. 2,100 mg sodium sulfite/kg bw per day or 560 mg SO_2 equivalent/kg bw per day and the NOAEL for fetal toxicity below 0.32% (approx. 300 mg sodium sulfite/kg bw per day or 81 mg SO_2 equivalent/kg bw per day). The Panel noted that no adverse effects were seen in the live-birth part of the study. Furthermore, the Panel noted that in the prenatal phase of this study only 10–12 pregnant females per dose group were used and in the postnatal phase only four to five dams and in this latter phase only two dose levels were tested.

Sodium bisulfite

An unpublished study (CIVO/TNO, 1965) was reported by JECFA (1987). Groups of 20 Wistar rats (10 of each sex) were fed diets containing 0.125, 0.25, 0.5, 1.0 or 2.0% **sodium bisulfite** (0.077–1.23% as SO_2) for 17 weeks. A group of 20 rats on untreated diet served as controls. Immediately after preparation, all diets were stored at -18°C in closed glazed earthenware containers for not longer than 2 weeks. Measurements of loss of SO_2 on keeping each diet in air for 24 h at room temperature revealed losses amounting to 12.5, 10.0, 14.3, 8.2 and 2.5% of the sulfite present in the respective diets as listed above, SO_2 loss was inversely proportional to sulfite content. After 124 days, there was no effect on the growth of male and female rats. The control and 2.0% female groups, which were used for fertility studies, gave birth to litters during the course of the test. The other female groups on lower levels of dietary sulfite were not mated and showed significant depression of growth (as compared with controls that had been mated). Haematological measurements at 7–8 weeks (all groups) and at 13 weeks (2% group and controls) revealed no effect of sulfite. Thiamine could not be measured in the diet containing 2% sulfite after being stored for 14 days at -18°C; at 0.25 and 1.0% sulfite, there was some loss of thiamine, but this cannot be assessed precisely since the initial values were not quoted. Measurements of urinary thiamine excretion revealed substantial reduction at 1 week and mainly at 13 weeks in all groups receiving more than 0.125% sulfite in the diet. Urine concentration tests were not carried out on a sufficient number of animals to permit firm conclusions to be drawn. Males and females of the control and 2% groups were mated with rats obtained from the main colony. The only adverse findings observed in females of the 2% group were lower weight of the offspring at 7 and 21 days of life and 44.3% mortality as compared with mortalities of 0, 2.8 and 3.8% in the other groups of young rats. It was claimed that no changes were found in relative organ weights (liver, heart, spleen, kidneys, adrenals and testes) or in microscopic appearance (above-mentioned organs and the stomach, intestine, uterus, teeth and eyes). This unpublished report has not been available to the Panel. The critical effect was lower weight and increased mortality in the offspring. It may be concluded from this description that the NOAEL in this study was 1% sodium bisulfite (or 0.615% as SO_2) in the diet, which is equal to approximately 1000 mg/kg bw per day (or 615 mg SO_2 equivalent/kg bw).

The Food and Drug Research Laboratories (FDRL, 1972a,b,c,d [Docs. provided to EFSA n. 25, 26, 27 and 28]) also carried out a prenatal developmental toxicity study on **sodium bisulfite** in mice, rats and hamsters. The animals were dosed from implantation until the end of organogenesis. In all studies, animals were administered by gavage different doses sodium bisulfite diluted in water (dose volume 1 mL/kg bw per day in all groups); the control groups were vehicle treated. Body weights were recorded at regular intervals during gestation and all animals were observed daily for appearance and behaviour. To test the methodology and the sensitivity of the laboratory animals, positive controls were included. All dams were subjected to caesarean section, and the numbers of implantation sites, resorption sites, live and dead fetuses and body weights of live pups were recorded. All fetuses were examined grossly for sex distribution and for external abnormalities (for the mice, rat and hamster studies, one-third of the fetuses were used for detailed visceral examination and two-third stained and examined for skeletal defects).

In the mice study, groups of 23–24 virgin adult female albino CD-1 outbred mice were mated with young adult males and dosed on GD 6–15 by gavage with doses of 0, 2, 7, 32 or 150 mg/kg bw per day. A caesarean section was performed on GD 17. No treatment-related effects were observed. The NOAEL of the mice study was the highest dose tested, 150 mg sodium bisulfite/kg bw per day, corresponding to a maximum of 92 mg SO_2 equivalent/kg bw per day. Groups of 23–24 virgin adult female Wistar rats were mated with young adult males and dosed on GD 6–15 by gavage with doses of 0, 1.0, 5.0, 24.0 or 110 mg sodium bisulfite/kg bw per day. A caesarean section was performed on GD 20. The treatment had no effect on implantation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number in the vehicle controls. Hence, the NOAEL in this study was the highest dose tested, 110 mg sodium bisulfite/kg bw per day, corresponding to 68 mg SO_2 equivalent/kg bw per day.

In the hamster study, groups of 22 virgin female golden hamsters from an outbred strain were mated with mature males and dosed on GD 6–10 by gavage with doses of 0, 1, 6, 26 or 120 mg sodium bisulfite/kg bw per day. A caesarean section was performed on GD 14. No treatment related effects were observed. The NOAEL of the hamster study was the highest dose tested, 120 mg sodium bisulfite/kg bw per day corresponding to 74 mg SO_2 equivalent/kg bw per day.

Sodium and potassium metabisulfite

In a study by Cluzan et al. (1965), a solution containing 1.2 g of **potassium metabisulfite** per litre of water (700 mg SO_2 equivalent/L) was given as drinking beverage to 40 Wistar rats/sex aged 28–32 days over a period of 20 months. Control rats (40 rats/sex) were given distilled water. After 9 months, 21–22 females per group were mated. The intake of fluid by the test group was the same as that of the controls (no measurements of SO_2 loss from the metabisulfite solution appear to have been made). As calculated by the authors, the intake of SO_2 ranged from 190 mg/kg bw per day (days 1–15) to 58 mg/kg bw per day (month 2), 35 mg/kg bw per day (month 9) and 29 mg/kg bw per day (month 20) for males and 190 mg/kg bw per day (days 1–15) to 78 mg/kg bw per day (month 2), 54 mg/kg bw per day (month 9) and 40 mg/kg bw per day (month 20) for females. Reproduction studies over two generations revealed no effects of treatment except for a slightly smaller number of young in each litter from test animals and a smaller proportion of males in each of these litters in the first generation. Growth of the offspring up to three months was almost identical in test and control groups. The usefulness of this study for the risk assessment of the reprotoxic potential of potassium metabisulfite is limited as only one dose group was tested and the reporting of the study was limited.

In the study by Lanteaume et al. (1965), four groups of 10 rats/sex were given daily doses (30 mL/kg bw) of red wine containing 100 or 450 mg SO_2/L, an aqueous solution of **potassium metabisulfite** (450 mg SO_2/L), or pure water by oral gavage on 6 days each week for four successive generations. The females were treated for 4 months, the males for 6 months and the second generation was treated for 1 year. The only effect seen at the highest dose (13.5 mg SO_2/kg bw) was a slight reduction in hepatic cellular respiration. All other parameters examined, which comprised weight gain, weight and macroscopic or histological appearance of various organs, appearance and behaviour, proportion of parturient females, litter size and weight, showed no changes attributable to potassium metabisulfite. The Panel noted that the doses used in this study were low and that the data were poorly reported.

In the Til et al. (1972b) study, six groups of 20 rats/sex were mated after 21 weeks on diets containing 0, 0.125, 0.25, 0.5, 1.0 or 2.0% **sodium metabisulfite** and supplemented with thiamine; 10 males and 10 females were mated again at 34 weeks. Ten male and 10 female F_{1a} rats were mated at 12 and 30 weeks of age to give F_{2a} and F_{2b} offspring. Ten males and 15 females of the F_{2a} generation were then mated at 14 and 22 weeks to give F_{3a} and F_{3b} offspring. F_{1a} parents and F_{2a} parents were kept on the diet for 104 and 30 weeks, respectively. Pregnancy incidence, birth weight and postnatal survival were all normal. In the F_0 first mating, the body weight gain of offspring was decreased at 2% sodium metabisulfite and in the F_1 mating it was decreased at 1 and 2% sodium metabisulfite. The F_2 first mating showed decreased weight gain of offspring in all test groups at weaning, but little effect was seen in offspring of the second F_2 mating. Litter size was significantly decreased at 0.5% sodium

metabisulfate and above in the first F_2 mating. The body weights of F_0 adults were unaffected, while high-dose F_1 females and high-dose F_2 males and females both showed slightly decreased body-weight gains. According to the authors, the NOAEL corresponded to the diet containing 1% potassium metabisulfite corresponding to 262 mg SO_2 equivalent/kg bw per day when considering the loss. The Panel agreed with this conclusion.

The Food and Drug Research Laboratories (FDRL, 1972e,f,g,h,1974 [Docs. provided to EFSA n. 29, 30, 31, 32 and 33]) carried out a prenatal developmental toxicity study on **sodium metabisulfite** in mice, rats, hamsters and rabbits. The animals were dosed from implantation until the end of organogenesis. In all studies, animals were administered by gavage different doses sodium metabisulfite diluted in water (dose volume 1 ml/kg bw per day in all groups); the control groups were vehicle treated. Body weights were recorded at regular intervals during gestation and all animals were observed daily for appearance and behaviour. To test the methodology and the sensitivity of the laboratory animals, positive controls were also tested. All dams were subjected to caesarean section, and the numbers of implantation sites, resorption sites, live and dead fetuses, and the body weights of live pups were recorded. All fetuses were examined grossly for sex distribution and for external abnormalities (for the mice, rat and hamster studies one-third of the fetuses were used for detailed visceral examination and two-third stained and examined for skeletal defects). In the rabbit studies, all live fetuses were placed in an incubator for 24 h for the evaluation of neonatal survival. Thereafter, all pups were examined for visceral and skeletal abnormalities.

In the mice study, groups of 22–29 virgin adult female albino CD-1 outbred mice were mated with young adult males and dosed on GD 6–15 by gavage with doses of 0, 2, 7, 34, or 160 mg/kg bw per day. A caesarean section was performed on GD 17. A decrease in the number of corpora lutea and a corresponding decrease in the number of implantations were observed in the high-dose group. As dosing started at implantation, this was not considered as a treatment-related effect. The NOAEL of the mice study was the highest dose tested, 160 mg sodium metabisulfite/kg bw per day, corresponding to a maximum of 108 mg SO_2 equivalent/kg bw per day. Groups of 24 virgin adult female Wistar rats were mated with young adult males and dosed on GD 6–15 by gavage with doses of 0, 1, 5, 24 or 110 mg sodium metabisulfite/kg bw per day. A caesarean section was performed on GD 20. The treatment had no effect on implantation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number in the vehicle controls. Hence, the NOAEL in this study was the highest dose tested, 110 mg sodium metabisulfite/kg bw per day, corresponding to 74 mg SO_2 equivalent/kg bw per day.

Groups of 24 virgin adult female Wistar rats were mated with young adult males and dosed on GD 6–15 by gavage with doses of 0, 1, 5, 24 or 110 mg sodium metabisulfite/kg bw per day. A caesarean section was performed on GD 20. The treatment had no effect on implantation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number in the vehicle controls. Hence, the NOAEL in this study was the highest dose tested, 110 mg sodium metabisulfite/kg bw per day, corresponding to 74 mg SO_2 equivalent/kg bw per day.

In the hamster study, groups of 22–25 virgin female golden hamsters from an outbred strain were mated with mature males and dosed on GD 6–10 by gavage with doses of 0, 1, 6, 26 or 120 mg/kg bw per day. A caesarean section was performed on GD 14. No treatment-related effects were observed. The NOAEL of the hamster study was the highest dose tested, 120 mg sodium metabisulfite/kg bw per day corresponding to 81 mg SO_2 equivalent/kg bw per day.

Groups of 15–20 virgin Dutch-belted rabbits were inseminated and dosed from GD 6–18 with doses of 0, 1.23, 5.71, 26.5 or 123 mg/kg bw per day. A caesarean section was performed on GD 29. No treatment-related effects were observed. The NOAEL of the rabbit study was the highest dose tested, 123 mg sodium metabisulfite/kg bw per day corresponding to 83 mg SO_2 equivalent/kg bw per day.

The Food and Drug Research Laboratories (FDRL, 1975 [Doc. provided to EFSA n. 34]; FDA, 1991 [Doc. provided to EFSA n. 23]) carried out a prenatal developmental toxicity study on **potassium metabisulfite** in mice and rats. The animals were dosed from implantation until the end of organogenesis. In all studies, animals were administered by gavage different doses potassium metabisulfite diluted in water (dose volume 1 ml/kg bw per day in all groups); the control groups were vehicle treated. Body weights were recorded at regular intervals during gestation and all animals were observed daily for appearance and behaviour. To test the methodology and the sensitivity of the laboratory animals, positive controls were tested. All dams were subjected to caesarean section and the numbers of implantation sites, resorption sites, live and dead fetuses, and body weights of live pups were recorded. All fetuses were examined grossly for sex distribution and for external abnormalities (for the mice, rat and hamster studies one-third of the fetuses were used for detailed visceral examination and two-third stained and examined for skeletal defects).

In the mice study, groups of 27–32 virgin adult female albino CD-1 outbred mice were mated with young adult males and dosed from GD 6–15 by gavage with doses of 0, 1.25, 5.47, 26.9 or 125 mg potassium metabisulfite/kg bw per day. A caesarean section was performed on GD 17. No treatment related effects were observed. The NOAEL for this mice study was the highest dose tested, corresponding to 72 mg SO_2 equivalent/kg bw per day (FDRL, 1975 [Doc. provided to EFSA n. 34]).

Groups of 22–25 pregnant albino rats were fed doses of 0, 1.55, 7.19, 33.4 or 155.0 mg **potassium metabisulfite**/kg bw per day by gavage from GD 6–15 (FDA, 1991 [Doc. provided to EFSA n. 23]). A caesarean section was performed on GD 20. The treatment had no effect on implantation or on maternal or fatal survival. Body weight was slightly reduced at the highest dose level. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number observed in the vehicle controls. Hence, the NOAEL in this study was the highest dose tested, corresponding to 89 mg SO_2 equivalent/kg bw per day.

Dulak et al. (1984) investigated the reproductive toxicology of **sodium metabisulfite** in female rats (14–29/group) with induced deficiencies of sulfite oxidase. Induction of SOX deficiency was accomplished by administration of a diet with high tungsten to molybdenum ratio. The rat was chosen because it enables exposure of tissues to high systemic sulfite concentrations without debilitating side effects. The reproductive performance of female sulfite oxidase-deficient rats, exposed continuously to sulfite from about 3 weeks before mating until the experiment was terminated on GD 20, was compared to that of unexposed rats with normal SOX activity. There was no treatment-related trend in any of the parameters observed, including mating and pregnancy rates, gestational weight gain, pre-implantation loss, resorbed and dead fetuses, litter size, fetal weight and malformations. Of the spectrum of malformations observed in control and treated animals, only anophthalmia may have been treatment related. The Panel considered the two fetuses with anophthalmia seen in the treated group not as a treatment-related effect as also one fetus with the same malformation was observed in the control group. The authors concluded that there was no evidence to indicate that exposure of female rats to sulfite poses a significant reproductive hazard. The Panel agreed with this conclusion.

Ema et al. (1985) carried out a prenatal developmental toxicity study in Wistar rats fed **potassium metabisulfite** in the ad libitum diet on GD 7–14 at concentrations of 0, 0.1, 1 or 10% (according to the authors equal to 0, 130, 1,320 and 2,860 mg potassium metabisulfite/kg bw per day). On GD 20, a caesarean section was performed on 12–13 pregnant dams /group; six to seven dams of the control, 0.1 and 10% group were allowed to deliver and rear their litters until weaning. External, skeletal and internal examinations of the fetuses revealed no abnormalities of teratogenesis in any group. No adverse effects on the pre- and postnatal development of the offspring were found in the 1 and 0.1% groups. The fetal body weight was significantly lower than that of the control group and the postnatal survival rate of the offspring was slightly decreased in the 10% group. In this group, maternal body weight gain and food intake during pregnancy were decreased. Maternal malnutrition during pregnancy might be one of the reasons for these adverse effects in the 10% group. The Panel considered the mid-dose (1320 mg potassium metabisulfite/kg bw per day) as a NOAEL corresponding to 759 mg SO_2/kg bw per day. Furthermore, the Panel noted that in the prenatal phase

of this study only 12–13 pregnant females per dose group were used, only six to seven dams in the postnatal phase, and in this latter phase, only one dose level (the 10% group) was tested.

Overall, potassium metabisulfite has been the subject of a two- and four-generation toxicity study in rats with no developmental effects and reproductive effects, however, these studies were poorly reported and did not meet the current requirements for that kind of assays. In addition, the doses tested were very low in one study. It has also been tested in prenatal developmental toxicity studies by gavage in mice and rats with no developmental effects up to 72 mg SO_2 equivalent/kg bw per day and 89 mg SO_2 equivalent/kg bw per day (the highest dose tested) for 10 days in mice and rats, respectively. Sodium metabisulfite and sodium bisulfite were tested in dietary two-generation reproductive toxicity studies in rats and NOAELs corresponding to 615 and 576 mg SO_2 equivalent/kg bw per day were observed. In addition, sodium metabisulfite and sodium bisulfite were tested in mice, rats, hamsters and rabbits (rabbits only tested for sodium metabisulfite) by gavage and no developmental effects were observed when dams were dosed during organogenesis with doses up to 68 mg SO_2 equivalents/kg bw per day (the highest dose tested) for 10 days.

Studies on reproductive and developmental toxicity were lacking for calcium sulfite, calcium bisulfite and potassium bisulfite.

3.3.6. Immunotoxicity, hypersensitivity/allergy and intolerance

In vitro *studies*

Winkler et al. (2006) subjected mitogen-stimulated human peripheral blood monocytes to 0, 1, 10 or 50 mM **sodium sulfite**. This resulted in statistically significant dose-dependent decreased tryptophan degradation and decreased neopterin production, which suggested a suppressive effect of sodium sulfite on the activated Th-1 immune response. According to the authors, this is in line with the hypothesis that sulfite exposure may favour a Th2-type allergic response after contact with an allergen. The Panel noted that the doses used in this study exceeded the physiological concentration.

Animal studies

Three groups of female Wistar rats were treated with 0.25, 1 or 4% of **sodium metabisulfite** in their drinking water for 90 days (El Kadi et al., 2014). An immunisation protocol was conducted during the experiment. Mortality, weight, and food and water consumption were recorded. At the end of the experiment, serum immunoglobulin levels were evaluated; biochemical and haematological parameters were also investigated. Administration of sodium metabisulfite at 1% and 4% had significant effects on body weight, food and water consumed. There was an increase in biochemical parameters (calcium, urea, creatinine, uric acid, transaminases) and decrease in immunoglobulin levels. Haematology revealed a decrease in red blood cells and haemoglobin, as well as leucocytosis. A physiological study showed enlarged spleen, kidney, liver and stomach. The authors concluded that subchronic intake of sodium metabisulfite 1% and 4% seemed to alter immune function, biochemical, haematological and physiological parameters in Wistar rats. The Panel noted that the description of the study is limited and that the anti-vitamin B_1 effect of sulfites was not examined in this study.

Human studies

Simon and Wasserman (1986) reported that oral administration (prior to ingestion of sulfite) of 1–5 mg of vitamin B_{12} (cyanocobalamin) completely or partially blocked bronchoconstriction from sulfite sensitivity in a study of six patients. The authors proposed that this might be due to the role of vitamin B_{12} as a co-factor in sulfite oxidation, where sulfites are converted to sulfates. The Panel noted that this dose of vitamin was 1000 times higher than the Dietary Reference Values proposed by EFSA (EFSA NDA Panel, 2015).

A study by Acosta et al. (1989) described a case of a 38-year-old nurse with asthma and probable sensitivity to sulfites. A double-blind challenge with sulfite and placebo was performed. Pulmonary

function tests were measured along with plasma levels of sulfites. A deficiency in sulfite-oxidase was suggested as the trigger mechanism of bronchospasm.

Bronchoconstriction induced by sulfites in asthmatics have been confirmed in asthma-sufferers of all ages, even 2 year olds (Frick and Lemanske, 1991). Six out of fifty-six 6- to 14-year-old asthma-sufferers reacted with bronchoconstriction to challenge with varying doses of potassium metabisulfite either in capsules or in solution. Two of these children reacted only to doses of 50 or 100 mg given in solution, not when given in capsules (Boner et al., 1990). In another study of 20 7- to 14-year-old asthmatics, the prevalence was 20% (Sanz et al., 1992).

A case of recurrent severe urticaria and vasculitis in a 47-year-old man was diagnosed after placebo-controlled oral challenge test with a capsule containing 50 mg sodium metabisulfite (Wuthrich et al., 1993). Recurrent episodes of urticaria and angiooedema in a 22-year-old woman were found to be due to exposure to sulfites in foods (Belchi-Hernandez et al., 1993). Each episode was accompanied by urticaria, vomiting, throat tightness and oedema of lips and pharynx (Bellido et al., 1996). Sulfite-containing pickled onions were also reported to induce asthma attacks in 18 young male asthmatics (Gastaminza et al., 1995).

The practice of adding sulfites to hops for producing beer may result in low concentrations of sulfites in beer. As little as 100 mL beer with 3–4 mg SO_2/L beer gave symptoms, such as facial erythema and oedema, in a 25-year-old-man. The symptoms could be reproduced with 10 mg of sodium metabisulfite (Gall et al., 1996).

The ATSDR (1998) found that the available data indicated that sensitive asthmatics may respond to concentrations of sulfur dioxide as low as 0.1 ppm in the air.

In a study by Vally and Thompson (2001) four of 24 self-reported wine sensitive asthmatic patients were found to respond to sulfite in wine when challenged in a single dose fashion. In a double blind dose–response study, all four had a significant fall in forced expiratory volume in one second (FEV (1)) (>15% from baseline) following exposure to 150 ml of wine containing 300 ppm sulfite, but did not respond to wines containing 20, 75 or 150 ppm sulfite. Responses were maximal at 5 min after ingestion (mean (SD) maximal decline in FEV(1) 28.7 (13)%) and took 15–60 min to return to baseline levels. In a cumulative dose–response study, no significant difference was observed in any of the lung function parameters measured (FEV (1), peak expiratory flow PEF, mid-phase forced expiratory flow (FEF (25–75))) between wine sensitive and normal asthmatic subjects. The authors suggested that the role of sulfites and/or wine in triggering asthmatic responses has been overestimated, and that cofactors or other components in wine may play an important role in wine induced asthma.

In a selection of 20 patients with chronic urticaria, one patient was found to react to 10 mg sodium metabisulfite by oral double-blind placebo-controlled challenge. The authors found that the release of histamine, leukotrienes and other mediators induced by challenge with sulfite was not IgE-mediated (Di Lorenzo et al., 2002).

As reported by Nair et al. (2003), FDA analysed 767 reports of adverse reactions ascribed to ingestion of sulfite-treated foods. Most of the reactions occurred in steroid-dependent asthmatics, and many involved respiratory distress or failure, or anaphylaxis. FDA analysed 22 deaths allegedly associated with sulfite ingestion and determined that nine fatalities (all severe asthmatics) were probably and five fatalities (also asthmatics) were possibly due to sulfite ingestion.

A case of severe pruritus on the trunk, upper limbs and head lasting for 6 months in a 56-year-old man was diagnosed as sulfite intolerance by open and double-blind placebo-controlled challenge with 10 mg of sodium metabisulfite after 1 month on an elimination diet (Asero, 2005).

In a study on eight self-reported wine-sensitive asthmatic patients completing double-blind challenges with high- and low-sulfite wines on separate days, Vally et al. (2007) found that one of the eight subjects demonstrated a clinically significant reduction in FEV (1) following challenge with either high- or low-sulfite wine. In contrast, one patient demonstrated a clinically significant increase in bronchial hyperresponsiveness (BHR) following challenge with both high- and low-sulfite wines, and a smaller increase in BHR following placebo challenge. A second patient showed a significant increase, while another showed a significant decrease in BHR following challenge with low-sulfite wine. A fourth patient showed marginal increases in BHR following challenge with both high- and low-sulfite wines. Hence, this study did not support a major role for the sulfite additives in wine-induced asthmatic responses in the patients examined during this study.

In a random sample of 6,000, 18–69 year olds in greater Copenhagen, the self-reported incidence of alcohol-induced symptoms from the upper airways, or lower airways and skin was 7.6% and 7.2%, respectively. All types of alcoholic beverages were reported as triggers, most commonly red wine. Alcohol-induced symptoms from the upper and lower airways were significantly more prevalent, among allergic rhinitis and asthma sufferers (odds ratio between 3.0 and 8.1, $P<0.001$) (Linneberg et al., 2008). According to the authors, sulfite additives have been implicated in wine-induced asthmatic reactions, but only a small number of wine-sensitive asthmatics respond to sulfite challenge under laboratory conditions suggesting that cofactors or other components may be needed for the reaction to occur.

Vally et al. (2009) found that most studies report a 3–10% prevalence of sulfite sensitivity among asthmatic subjects following ingestion. The severity of these reactions varies, and steroid-dependent asthmatics, those with marked airway hyperresponsiveness, and children with chronic asthma, appear to be at greater risk. In addition to episodic and acute symptoms, sulfites may also contribute to chronic skin and respiratory symptoms.

Vally and Misso (2012) in a review reported that exposure to sulfites arises mainly from the consumption of foods and drinks that contain these food additives; however, exposure may also occur through the use of pharmaceutical products, as well as in occupational settings.

Ranguelova et al. (2012) studied the effect of bisulfite on human neutrophils and reported that these cells were able to produce reactive free radicals due to bisulfite oxidation. According to the authors, the potential toxicity of bisulfite during pulmonary inflammation or lung-associated diseases, such as asthma may be related to free radical formation.

The Panel noted that sulfites were frequently identified as responsible for 'allergic/sensitivity' reactions when used in pharmaceutical and cosmetic products. García-Gavín et al. (2012) reported that 124 (4.5%) of 2,763 patients patch tested positively to sodium metabisulfite. A total of 13 cases (10.5%) were occupational and 10 of them presenting with hand eczema. Sodium metabisulfite was the single allergen found in 76 cases (61.3%). The reactions were considered to be relevant in 80 cases (64.5%), of which 11 were occupational.

Overall, the Panel noted that:

- IgE tests were usually negative, which means that the reactions were not immune-mediated;
- sensitivity reactions, which were reported when sulfites were used as food additives were mostly intolerance reactions;
- reactions to sulfites are more frequent in people with an atopic background (mainly in asthmatics), they can result in symptoms in various tissues and organs (lung, skin) possibly due to a specific genetic background;
- exposure to sulfites can increase the number, symptoms and severity of hyperreactivity in

animal models of allergy or in asthmatic individuals;

- the possible role of partial SOX deficiency in the sensitivity to sulfites is not substantiated.

3.3.7. Other studies

The description of the studies referred in this section is presented in Appendix M.

3.3.7.1. Neurotoxicity

Overall, 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.

3.3.7.2. Anti vitamin B_1 effect of sulfites

Several studies reported that SO_2 can destroy some of the thiamine content of wine, however, in studies were human volunteers received SO_2 approximately 10 times the current group ADI for 50 days, no effect on the thiamine status (blood thiamine levels, urinary thiamine excretion and by determination of thiamine-dependent enzyme activity) was reported. Clinical, neurophysiological and biochemical investigations produced no indication of adverse effects from sulfite.

The Panel noted that from the animal data available, there is a great variability between animal species in the sensitivity to the anti-vitamin B_1 effect of sulfites; cats and dogs being highly sensitive. The only study in humans available to the Panel was conducted with doses of sulfites equivalent to 3.5 mg/kg bw per day (5 times the current group ADI) administered for 25 days to the subjects and no effects were reported.

3.3.7.3. Nephrotoxicity

The Panel noted that *in vitro* sulfites are cytotoxic to kidney cell lines at high dose of exposure. In animal studies, rat exposed to 10 times the current group ADI, effects on the kidney function were indicated by the reported alteration of some markers (increased urinary excretion of protein and alkaline phosphatase activity).

In human, increased serum sulfites levels were reported in patients with chronic renal failure (CRF) who have therefore a reduced glomerular filtration of sulfites. The authors hypothesised that this increase level may contribute to the tissue and organ dysfunctions in patients with CRF.

3.3.7.4. Hepatotoxicity

The only available data to the data are from *in vitro* studies, which reported that cultured hepatocytes are susceptible to oxidative damage resulting from the reactive oxygen species (ROS) formation induced by sulfites. However, no increase in the expression of p53 and p-p53 (Ser15) was reported in a hepatic cell line. Therefore these data are difficult to consider in the risk assessment of sulfites.

3.3.7.5. Potential roles of hydrogen sulfide

The Panel noted that hydrogen sulfide (H_2S) and sulfites have close interactions and can be produced from each other. Overall, the Panel noted that the reported effects of hydrogen sulfide suggested that this compound might have various physiological roles, which deserve consideration in the evaluation of sulfites, however, further research on the relationship between H_2S and the use of sulfites as food additives are needed before a conclusion can be drawn on their beneficial or detrimental roles in modulating H_2S activities, if any.

3.3.7.6. Sulfites, obesity and metabolic syndrome

The Panel noted that the effects reported *in vivo* in mice were not consistent with the effects reported *in vitro*, and did not support, at least in mice exposed to sulfites in drinking water, the hypothesis of an inflammatory effect of these substances on the GIT.

3.3.7.7. Sulfites and calcium metabolism

In animals and man, it has been reported that sulfites may affect calcium metabolism by limiting its absorption and increasing its urinary excretion. This was reported even at doses that are compatible with the use of sulfites as food additives (Hugot et al. 1965).

3.3.7.8. Sulfites and the glutathione system

Sulfur dioxide and sulfites may react with disulfide bond containing proteins, such as albumin and fibronectin (Menzel et al. 1986); this reaction also concerns GSSG. These results suggest that SO_2 may affect the detoxification of xenobiotic compounds by inhibiting the enzymatic conjugation of GSH and reactive electrophiles. Decreased glutathione levels in the lungs of rats exposed to sulfur dioxide suggest that glutathione may be involved in its detoxification process (Langley-Evans et al., 1996). *In vitro* experiments have demonstrated that sulfites, react with GSH to form S-sulfoglutathione in a reaction which is catalysed by thiol transferase (Kagedal et al., 1986). Conversion of S-sulfoglutathione by γ-glutamyltranspeptidase yields S-sulfocysteinylglycine, which is hydrolysed to S-sulfocysteine by renal peptides. S-sulfoglutathione has been detected in lenses and intestinal mucosa of animals and S-sulfocysteine has been observed in body fluids.

3.3.8. Biological and toxicological data on reaction products of sulfites.

As described in Section 2.5, sulfur dioxide and sulfites may react with aldehydes, ketones and sulfhydryl moieties, and therefore can form reversible and irreversible adducts with a wide range of food components, including (reducing) sugars, anthocyanins, thiamine, nucleic acids, cysteine residues in proteins, etc.

Non-enzymatic reactions of sulfite with tissue components include lysis of disulfide bonds, with the formation of *S*-sulfonates and thiols (Cecil, 1963). Under conditions of sulfite loading, appreciable amounts of S-sulfonates may be formed, and cysteine-*S*-sulfonate has been found in urine (Gunnison and Palmes, 1974), while glutathione-*S*-sulfonate has been detected in bovine ocular lenses (Waley, 1969). Only interchain disulfide bridges of native proteins undergo sulfitolysis (Cecil and Wake, 1962) and the protein S-sulfonates formed slowly released sulfite ions in the presence of sulfydryl compounds (Swan, 1959). Sulfites are strongly bound in the form of S-sulfonates by plasma proteins and are gradually cleared from the blood by mechanisms, which are not totally clear (Gunnison, 1981; Gunnison and Palmes, 1974).

3.3.8.1. ADME of reaction products of sulfites.

Only limited information exists on the ADME of the reaction products resulting from the reaction of sulfites with food components.

In one study, the excretion of single intragastric doses of ^{14}C-labelled 3-deoxy-4-sulfohexosulose (DSH) was studied in male CF1 mice, and male and female Wistar albino rats (Walker et al., 1983). In mice 29% and in rats between 16.5 and 31% of the administered radioactivity was excreted in the urine. All urinary elimination was as unchanged DSH. In mice, 50% of the dose was eliminated in the faeces and between 58.5 and 73% in the faeces of rats. Similar results were obtained with ^{35}S-labelled DSH. Organ analyses from 0.25 to 24 h after intragastric administration of [^{14}C] DSH/kg bw to male rats and mice showed that at all times most of the ^{14}C activity was associated with the gastrointestinal tract in both species. Maximum tissue levels were 2.16% of the dose in the rat liver 0.5 h after dosing and 1.57% in the mouse kidney after 0.25 h.

Sodium sulfite solutions were administered to Sprague–Dawley rats in which the portal vein and vena cava were cannulated for blood sampling. The examination of plasma showed the presence of S-sulfonates in both pre- and post-hepatic blood, whereas free sulfite was detected only in portal blood. The author concluded that the sulfite was absorbed and rapidly metabolised by oxidation or the formation of S-sulfonates (Wever, 1985).

The hydroxysulfonates from the reaction between sulfites and carbonyls (e.g., acetaldehyde in wine) are chemically stable over the pH range 1–8 and their dissociation in the stomach and small intestine would not be expected to be rapid (Taylor et al., 1986). Instead, the sugar hydroxysulfonates would be stable in the stomach but would be predicted to dissociate in the small intestine with the release of a mixture of sulfite and bisulfite ions. Generally, there is little information about the stability and the toxicokinetic properties of the sulfite adducts with proteins, amino acids, vitamins, nucleic acids and nucleotides (Taylor et al., 1986).

3.3.8.2. Toxicological data for reaction products of sulfites.

A review of the reported toxic effects of some of these reaction products has been conducted by Taylor et al. (1986). No further significant data have been identified in the literature since then.

The acute toxicity of 3-deoxy-4-sulfohexulose has been tested in Wistar rats and CF1 mice administered by gavage a single dose of 5 g/kg of the pure synthetic compound in solution in water. After 14 days, the only adverse effect reported was transient diarrhoea in both species. The compound was not mutagenic in an Ames test (four strains, with and without metabolic activation) (Walker and Mendoza-Garcia, 1983).

A subchronic oral toxicity study of acetaldehyde hydroxysulfonate was conducted in groups of 8 normal or SOX-deficient female Sprague–Dawley rats. The authors calculated that the rats were given doses of 0, 7, 70 mg SO_2 equivalent/kg bw per day; or a higher dose with the drinking water which was available ad libitum for 8 weeks. In all groups, drinking water was fortified with 50 mg/L thiamine. Liver lesions were noted in rats treated with acetaldehyde hydroxysulfonate. These lesions may possibly be attributable to the effects of free acetaldehyde. The NOAEL for acetaldehyde hydroxysulfonate was 7 mg SO_2 equivalent/kg bw per day for sulfite oxidase-deficient rats and 70 mg SO_2 equivalent/kg bw per day for normal rats. An increase in urinary sulfite levels in sulfite oxidase-deficient rats was observed after dosing with acetaldehyde hydroxysulfonate. These findings suggest that acetaldehyde hydroxysulfonate is metabolised to acetaldehyde and free sulfite (Hui et al., 1989).

In the study by Ribera et al. (2001) described in Section 3.2.2., according to the authors, the sulfite-treated biscuit product used to feed mice was likely to contain a mixture of protein-*S*-sulfonates, sugar hydroxysulfonates, carbonyl hydroxysulfonates and possible other minor combined forms of sulfites. At the doses tested (up to 25 mg SO2/kg bw per day), there was no evidence of adverse effects of such compounds.

The overall conclusion of Taylor et al. (1986) was that '*further work is especially needed on the assessment of the toxicity of the combined sulfites. Since the bulk of sulfite ingestion is in the form of combined sulfites, the general lack of such information makes hazard evaluation virtually impossible*'. No such new data were available to the Panel.

4. Discussion

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that has become available since then and the data available following public calls for data. The Panel noted that not all of the original studies on which previous evaluations were based were available for re-evaluation by the Panel.

In its latest toxicological assessment, JECFA (1987) allocated a group ADI of 0–0.7 mg SO_2 equivalent/kg bw per day for sulfur dioxide and sulfites based on a NOAEL from a 2-year rat study

(Til et al., 1972b) of 0.25% sodium metabisulfite in the diet, equivalent to 70 mg SO_2 equivalent/kg bw per day. The critical effects in this study were the presence of occult blood in the faeces and hyperplasia or inflammation of the stomach. JECFA also noted the occurrence of several cases of 'idiosyncratic reactions' in humans towards sulfite-containing foods. Based on this, the Committee recommended that the frequency of idiosyncratic adverse reactions and the relative toxic effects of free and bound sulfur dioxide should be kept under review, and that information on the chemical forms of sulfur dioxide in food were also needed.

In 1996, the SCF similarly allocated a group ADI of 0.7 mg SO_2 equivalent/kg bw per day based on both the pigs and rats studies by Til et al. (1972a,b).

The group ADI allocated by JECFA and the SCF has in both cases been determined mainly based on irritating local effects and set '*under the assumption that results from all sulphiting substances can be compared when taking into consideration the amount of SO_2 being the theoretical result of dosing*'. The Panel noted that there are no data available to substantiate that the theoretical yield of SO_2 is independent on the matrix (food or drinks) to which the sulfiting agent is applied. The Panel noted that the sulfur dioxide yield may vary between different sulfites and the actual specified content may not reach the theoretical yields. These differences must be considered when evaluating and comparing toxicological studies carried out on different sulfites.

The EFSA NDA Panel (2014) noted that the '*sulphites can react with food constituents, including sugars, proteins and lipids, to form adducts or derivatives. Some of these reactions are reversible, while others are not. The former lead to compounds that may serve as reservoirs for free sulphite, while the latter remove sulphites permanently from the pool of available free SO_2. Since free SO_2 is the most likely cause of adverse reactions to sulphiting agents, these chemical reactions have significant implications regarding foods that may cause difficulty in sensitive patients (Bush et al., 1986b; Simon, 1998). The likelihood of a particular food provoking a reaction depends upon the ratio of free to bound sulphite. For example, lettuce has few components to which sulphites can react, therefore most of the sulphite in lettuce remains in the free inorganic state and this explains why lettuce (salad bars) seems to provoke sulphite sensitive reactions frequently (Martin et al., 1986; Simon, 1998). In contrast, sulphites added to shrimp and potatoes tend to be bound and are not as likely to produce reactions in sulphite sensitive subjects*'. The Panel agreed with this statement.

Sulfites are used as antioxidants for the improvement of food quality and appearance as well as extending shelf life. Sulfites are effective against most bacteria and are widely used antimicrobial agents in the production of meats, in fruit products, fruit juices, potatoes, biscuits and in alcoholic beverages. Thus, sulfites are used in a variety of foods, which have different physical (pH) and ingredients composition, which may influence their fate: stability, solubility, availability and loss during processing.

The Panel noted that endogenous sulfites can be generated as a consequence of the body's normal processing of sulfur-containing amino acids and that sulfites may occur as a consequence of fermentation and are naturally present in a number of foods and beverages.

Knowledge on the toxicokinetics of sulfites is primarily based on old data. Sulfites used in foods may be partially liberated as sulfur dioxide both during and after ingestion and the sulfur dioxide can be inhaled and absorbed through the lungs as sulfite. Sulfite is converted to sulfate, primarily in the liver, by the enzyme SOX. This enzyme is also present in numerous mammalian cells (Beck-Speier et al. 1985), and tissues, including the lungs, kidneys, heart, brain, spleen (Cabré et al., 1990) although levels of activity vary between differing cells and tissues. The Panel noted that the activity of this enzyme is lower (10–20 times) in the human liver compared to the rat and that this was the rationale for using rats with a deficient SOX activity in some toxicity studies. Other studies (Constantin et al., 1994, 1996) showed that an alternative pathway of metabolism exists, so that intermediate formation of sulfur trioxide radicals may occur. The Panel noted the absence of specific ADME studies measuring reaction products from the different sulfites. Furthermore, the Panel noted that it was not

possible to ascertain the relative contribution of the differing pathways of sulfite metabolism at realistic levels.

Short-term toxicity studies in SOX-competent or -deficient rats indicate a NOAEL of 70 mg SO_2 equivalents/kg bw per day. The critical effect was gastric lesions. In subchronic studies in pigs, a NOAEL of 72 mg SO_2 equivalents/kg bw per day was identified, and higher levels caused mucosal lesions in the stomach and the first part of the large intestine.

Based on the 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.

Only old long-term studies, restricted to sodium and potassium bisulfite, were available. No carcinogenic potential was detected in these studies and a NOAEL of 70 mg SO_2 equivalent/kg bw per day was identified. The Panel noted that a possible tumour promoting activity of sulfites in the pylorus of the glandular stomach was reported in two initiation–promotion studies in rats, which may be related to hyperplasia of the fundic glands induced by sodium metabisulfite.

Potassium sulfite has been the subject of both two- and four-generation toxicity studies in rats with no developmental effects at doses up to 13–29 mg SO_2 equivalent/kg bw per day. The Panel noted that these studies were poorly reported that they did not meet the current requirements for end points tested and that the doses used were low; they were therefore of limited use for this evaluation. Potassium sulfite has also been tested for induction of malformations in offspring in rats and mice with no apparent effects. In addition, sodium metabisulfite and sodium bisulfite have been tested in rats, mice and hamsters with no apparent effects when dams were dosed during organogenesis with doses up to 262 mg SO_2 equivalent/kg bw per day for 10 days. The Panel noted that studies on reproductive and developmental toxicity were lacking for calcium sulfite, calcium bisulfite and potassium bisulfite.

Sulfite sensitivity occurs mostly in asthmatics and may occur in a small number of non-asthmatic individuals. Numerous studies confirm that sensitivity to sulfites is prevalent and, after oral intake, may present as asthmatic attacks in people suffering from asthma, but also as urticaria and angiooedema in other individuals. It is estimated that 3–10% of all asthmatics are sensitive to sulfites. Both symptoms of bronchoconstriction and skin symptoms like urticaria are prevalent among people of virtually all ages: children, young and middle-aged. The Panel agreed with the EFSA NDA Panel statement (2014) that '*Minimal Eliciting Doses have not been systematically assessed and the smallest concentration of sulphites able to trigger a reaction in a sensitive person is unknown*'.

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. Ingested sulfites may cause irritation of the respiratory tract, pH appearing to be an important determinant for the asthmatic response. Sulfites may stimulate the parasympathetic system and provoke cholinergic-dependent bronchoconstriction, this being particularly prominent in individuals with low SOX activity. The EFSA NDA Panel (2014) concluded that '*Histamine and other bioactive mediators can be released through non Ig-E mediated mechanisms. Increased synthesis of prostaglandins can also induce bronchoconstriction. In addition, in experimental models, sulphites may contribute to the persistence of chronic asthma symptoms and enhance allergic sensitization and airway inflammation*' (EFSA, NDA Panel, 2014). The Panel agreed with this conclusion.

The human sensitivity reactions towards sulfited wines, which cannot always be reproduced by exposure to sulfites alone (Linneberg et al., 2008), indicated that they may also be dependent on some co-exposure to either reaction products or other constituents, such as alcohol, biogenic amines or contaminants arising from the wine processing, such as residual refining agents (egg white protein, casein). However, Vassilopoulou et al. (2011) reported that the amount of these allergens in the wines they studied was extremely low and concluded that current evidence indicates a very low risk for the

allergic consumer from the wine-fining agents used in the wines they studied. The Panel noted that some sensitivity reactions following ingestion of wine have also been reported as associated with the presence of other allergenic components such as hymenoptera venom from insects collected with the grapes (Armentia et al., 2007; Armentia, 2008).

The Panel noted that JECFA (1987) had the following requests for further studies that remain outstanding at this time:

- Studies to elucidate the frequency and magnitude of asymptomatic SOX deficiency and its role in sulfite intolerance.

- Studies on the ability of the various forms of bound sulfur dioxide in foods to elicit adverse reactions in sulfite-sensitive asthmatics.

Overall, when evaluating the available studies, the Panel noted that several elements of uncertainty were apparent. Doses were often given as percentage in ad libitum diet or drinking water and estimation of doses expressed as SO_2 equivalent/kg bw per day has been carried out by the Panel. The consequence of supplementing the diet of studied animals with thiamine may induce the formation of complexes thereby decreasing the dose of available sulfite and masking some of the effects of sulfites. The analytical data available do not adequately differentiate between free and bound sulfite. Finally, the database is generally old and does not contain studies performed according to the current guidelines.

To assess the dietary exposure to sulfur dioxide–sulfites (E 220–228) from their use as food additive, the exposure was calculated based on (1) the MPLs set out in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*) and (2) usage or analytical data (defined as the *refined exposure assessment scenario*).

In view of all the analytical data received, the Panel decided to assess the refined exposure to sulfur dioxide–sulfites (E 220–228) according to two sets of concentration data: a) reported use levels and analytical results (not exceeding the MPLs) for food categories for which direct addition of sulfur dioxide–sulfites (E 220–228) is authorised, according to Annex II to Regulation (EC) No 1333/2008 and Annex IB to Regulation (EC) No 606/2009; and b) in addition to the previous dataset, the available analytical data for foods categories which may contain sulfur dioxide–sulfites (E 220–228) 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.

Using the *regulatory maximum level exposure assessment scenario*, the mean and 95th percentile exposure to sulfur dioxide–sulfites (E 220–228) were above the current group ADI for all population groups (Table 12). This was also the case for the refined high level exposure estimate of sulfur dioxide–sulfites (E 220–228) when considering only food categories for which direct addition of sulfur (E 220–228) is authorised in the *brand-loyal scenario* (Table 12) and for the mean exposure in infants, toddlers, children and elderly. For the *non-brand-loyal scenario*, the exposure to sulfur dioxide–sulfites (E 220–228) at the high level was close to the current group ADI in infants, adolescents, adults and the elderly, and exceeded the current group ADI in toddlers and children. The main contributing food sources for both scenarios were: 'Processed potato products, except dehydrated potatoes', 'Fruit juices as defined by Directive 2001/112/EC and vegetable juices', 'Flavoured drinks' and 'Wine'.

The refined exposure to sulfur dioxide–sulfites (E 220–228), considering additional exposure taking into account the available analytical data for foods categories which may contain sulfur dioxide–sulfites (E 220–228) 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, also resulted in mean and high level exposure estimates above the current group ADI in all population groups in the *brand-loyal scenario*. For the *non-brand-loyal scenario*, the mean exposure to sulfur

dioxide–sulfites (E 220–228) was slightly above the current group ADI for toddlers, whereas the high exposure was above the current group ADI for all population groups. The main contributing food sources for both scenarios were: 'Processed potato products, except dehydrated potatoes', 'Meat, only chicken meat', 'Meat preparations as defined by Regulation (EC) No 853/2004' and 'Wine'.

Overall, the anticipated exposure estimates of sulfur dioxide–sulfites (E 220–228) exceeded the current group ADI in most population groups, regardless of the exposure scenario and concentration data used in the assessment. The possibility of exceeding the existing group ADI was consistent with the results of the second French total diet study (TDS) (ANSES, 2011).

OVERALL CONSIDERATIONS AND CONCLUSIONS

In its evaluation of sulfur dioxide (E 220) and sulfites (sodium sulfite, sodium bisulfite, sodium metabisulfite, potassium metabisulfite, potassium bisulfite, calcium sulfite and calcium bisulfite (E 221–228)), 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.

However, the Panel noted that the remaining sulfur dioxide, bisulfite and sulfite ions existed in a series of equilibria and that these would favour bisulfite ions at the pH of the stomach and sulfite ions at physiological pHs. Therefore the Panel considered that once ingested, based on their capacity to form sulfite ions, read across between the different sulfite sources is possible.

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.

However, the Panel noted that:

- the overall available database was limited;
- this database did not indicate any concern for genotoxicity and did not report effects in chronic, carcinogenicity and reprotoxicity studies after oral exposure in the diet, by gavage, or in the drinking water. A NOAEL of 70 mg SO_2 equivalent/kg bw per day was identified from a long term toxicity study in rats;
- although the majority of the available toxicological studies were performed using sodium or potassium metabisulfite, because exposure is predominantly to the sulfite ion irrespective of its source, read across of these data to other sulfites and sulfur dioxide is feasible.

In addition, the Panel observed that:

- the exposure to sulfur dioxide–sulfites was:

  - above the group ADI of 0.7 SO_2 equivalent/mg kg per bw in all population groups at both the mean and the high level in the brand-loyal scenario, and at the high level in the non-brand-loyal scenario, when calculated in the refined exposure scenario considering only direct addition of sulfur dioxide–sulfites to food;
  - above the group ADI in all populations at the high level for the non-brand loyal scenario in the refined exposure scenario considering additional exposure taking into account the available analytical data for foods categories which may contain sulfur dioxide–sulfites (E 220–228) 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.
- there are numerous reports of sensitivity/intolerance reactions in humans exposed to sulfited solid foods and beverages.

Overall, considering that**:**

- the group ADI allocated by JECFA and the SCF of 0–0.7 mg SO_2 equivalent/kg bw per day based on a NOAEL in both the pigs and rats studies was on the assumption that they can result from all sulfiting substances;
- the toxicological database on sulfites and their reaction products with food components was limited;
- based on the common exposure to sulfite ions, extrapolation between studies using various sulfite sources was possible;
- there were data suggesting that the critical effects of sulfites (and particularly sulfur dioxide) were site of contact effects, however, it was not possible to ascertain whether there were no systemic effects,
- improving the toxicological database might result in either an increase or a decrease in the group ADI, depending on, for example, the effects detected, the identified point of departure and the use of chemical specific rather than default uncertainty factors.

The Panel concluded that the current group ADI of 0.7 mg SO_2 equivalent/kg bw per day (derived using a default uncertainty factor of 100) would remain adequate but should be considered temporary, whilst the database was improved.

The Panel further concluded that exposure estimates to sulfur dioxide–sulfites were higher than the group ADI of 0.7 mg SO_2 equivalent/kg bw per day for all population groups.

RECOMMENDATIONS

The Panel recommended that:

- the database and the temporary group ADI should be re-evaluated. The Panel noted that the studies recommended below could require 5 years for completion;
- 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.

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APPENDICES

A. SUMMARY OF REPORTED USE LEVELS TO SULFUR DIOXIDE–SULFITES (E 220–228) EXPRESSED AS SULFUR DIOXIDE (MG/KG OR MG/L) IN FOODS PROVIDED BY THE INDUSTRY

Food category	Data providers	E code	MPL	N	Usage minimum level (min–max)	Usage typical level (min–max)	Usage maximum level (min–max)
04.2.1 Dried fruit	**FDE**	**E 220**	50–2000	2	0.4–1	0.5–1.5	0.6–2
04.2.2 Fruit and vegetables in vinegar, oil or brine	**FDE**	**E 223**	100–500	1	3.37	5.4	9.81
		E 224		1	79.63	79.63	79.63
04.2.3 Canned or bottled fruit and vegetables, only bottled, sliced lemon	**FDE**	**E 220**	50–250	1	50	200	250
04.2.4.1 Fruit and vegetable preparations, excluding compote, only horseradish pulp	**FDE**	**E 223**	50–800	2	200	400	400
04.2.6 Processed potato products	**FDE**	**E 223**	100–400	1	30.75	61.44	100.69
		E 224		1	10.1	10.1	10.1
04.2.6 Processed potato products, only dehydrated potatoes products	**FDE**	**E 223**		1	39	42	45
05.2 Other confectionery, including breath refreshening microsweets	**FDE**	**E 221**	50–100	2	0	5	50
06.2.2 Starches	**AAF**	**E 220**	50	1	10	10	50
	FDE	**E 220**		1	0	10	50
07.2 Fine bakery wares, only dried biscuits	**FDE**	**E 223**	50	1	9	25	50
08.2 Meat preparations as defined by Regulation (EC) No 853/2004 (M42), only	**BMPA**		450	2	355	355	450
	FDE	**E 220**		2	450	450	450

breakfast sausages; burger meat with a minimum vegetable and/or cereal content of 4% mixed within the meat		**E 221**		4	450	450	450
		E 222		4	450	450	450
		E 223		4	450	450	450
		E 224		4	450	450	450
		E 226		4	450	450	450
		E 227		4	450	450	450
		E 228		4	450	450	450
08.2 Meat preparations as defined by Regulation (EC) No 853/2004 (M42), only salsicha fresca, longaniza fresca and butifarra fresca	**FDE**	**E 220**		1	450	450	450
		E 221		1	450	450	450
		E 222		2	450	450	450
		E 223		2	450	450	450
		E 224		2	450	450	450
		E 226		2	450	450	450
		E 227		2	450	450	450
		E 228		2	450	450	450
11.1 Sugars and syrups as defined by Directive 2001/111/EC, only glucose syrup, whether or not dehydrated	**AAF**	**E 220**	10–20	1	10	10	20
	FDE	**E 220**		1	0	10	20
12.3 Vinegars	**FDE**	**E 220**	170	1	5.07	5.58	6.56
		E 223		1	50.41	50.41	50.41
		E 224		3	6.77–120	6.77–120	6.77–120
12.4 Mustard, excluding dijon mustard	**FDE**	**E 220**	250–500	1	11.86	74.79	94.21
12.4 Mustard, only dijon mustard	**FDE**	**E 223**		1	450	470	500
12.9 Protein products, excluding products covered in category 1.8, only gelatine	**GME**	**E 220**	50–200	1	10	30	50
		E 221		1	10	30	50
		E 222		1	10	30	50
		E 223		1	10	30	50
		E 224		1	10	30	50

		E 226		1	10	30	50
		E 227		1	10	30	50
		E 228		1	10	30	50
14.1.4 Flavoured drinks, only non-alcoholic flavoured drinks containing at least 235 g/L glucose syrup	**FDE**	**E 223**	20–350	14	10–45	20–45	24–50
	UNESDA	**E220–228**		-	-	0.1–45	20–45
14.1.4 Flavoured drinks, only other concentrates based on fruit juice or comminuted fruit; capilé groselha	**UNESDA**	**E220–228**		-	-	20–160	250
14.2.1 Beer and malt beverages	**FDE**	**E 220**	20–50	3	0	315	10–23
14.2.2 Wine and other products defined by Regulation (EEC) No 1234/2007, and alcohol-free counterparts	**FDE**	**E 220**	200	1	27	27	37
14.2.3 Cider and perry	**FDE**	**E 220**	200	2	100–119	158–166	200
		E 223		1	13	75	200

B. SUMMARY OF ANALYTICAL RESULTS (MIDDLE BOUND MG/KG OR MG/L AS APPROPRIATE) OF SULFUR DIOXIDE PROVIDED BY THE MEMBER STATES

						Range				All data			
Food category number	**Food category description**	**Specifications**	**MPL**	**N**	**% LC**	**LOD**		**LOQ**		**Min**	**Mean**	**P95**	**Max**
01.7.1	Unripened cheese, excluding products falling in category 16	Only cheese, processed spreadable[(a)]	-	51	39.2	1	1	2	5	0.5	3.1	7	8
01.8	Dairy analogues, including beverage whiteners[(a)]		-	3	100	4.1	4.1	13.3	13.3	2.1	2.1	2.1	2.1
02	Fats and oils and fat and oil emulsions	Only animal fat	-	2	100	8	8	8	8	4	4	4	4
04.1	Unprocessed fruit and vegetables[(a)]	Only figs	-	1	100	8	8	8	8	4	4	4	4
		Only globe artichokes	-	1	100	3.3	3.3	10	10	5	5	5	5
		Only nashi pear	-	1	100	5	5	15	15	2.5	2.5	2.5	2.5
		Only oyster mushroom	-	2	100	4	4	11.1	11.1	5.6	5.6	5.6	5.6
		Only plums	-	1	100	8	8	8	8	4	4	4	4
		Only sour cherry	-	1	100	10	10	20	20	5	5	5	5
		Only strawberries	-	1	0	2	2	10	10	10	10	10	10
		Only parsley root	-	1	0					12.8	12.8	12.8	12.8
		Only apricots	-	1	0	0	0	0	0	1.1	1.1	1.1	1.1
		Only celeriac	-	1	0	10	10	10	10	87	87	87	87
		Only chestnuts	-	1	0	8	8	8	8	14	14	14	14
		Only dates	-	3	66.7	8	100	8	300	4	19.3	50	50
		Only coconuts	-	2	50	8	8	8	8	3.5	3.8	4	4
		Only cucumbers	-	3	0	10	10	10	10	16.9	18.2	19.5	19.5

		Only pumpkins	-	4	0	3.3	3.3	10	10	30	40	45	45
04.1.1	Entire fresh fruit and vegetables	Grapes	10	33	54.5	2	10	5	20	0.7	13.5	93	140.5
		Lychee	100	17	76.5	5	8	8	24	2.5	8.6	42	42
04.1.2	Peeled, cut and shredded fruit and vegetables	Only garlic	300	2	100	10	100	10	300	5	27.5	50	50
		Only onions	300	34	55.9	2	10	5	20	2.5	27.6	159	187
04.1.3	Frozen fruit and vegetables	Only other fruit and vegetables not listed in Annex II[(a)]	-	4	100	1.5	10	5	30	0.8	3	5	5
		Only white vegetables, including mushrooms and white pulses	50	11	90.9	2	50	6	150	1	5.6	25	25
04.2.1	Dried fruit and vegetables	Only dried coconut	50	24	66.7	2	46	4	50	1	12.5	27	40.2
		Only dried ginger	150	1	0	12.4	12.4	20	20	49	49	49	49
		Only dried mushrooms	100	15	13.3	1.5	10	5	18	1	43.1	132	132
		Only dried peach	2000	7	14.3	4.1	120	10	360	60	922.8	1913	1913
		Only dried vegetables	400	24	58.3	2	10	5	20	2.5	115.1	595	1980
		Only dried apple	600	192	25.5	0	300	0	900	0	66	378	927.7
		Only dried apricot	2000	208	10.1	1	382.7	5	1148	0.8	1336.4	2743	4402.5
		Only dried banana	1000	37	94.6	1	100	3	300	0.5	7.5	50	58.2
		Only dried dates[(a)]	-	67	86.6	1.5	500	0	1500	0	11.4	57	250
		Only dried figs	2000	99	69.7	1	600	0	1800	0	167.8	933	1566

		Only dried fruits, excluding dried apples, pears, bananas, apricots, peaches, grapes, prunes and figs	500	1034	46.7	0	120	0	360	0	215.9	1269.8	18767
		Only dried grapes	2000	182	63.7	0	169.6	0	508.9	0	184.9	1239	2105
		Only dried mango	-	30	40	2	46	4	50	1	54.8	292.4	518.4
		Only nuts in shell nut	500	63	47.6	0.3	10	1	10	1.2	23	64	317
		Only dried pear	600	36	0	0	10	0	20	0.1	114.8	900	919.8
		Only dried prune	2000	1945	9.8	0	100	0	300	0	64.3	50	2760
		Only dried tomatoes	200	120	78.3	0.5	10	1	24	0.5	364.2	3416.5	8539.1
04.2.2	Fruit and vegetables in vinegar, oil, or brine	Only olives[(a)]	-	7	100	3.3	10	1.3	13.3	0.7	2.9	5	5
		Only golden peppers in brine	500	35	26.5	4.1	10	10	13.3	5	167	350.8	397.3
		Except olives and golden peppers in brine	100	191	40.8	0.4	1000	1	3000	0.5	26.168	87.3	100
04.2.3	Canned or bottled fruit and vegetables	Only fruit and vegetables not listed in Annex II[(a)]	-	102	70.6	0.8	46	2.4	72	0.4	74.5	189	1647
		Only white vegetables and pulses	50	23	43.5	3	12	6	36	4	177.2	457	517
		Only sweetcorn	100	1	100	5	5	15	15	2.5	2.5	2.5	2.5
04.2.4.1	Fruit and vegetable preparations, excluding compote	Only olives[(a)]	-	13	92.3	4.1	40	8	120	2.1	13.5	50	50
		Only tomatoes purée[(a)]	-	7	71.4	3	30	8	90	4	9.7	24	24
		Other fruit products[(a)]	-	18	61.1	3	10	8	30	2.1	41.8	447	447

		Other vegetable products[(a)]	-	35	57.1	0	100	0	100	1	31.3	120	389.7
		Only coconut milk[(a)]	-	12	50	1	46	2	50	2.5	10.4	33	33
		Only chilli pepper[(a)]	-	40	45	1.5	8	5	20	0.8	30.6	120	129
		Only white vegetables	50	6	33.3	2	10	6	13.3	1.5	32.1	138	138
		Only onion, garlic and shallot pulp	300	29	34.5	4.1	100	8	300	2.5	76.9	216	259
		Only horseradish pulp	800	146	0.7	0.3	10	1	10	3.8	333.9	696	1380
04.2.4.2	Compote, excluding products covered by category 16	Only fruit compote[(a)]	-	25	96	1	4	1.2	11.1	0.6	9.9	5.6	153
04.2.5.1	Jam, jellies and marmalade and sweetened chestnut		100	327	83.2	0	100	0	300	0	18.4	62	1307
04.2.6	Processed potato products	Except dehydrated potatoes	100	142	19.0	0.0	30	0.0	90.0	0.5	21.6	56.4	177.6
		Dehydrated potato	400	420	50.5	0.0	100	0.0	300.0	0.5	25.7	89.2	320.0
05.1	Cocoa and chocolate products as covered by Directive 2000/36/EC	Only chocolate with nuts or fruits[(a)]	-	1	100	5	5	15	15	2.5	2.5	2.5	2.5
05.2	Other confectionery including breath	Only glucose syrup-based confectionary	50	36	69.4	1	8	5	13.3	0.9	71.3	543	765

	refreshing microsweets	Only candied, crystallised or glacé fruit, vegetables, angelica and citrus peel	100	161	63.4	0.3	100	0	300	0	18.5	57	358.6
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4		40–100	1	100	8	8	10	10	4	4	4	4
06.2.2	Starches	Excluding starches for infant formulae, follow on formulae and processed cereal-based foods and baby foods	50	3	33.3			10	10	5	7.9	12.5	12.5
06.3	Breakfast cereals	Only cereal flakes[(a)]	-	8	50	1	8	2	10	4	8.6	21.2	21.2
07.1	Bread and rolls			20	85	1	8	2	10	0.8	12.1	88.3	159
07.2	Fine bakery wares	Only pastries and cakes[(a)]	-	3	66.7	3.3	3.3	10	10	5	32.7	88	88
		Only dry biscuits	50	355	83.7	1	50	0	150	0	8.6	28	224
08	Meat	Only lamb meat[(a)]	-	1	100	23.4	23.4	25	25	12.5	12.5	12.5	12.5
		Other meat specialties[(a)]	-	3	100	3.3	3.3	10	10	5	5	5	5
		Only turkey meat[(a)]	-	1	0	3.3	3.3	10	10	232	232	232	232
		Only chicken meat[(a)]	-	12	50	3.3	3.3	10	10	5	63.1	359	359

08.2	Meat preparations as defined by Regulation (EC) No 853/2004	Only breakfast sausages; burger meat with a minimum vegetable and/or cereal content of 4% mixed within the meat	450	81	8.6	3.3	10	10	30	5	252.4	947	1378
		Only salsicha fresca, longaniza fresca and butifarra fresca	450	196	6.1	1	36.5	10	50	5	297.9422	628	899
09.1.1	Unprocessed fish[(a)]		-	4	100	3.3	3.3	10	10	5	5	5	5
09.1.2	Unprocessed molluscs and crustaceans		300	684	60.5	0	10	0	30	0	41.1	173	885.2
09.2	Processed fish and fishery products including molluscs and crustaceans		300										
11.1	Sugars and syrups as defined by Directive 2001/111/EC	Only sugars, except glucose syrup	10	14	78.6	1.8	10	5.3	30	0.9	5.2	47	47
11.2	Other sugars and syrups	Only molasses	70	13	100	10	10	10	10	5	5	5	5
11.3	Honey as defined in Directive 2001/110/EC[(a)]		-	2	100	10	10	10	10	5	5	5	5
12.2.1	Herbs and spices	Only capers	-	9	88.9	3	4.1	6	13.3	1.5	5.2	31.9	31.9
		Only ginger	-	37	45.9	8	10	8	10	5	83.4	313.6	316.8

		Only cinnamon	150	2	100	10	10	4	10	2	3.5	5	5
12.3	Vinegars		170	512	34.4	0.1	11	0.1	35	0.1	31.34	109.4	316
12.4	Mustard		250	169	61.5	0	100	0	300	0	31.2	186	440.5
12.5	Soups and broths[(a)]		-	39	74.4	1	8	3	13.3	0.5	7.7	30.2	64.5
12.6	Sauces	Only chilli powder[(a)]	-	1	100			10	10	5	5	5	5
		Only chutney and pickles[(a)]	-	9	77.8	0.9	10	0	30	0	4	14	14
		Only dressings[(a)]	-	25	76	0.3	100	1	300	0.5	21.5	50	180
		Only savoury sauces[(a)]	-	35	71.4	0.5	100	1	300	0.5	32.4	100	555
12.7	Salads and savoury based sandwich spreads	Only prepared salads	-	63	68.3	1.5	100	0	300	0	7.4	26	50
12.9	Protein products, excluding products covered in category 1.8	Only gelatine	50	9	77.8	1	4	5	11.1	0.9	10.7	38	38
13.1	Foods for infants and young children[(a)]		-	5	100	3	10	2.8	30	1.4	3.6	5	5
14.1	Non-alcoholic beverages	Only cola drinks[(a)]	-	4	100			5	5	2.5	2.5	2.5	2.5
14.1.1	Water, including natural mineral water as defined in Directive 2009/54/EC and spring water and all other bottled or packed waters[(a)]		-	1	100	20	20	60	60	10	10	10	10
14.1.2	Fruit juices as defined by	Only juices not listed in Annex II	-	15	73.3	0.8	46	2.4	50	0.4	11.9	85	85

	Directive 2001/112/EC and vegetable juices	Apple juice	50	19	94.7	1.7	12	2.5	18	1.3	3.5	7	7
		Fruit juice	50	72	86.1	0	46	0	100	0	14.2	80	255.3
		Grape juice	70	241	54.4	0.3	12	1	18	0.5	9.3	49	392
		Lemon juice	350	18	16.7	2.5	8	6	15.6	1.5	110.3	270.4	270.4
		Lime juice	350	2	0	46	46	50	50	88	91	94	94
		Orange juice	50	32	93.8	0.8	46	2.4	50	0.4	9.3	76	79
		Pineapple juice	50	3	66.7	3.3	3.3	10	10	1.5	3.8	5	5
14.1.4	Flavoured drinks	Only carry-over from concentrates in non-alcoholic flavoured drinks containing fruit juice	20	158	83.5	0.3	100	1	300	0.4	11.6	74.9	131.3
		Only non-alcoholic flavoured drinks containing at least 235 g/L glucose syrup	50										
14.1.5	Coffee, tea, herbal and fruit infusions, chicory; tea, herbal and fruit infusions and chicory extracts; tea, plant, fruit and cereal preparations for infusions, as well as mixes and instant mixes of these products	Only tea and infusions[(a)]	-	3	100	8	10	8	10	4	4.3	5	5
14.2.1	Beer and malt beverages		20	207	35.7	0.2	5	0.5	15	0	3.3	8.2	48

14.2.3	Cider and perry		200	1078	11.6	0.5	46	1	66	0.5	82.9	176	660
15.1	Potato-, cereal-, flour- or starch-based snacks - Only cereal-based snack foods	Only cereal- and potato-based snakes	50	38	86.8	1	100	5	300	1.6	10.4	63	100
15.2	Processed nuts	Only marinated nuts	50	75	88	1.8	50	0	150	0	7.1	25	34
16	Desserts, excluding products covered in categories 1, 3 and 4	Only ices and desserts[(a)]	-	12	91.7	1	8	1.3	10	0.5	9.8	80	80
17	Food supplements as defined in Directive 2002/46/EC of the European Parliament and of the Council (5) excluding food supplements for infants and young children[(a)]		-	3	0	1.7	1.7	5	5	325	562.2	743.5	743.5
18	Processed foods not covered by categories 1 to 17, excluding foods for infants and young children	Only legume-based meals[(a)]	-	4	100	4.1	8	10	13.3	2.1	2.5	4	4
		Only pasta[(a)]	-	13	100	4.1	10	8	30	2.1	2.8	5	5
		Only pizza and sandwiches[(a)]	-	12	100	4.1	4.1	13.3	13.3	2.1	2.6	6.7	6.7
		Only vegetable based-meals[(a)]	-	17	94.1	3.3	10	10	13.3	2.1	4.6	12	12

Liqueur wines[(b)]	Fortified and liqueur wines (e.g. Vermouth, Sherry, Madeira)	200	212	10.4	0.7	17.3	2	58	0.5	55.6	136	234
	Liqueur		22	59.1	1.7	4.8	3	10	1.5	46	172	500
Wine[(b)]		250	15268	5.6	0.3	33.3	1	100	0.1	104.5	193	2471

MPL: maximum permitted level; LOD: limit of detection; LOQ: limit of quantification.

(a) Food categories for which the direct addition of sulfur dioxide–sulfites (E 220–228) is not authorised according to Annex II to Regulation (EC) No 1333/2008.

(b) Food categories authorised according to Annex IB to Regulation (EC) No 606/2009.

C. Concentration levels of sulfur dioxide–sulfites (E 220–228) expressed as sulfur dioxide (mg/kg or mL/kg as appropriate) used in the 'regulatory maximum level exposure assessment scenario' and in the refined exposure scenario considering only food categories listed in Annex II to Regulation (EC) No 1333/2008 and Annex IB to Regulation (EC) No 606/2009 (dataset 1)

Food category number	Food category description	Specifications	MPL scenario	Concentration levels used in the refined exposure assessment scenario			
				Concentration levels exceeding the MPLs excluded		Concentration levels exceeding the MPLs included[(a)]	
				Mean	Max	Mean	95th percentile[c]
04.1.1	Entire fresh fruit and vegetables	Only tables grapes	10	4.3	8.0	13.5	93.0
		Only fresh lychees		3.1	4.0	8.6	42.0
04.1.2	Peeled, cut and shredded fruit and vegetables	Only garlic	300	27.5	50.0	27.5	50.0
		Only onions		27.6	187.0	27.6	187.0
		Only shallots pulp		N.A.	N.A.	N.A.	N.A.
04.2.1	Dried fruit	Only dried tomatoes	200	6.8	73.0	364.2	3416.5
		Only dried fruits, excluding dried apples, pears, bananas, apricots, peaches, grapes, prunes and figs	500	34.7	500.0	215.9	1269.8
		Only nuts in shell nuts		23.0	317.0	23.0	317.0
		Only dried apples	600	61.5	420.0	66.0	420.0
		Only dried pears		68.0	528.0	114.8	900.0
		Only dried bananas	1000	7.5	58.2	7.5	58.2
		Only dried apricots	2000	1088.6	1987.8	1336.4	2743.0
		Only dried grapes		4.3	8.0	184.9	1239.0
		Only dried prunes		52.4	1980.0	64.3	1980.0
		Only dried figs		167.8	1566.0	167.8	1566.0
04.2.2	Fruit and vegetables in vinegar, oil and brine	Except olives and golden peppers in brine	100	26.2	100.0	26.2	100.0
04.2.4.1	Fruit and vegetable preparations excluding compote	Only processed white vegetables and mushrooms	50	N.A.	N.A.	N.A.	N.A.

		Only onion, garlic and shallot pulp	300	76.9	259.0	76.9	259.0
		Only horseradish pulp	800	305.2	768.0	333.9	768.0
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut	Only jams, jellies and marmalades with sulfited fruit	100	7.3	84.5	18.4	84.5
04.2.5.3	Other similar fruit or vegetable spreads	Other fruit spreads	50	N.A.	N.A.	N.A.	N.A.
04.2.6	Processed potato products	Except dehydrated potatoes	100	21.5	100.0	25.7	100.0
		Only dehydrated potatoes products	400	21.6	177.6	21.6	177.6
05.2	Other confectionery including breath refreshening microsweets	Only candied, crystallised or gacé fruit, vegetables, angelica and citrus peel	100	15.4	99.0	18.5	99.0
		Only glucose syrup-based confectionery (carry-over from the glucose syrup only)	50	6.9	37.0	71.3	543.0
06.2.2	Starches[(b)]	Excluding starches for infant formulae, follow-on formulae and processed cereal-based foods and baby foods	50	10.0	50.0	10.0	50.0
07.2	Fine bakery wares	Only dry biscuits	50	6.6	50.0	8.0	50.0
08.2	Meat preparations as defined by Regulation (EC) No 853/2004	Only breakfast sausages; burger meat with a minimum vegetable and/or cereal content of 4% mixed within the meat	450	163.6	448.2	252.4	947.0
		Only salsicha fresca, longaniza fresca and butifarra fresca	450	240.4	447.0	297.9	628.0
09.1.2	Unprocessed molluscs and crustaceans		270	34.1	296.0	41.1	296.0

09.2	Processed fish and fishery products including molluscs and crustaceans						
11.1	Sugars and syrups as defined by Directive 2001/111/EC[6]	Only sugars, except glucose syrup	10	2.0	5.0	5.2	47.0
11.2	Other sugars and syrups		40	N.A.	N.A.	N.A.	N.A.
12.2.1	Herbs and spices	Only cinnamon	150	3.5	5.0	3.5	5.0
12.3	Vinegars		170	29.0	167.0	31.3	167.0
12.4	Mustard		250	27.4	219.6	31.2	219.6
12.9	Protein products, excluding products covered in category 1.8	Only gelatine	50	10.7	38.0	10.7	38.0
14.1.2	Fruit juices as defined by Directive 2001/112/EC[14] and vegetable juices	Fruit juice, not specified	50	4.1	32.0	14.2	80.0
		Only orange		4.8	5.0	9.3	76
		Only grapefruit		4.2	49.0	9.3	49.0
		Only apple		3.5	7.0	3.5	7.0
		Only pineapple		3.8	5.0	3.8	5.0
		Only lemon	350	110.3	270.4	110.3	270.4
		Only lime		91.0	94.0	91.0	94.0
14.1.4	Flavoured drinks	Only carry-over from concentrates in non-alcoholic flavoured drinks containing fruit juice	50	5.2	50.0	11.6	74.9
		Only non-alcoholic flavoured drinks containing at least 235 g/L glucose syrup					
		Only other concentrates based on fruit juice or comminuted fruit; capilé, groselha	250	N.A.	N.A.	N.A.	N.A.
14.2.1	Beer and malt beverages		20	2.8	13.2	3.3	48.0

14.2.3	Cider and perry		200	78.1	200.0	82.9	200.0
15.1	Potato-, cereal-, flour- or starch-based snacks - only cereal-based snack foods	Only cereal- and potato-based snacks	50	5.2	25.0	10.4	63.0
	Liqueur wines[(c)]	Fortified and liqueur wines	200	53.9	172.8	55.6	172.8
		Liqueurs		24.4	172.0	46.0	172.0
	Wine[(c)]		250[(d)]	102.0	396.0	104.5	396.0

MPL: maximum permitted level; N.A.: Not taken into account because data were not available.

(a) When concentration levels exceeding the MPLs were included, the 95th percentile level was used instead of the maximum value in order to minimise the impact of possible outliers. However, the maximum value was used in case the 95th percentile level resulted below the MPL.

(b) Usage level.

(c) Food categories authorised according to Annex IB to Regulation (EC) No 606/2009.

(d) In special cases, levels of sulfur dioxide–sulfites (E 220–228) in wine are authorised up to 400 mg/L, this threshold has been used to identify analytical results above the MPL.

D. Concentration levels of sulfur dioxide–sulfites (E 220–228) expressed as sulfur dioxide (mg/kg or mL/kg as appropriate) used for foods categories which may contain sulfur dioxide–sulfites (E 220–228) due to carry-over and 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, and for which analytical data were available

Food category number	Food category description	Specifications	Concentration levels used in the refined exposure	
			Mean	95th percentile
1.7.1	Unripened cheese, excluding products falling in category	Only cheese, processed	3.1	7.0
04.1	Unprocessed fruit and vegetables	Only dates	19.3	50.0
		Only coconuts	3.8	4.0
		Only cucumbers	18.2	19.5
		Only pumpkins	40.0	45.0
04.2.4.1	Fruit and vegetables preparation, excluding compote	Only table olives	13.5	50.0
		Only tomato purée	9.7	24.0
		Only coconut milk	10.4	33.0
		Other fruit products	41.8	447.0
		Other vegetable products	31.3	120.0
		Only chilli pepper	30.6	120.0
04.2.4.2	Fruit compote, excluding products covered by category	Only fruit compote	9.9	5.6
06.3	Breakfast cereals	Only cereal flakes	8.6	21.2
07.1	Bread and rolls		12.1	88.3
07.2	Fine bakery wares	Pastries and cakes	32.7	88.0
08	Meat	Only chicken meat	63.1	359.0
12.2.1	Herbs and spices	Only capers	5.2	31.9
		Only ginger	83.4	313.6
12.5	Soups and broths	Ready to eat soups	7.7	30.2
12.6	Sauces	Only chutney and pickles	4.0	14.0
		Only dressing	21.5	50.0
		Only savoury sauces	32.4	100.0
12.7	Salads and savoury-based sandwiches	Only prepared salads	7.4	26.0
14.1.2	Fruit juices as defined by Directive 2001/112/EC[14] and vegetable juices	Only cranberry	11.9	85.0
		Only pear	11.9	85.0
		Only blackcurrant	11.9	85.0

		Only elderberry	11.9	85.0
		Only tomato	11.9	85.0
		Only pomegranate	11.9	85.0
16	Desserts, excluding products covered in categories 1,3	Only ices and desserts	9.8	80.0
17	Food supplements as defined in Directive 2002/46/EC		562.2	743.5
18	Processed food	Only vegetable-based meals	5.1	10.0

E. Summary of exposure to sulfur dioxide–sulfites (E 220–228) from their use as food additives considering concentration levels above the MPLs for food categories listed in Annex II to Regulation (EC) No 1333/2008 and Annex IB to Regulation (EC) No 606/2009 and, in addition, the available analytical data for foods categories which may contain sulfur dioxide–sulfites (E 220–228) 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 (min–max across the dietary surveys in mg/kg bw per day).

	Infants (4–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (> 65 years)
Brand-loyal scenario						
Mean	0.33–1.01	0.98–2.12	0.79–1.85	0.45–1.20	0.54–0.95	0.53–1.01
95th perc	1.89–3.63	2.65–6.11	1.63–4.55	0.89–2.65	1.38–2.38	1.10–2.48
Non-brand-loyal scenario						
Mean	0.1–0.39	0.35–0.94	0.3–0.85	0.17–0.54	0.21–0.40	0.23–0.39
95th perc	0.57–1.08	0.95–2.59	0.66–2.08	0.35–1.15	0.57–1.01	0.54–0.82

F. Summary of exposure to sulfur dioxide–sulfites (E 220–228) from their use as food additives for the 'regulatory maximum level exposure assessment scenario' and in the 'refined exposure scenarios' per population group and survey: mean and 95th percentile (mg/kg bw per day)

Population group	Country (survey)	N of subjects	MPL[1] scenario		Refined exposure assessment scenario using dataset 1[(a)]				Refined exposure assessment scenario using dataset 2[(b)]			
					Brand loyal		Non brand		Brand loyal		Non brand loyal	
			Mean	95th perc.	Mean	95th perc.	Mean	95th perc.	Mean	95th perc.	Mean	95th perc.
Infants	Bulgaria (NUTRICHILD)	659	0.42	1.33	0.19	0.68	0.06	0.16	0.58	2.22	0.17	0.60
	Germany (VELS)	159	0.52	1.77	0.42	1.40	0.16	0.65	0.52	1.56	0.21	0.85
	Denmark (IAT 2006_07)	826	1.03	3.08	0.65	1.70	0.23	0.70	0.80	1.81	0.31	0.85
	Finland (DIPP_2001_2009)	500	1.10	3.95	0.91	3.48	0.15	0.36	0.99	3.61	0.20	0.45
	United Kingdom	1369	0.42	1.81	0.25	1.10	0.09	0.47	0.44	1.40	0.16	0.62
	Italy (INRAN_SCAI_2005_06)	12	0.23		0.13	.	0.03	.	0.25		0.08	
Toddlers	Belgium (Regional_Flanders)	36	1.80		0.67	.	0.31	.	1.09		0.54	
	Bulgaria (NUTRICHILD)	428	0.94	2.36	0.49	1.55	0.21	0.85	1.35	2.84	0.52	1.20
	Germany (VELS)	348	1.60	3.28	1.13	2.75	0.48	1.20	1.36	2.94	0.65	1.41
	Denmark (IAT 2006_07)	917	2.13	4.51	0.92	2.26	0.45	1.07	1.21	2.41	0.60	1.24
	Spain (enKid)	17	1.17		0.73	.	0.33	.	1.45		0.57	
	Finland (DIPP_2001_2009)	500	1.20	2.90	0.81	1.89	0.24	0.61	1.01	2.31	0.34	0.79
	United Kingdom (NDNS-	185	1.45	3.14	0.70	1.79	0.32	0.93	0.97	1.98	0.48	1.15
	United Kingdom	1314	1.16	3.41	0.53	1.71	0.23	0.85	0.82	2.01	0.38	1.03
	Italy (INRAN_SCAI_2005_06)	36	0.75		0.41	.	0.14	.	0.74		0.26	
	Netherlands (VCP_kids)	322	2.21	6.92	1.22	4.50	0.56	2.26	1.60	4.64	0.74	2.40
Other children	Austria (ASNS_Children)	128	1.04	2.01	0.56	1.27	0.29	0.71	0.87	1.56	0.46	1.00
	Belgium (Regional_Flanders)	625	1.49	3.31	0.65	1.55	0.28	0.74	0.95	1.86	0.45	0.94
	Bulgaria (NUTRICHILD)	433	1.01	2.76	0.59	1.99	0.28	1.02	1.34	2.71	0.60	1.52
	Czech Republic (SISP04)	389	0.97	2.38	0.64	1.82	0.27	0.95	1.10	2.64	0.46	1.09
	Germany (EsKiMo)	835	1.32	2.76	0.77	1.89	0.41	1.03	0.96	1.98	0.53	1.15
	Germany (VELS)	293	1.44	2.71	1.00	2.27	0.45	0.94	1.23	2.40	0.63	1.18
	Denmark (DANSDA 2005–08)	298	1.33	2.72	0.61	1.18	0.32	0.66	0.90	1.46	0.46	0.83
	Spain (enKid)	156	1.09	2.83	0.69	1.67	0.31	0.98	1.18	2.43	0.52	1.17

	Spain (NUT_INK05)	399	0.94	2.14	0.60	1.61	0.31	0.83	1.02	2.20	0.48	1.07
	Finland (DIPP_2001_2009)	750	1.29	2.74	0.74	1.85	0.39	1.01	0.97	2.05	0.50	1.14
	France (INCA2)	482	0.81	1.81	0.38	0.96	0.18	0.50	0.67	1.30	0.37	0.74
	United Kingdom (NDNS-	651	1.10	2.43	0.59	1.44	0.27	0.76	0.83	1.73	0.43	0.95
	Greece (Regional_Crete)	838	0.63	1.55	0.25	0.70	0.10	0.34	0.57	1.18	0.25	0.55
	Italy (INRAN_SCAI_2005_06)	193	0.76	1.88	0.44	1.27	0.19	0.59	0.76	1.65	0.32	0.79
	Latvia (EFSA_TEST)	187	0.90	2.62	0.64	2.40	0.31	1.06	0.96	2.55	0.49	1.25
	Netherlands (VCP_kids)	957	1.86	5.11	1.16	3.63	0.53	1.65	1.45	3.78	0.69	1.83
	Netherlands	447	1.06	2.33	0.82	1.94	0.29	0.79	1.09	2.11	0.47	1.02
	Sweden (NFA)	1473	1.36	2.92	0.90	2.01	0.41	0.96	1.11	2.24	0.59	1.20
Adolescents	Austria (ASNS_Children)	237	0.66	1.43	0.38	0.90	0.17	0.48	0.59	1.20	0.28	0.61
	Belgium (Diet_National_2004)	576	0.53	1.34	0.33	0.97	0.12	0.38	0.52	1.14	0.23	0.52
	Cyprus (Childhealth)	303	0.35	0.85	0.16	0.42	0.06	0.15	0.37	0.74	0.14	0.30
	Czech Republic (SISP04)	298	0.88	2.31	0.59	1.63	0.26	0.77	0.88	2.09	0.38	0.90
	Germany	1011	0.63	1.67	0.37	1.09	0.18	0.58	0.52	1.22	0.26	0.68
	Germany (EsKiMo)	393	1.02	2.03	0.62	1.54	0.31	0.79	0.77	1.66	0.40	0.88
	Denmark (DANSDA 2005–08)	377	0.69	1.51	0.33	0.72	0.15	0.34	0.48	0.89	0.22	0.46
	Spain (AESAN_FIAB)	86	0.43	0.97	0.25	0.75	0.14	0.43	0.50	1.18	0.23	0.49
	Spain (enKid)	209	0.71	1.64	0.46	1.26	0.22	0.65	0.78	1.85	0.36	0.85
	Spain (NUT_INK05)	651	0.60	1.40	0.39	1.08	0.19	0.55	0.65	1.37	0.30	0.69
	Finland (NWSSP07_08)	306	0.61	1.32	0.29	0.68	0.14	0.38	0.41	0.99	0.20	0.47
	France (INCA2)	973	0.43	1.04	0.21	0.53	0.10	0.27	0.39	0.78	0.20	0.42
	United Kingdom (NDNS-	666	0.60	1.32	0.35	0.82	0.14	0.42	0.50	1.03	0.23	0.52
	Italy (INRAN_SCAI_2005_06)	247	0.46	1.11	0.27	0.75	0.11	0.41	0.43	0.87	0.19	0.49
	Latvia (EFSA_TEST)	453	0.71	1.63	0.48	1.27	0.25	0.70	0.70	1.55	0.38	0.86
	Netherlands	1142	0.71	1.68	0.51	1.18	0.18	0.50	0.71	1.47	0.30	0.66
	Sweden (NFA)	1018	0.99	2.16	0.63	1.41	0.28	0.66	0.76	1.52	0.39	0.81
Adults	Austria (ASNS_Adults)	308	0.42	1.31	0.33	1.07	0.12	0.42	0.53	1.23	0.22	0.53
	Belgium (Diet_National_2004)	1292	0.63	1.79	0.57	1.97	0.19	0.62	0.73	2.06	0.27	0.70
	Czech Republic (SISP04)	1666	0.77	2.02	0.59	1.81	0.26	0.76	0.75	1.94	0.34	0.87
	Germany	10419	0.67	1.77	0.52	1.76	0.21	0.64	0.65	1.84	0.29	0.73

	Denmark (DANSDA 2005–08)	1739	0.85	1.78	0.67	1.89	0.24	0.59	0.74	1.93	0.29	0.64
	Spain (AESAN)	410	0.42	1.11	0.30	1.05	0.12	0.43	0.48	1.23	0.19	0.51
	Spain (AESAN_FIAB)	981	0.48	1.26	0.37	1.21	0.15	0.44	0.56	1.42	0.22	0.53
	Finland (FINDIET2012)	1295	0.47	1.35	0.34	1.13	0.15	0.51	0.52	1.35	0.24	0.62
	France (INCA2)	2276	0.59	1.62	0.52	1.97	0.18	0.59	0.64	2.03	0.26	0.67
	United Kingdom (NDNS-	1266	0.68	1.75	0.55	1.91	0.20	0.62	0.67	2.01	0.27	0.71
	Hungary (National_Repr_Surv)	1074	0.64	1.49	0.46	1.25	0.23	0.60	0.60	1.35	0.30	0.69
	Ireland (NANS_2012)	1274	0.67	1.84	0.51	1.84	0.19	0.63	0.73	1.97	0.30	0.75
	Italy (INRAN_SCAI_2005_06)	2313	0.53	1.40	0.52	1.70	0.17	0.52	0.61	1.75	0.22	0.58
	Latvia (EFSA_TEST)	1271	0.44	1.20	0.32	0.98	0.16	0.50	0.51	1.18	0.26	0.63
	Netherlands	2057	0.60	1.53	0.48	1.61	0.17	0.51	0.64	1.76	0.26	0.62
	Romania (Dieta_Pilot_Adults)	1254	0.58	1.29	0.36	0.97	0.17	0.46	0.52	1.16	0.23	0.54
	Sweden (Riksmaten 2010)	1430	0.55	1.32	0.46	1.39	0.17	0.47	0.58	1.50	0.25	0.57
Elderly and very elderly	Austria (ASNS_Adults)	92	0.47	1.03	0.44	1.30	0.15	0.40	0.58	1.34	0.24	0.49
	Belgium (Diet_National_2004)	1215	0.50	1.64	0.48	1.90	0.15	0.56	0.63	1.96	0.23	0.65
	Germany	2496	0.60	1.62	0.50	1.83	0.20	0.59	0.63	1.90	0.27	0.66
	Denmark (DANSDA 2005–08)	286	0.97	2.01	0.89	2.41	0.30	0.69	0.95	2.46	0.34	0.74
	Finland (FINDIET2012)	413	0.37	1.25	0.28	1.09	0.11	0.41	0.45	1.26	0.20	0.49
	France (INCA2)	348	0.67	1.79	0.67	2.16	0.21	0.62	0.78	2.21	0.28	0.71
	United Kingdom (NDNS-	305	0.52	1.47	0.40	1.57	0.14	0.49	0.50	1.69	0.20	0.57
	Hungary (National_Repr_Surv)	286	0.52	1.24	0.39	1.15	0.19	0.56	0.53	1.31	0.25	0.63
	Ireland (NANS_2012)	226	0.44	1.21	0.35	1.35	0.11	0.39	0.55	1.49	0.21	0.52
	Italy (INRAN_SCAI_2005_06)	518	0.59	1.64	0.65	2.09	0.19	0.61	0.74	2.18	0.24	0.65
	Netherlands	173	0.59	1.58	0.55	1.91	0.18	0.60	0.69	1.98	0.27	0.67
	Netherlands (VCP-Elderly)	739	0.50	1.35	0.44	1.49	0.15	0.45	0.58	1.55	0.23	0.55
	Romania (Dieta_Pilot_Adults)	128	0.50	1.05	0.32	0.78	0.14	0.40	0.49	0.94	0.21	0.48
	Sweden (Riksmaten 2010)	367	0.58	1.40	0.53	1.50	0.19	0.52	0.65	1.59	0.28	0.63

MPL: maximum permitted level; perc.: percentile.

(a) Dataset 1 includes reported use levels and analytical results (not exceeding the MPLs) for food categories for which direct addition of sulfur dioxide–sulfites (E 220–228) is authorised, according to Annex II to Regulation (EC) No 1333/2008 and Annex IB to Regulation (EC) No 606/2009.

(b) Dataset 2 includes in addition to dataset 1, the available analytical data for foods categories which may contain sulfur dioxide–sulfites (E 220–228) 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.

G. Main food categories contributing to exposure to sulfur dioxide–sulfites (E 220–228) from their use as food additives in the 'Regulatory maximum level exposure scenario' (> 5% to the total mean exposure) and number of surveys in which the food category is contributing

Food category number	Food category description	Specifications	Min–max % of contribution (n surveys)					
			Infants	Toddlers	Other children	Adolescents	Adults	Elderly
04.1.2	Peeled, cut and shredded fruit and vegetables	Only garlic Only onions Only shallots pulp	23.4–33.4 (3)	6.2–31.1 (6)	5.6–24.8 (9)	5.1–15.1 (12)	5.2–31.9 (13)	5.5–38.0 (9)
04.2.2	Fruit and vegetables in vinegar, oil and brine	Exept olives and golden peppers in brine					5.98 (1)	6.5–6.5 (1)
04.2.6	Processed potato products	Except dehydrated potatoes	8.9–43.4 (5)	9.3–48.5 (8)	7.4–34.3 (14)	5.7–33.2 (14)	5.1–14.4 (11)	5.2–17.5(9)
		Only dehydrated potatoes products		10.6 (1)	6.6–28.6 (2)	12.1–27.3 (2)	11.0 (1)	8.9 (1)
04.2.1	Dried fruit	Only dried tomatoes Only dried fruits, excluding dried apples, pears, bananas, apricots, peaches, grapes, prunes and figs Only nuts in shell nuts Only dried apples Only dried pears Only dried bananas Only dried apricots Only dried grapes Only dried prunes Only dried figs	36.3–46.4 (3)	7.6–47.0 (6)	11.1–18.9 (3)	5.8–8.7 (2)	5.5–8.9 (3)	5.0–18.5 (10)
07.2	Fine bakery wares	Only dry biscuits	9.8 (1)	5.6 (1)				
08.2	Meat preparations as defined by Regulation (EC) No 853/2004	Only breakfast sausages; burger meat with a minimum vegetable and/or cereal content of 4% mixed within the meat	10.4–29.6 (4)	7.8–39.2 (10)	10.6–57.4 (18)	6.5–55.8 (17)	11.5–58.9 (17)	7.3–44.1 (14)

		Only salsicha fresca, longaniza fresca and butifarra fresca						
09.1.2	Unprocessed molluscs and crustaceans							
09.2	Processed fish and fishery products, including molluscs and crustaceans					6.0–6.4 (2)		
11.1	Sugars and syrups as defined by Directive 2001/111/EC[6]	Only sugars, except glucose syrup	5. 5 (1)					
14.1.4	Flavoured drinks	Only carry-over from concentrates in non-alcoholic flavoured drinks containing fruit juice Only non-alcoholic flavoured drinks containing at least 235 g/L glucose syrup Only other concentrates based on fruit juice or comminuted fruit; capilé, groselha	8.5 (1)	6.1–14.4 (5)	5.2–27.8 (14)	5.1–30.23 (14)	5.0–11.2 (9)	5.5 (1)
14.1.2	Fruit juices as defined by Directive 2001/112/EC[14] and vegetable juices	Fruit juice, not specified Only orange Only grapefruit Only apple Only pineapple Only lemon Only lime	10.4–37.7 (3)	5.2–47.2 (9)	6.1–54.8 (17)	7.1–37.1 (15)	5.4–18.2 (12)	5.1–13.0 (7)
14.2.1	Beer and malt beverages						5.1–12.7 (9)	5.0–7.2 (3)
14.2.3	Cider and perry						6.4–12.8 (3)	

Wine[(a)]		10.6–46.0 (16)	11.8–57.7 (14)
Liqueur wines [(a)]	Fortified and liqueur wines liqueurs		5.3 (1)

(a) Food categories authorised according to Annex IB to Regulation (EC) No 606/2009

H. Main food categories contributing to exposure to sulfur dioxide–sulfites (E 220–228) from their use as food additives (> 5% to the total mean exposure) and number of surveys in which the food category contributed in the 'brand-loyal refined exposure scenario' considering dataset 1[(a)].

Food category number	Food category description	Specifications	Min–max % of contribution (n surveys)					
			Infants	Toddlers	Other children	Adolescents	Adults	Elderly
04.1.2	Peeled, cut and shredded fruit and vegetables	Only garlic Only onions	9.4–40.2 (3)	7.7–27.4 (2)	16.3 (1)		6.3–14.2 (3)	19.8 (1)
04.2.1	Dried fruit	Only dried tomatoes Only dried fruits, excluding dried apples, pears, bananas, apricots, peaches, grapes, prunes and figs Only nuts in shell nuts Only dried apples Only dried pears Only dried bananas Only dried apricots Only dried grapes Only dried prunes Only dried figs	7.0–51.6 (4)	10–26.5 (4)	5.1–7.9 (2)		8.0 (1)	5.1–17.9 (6)
04.2.6	Processed potato products	Except dehydrated potatoes	9.0–58.4 (5)	7.9–65.4 (8)	5.1–46.3 (11)	5.4–40.1 (9)	7.7–8.9 (3)	6.0–10.7 (4)
		Only dehydrated potatoes products			14.2 (1)	14.2 (1)		
07.2	Fine bakery wares	Only dry biscuits	14.1 (1)		5.1 (1)			
08.2	Meat preparations as defined by Regulation (EC) No 853/2004	Only breakfast sausages; burger meat with a minimum vegetable and/or cereal content of 4% mixed within the meat	20.9–33.3 (4)	9.8–56.8 (10)	25.8–82.4 (18)	12.7–81.2 (17)	10.1–77.8 (17)	5.1–53.2 (14)

		Only salsicha fresca, longaniza fresca and butifarra fresca						
09.1.2	Unprocessed molluscs and crustaceans							
09.2	Processed fish and fishery products, including molluscs and crustaceans					7.8 (1)		
14.1.4	Flavoured drinks	Only carry-over from concentrates in non-alcoholic flavoured drinks containing fruit juice Only non-alcoholic flavoured drinks containing at least 235 g/L glucose syrup	5.5–19.5 (2)	6.9–39.1 (6)	5.3–49.9 (15)	7.3–44.4 (14)	5.9–14.7 (5)	
14.1.2	Fruit juices as defined by Directive 2001/112/EC[14] and vegetable juices	Fruit juice, not specified Only orange Only grapefruit Only apple Only pineapple Only lemon Only lime	9.2–37.6 (2)	5.1–50.2 (5)	5.1–56.5 (6)	6.0–13.3 (3)		
14.2.3	Cider and perry						8.2–16.2 (3)	
	Wine[(b)]					5.1–6.6 (3)	6.8–75.2 (17)	24.1–84.8 (14)

(a) Dataset 1 includes reported use levels and analytical results (not exceeding the MPLs) for food categories for which direct addition of sulfur dioxide–sulfites (E 220–228) is authorised, according to Annex II to Regulation (EC) No 1333/2008 and Annex IB to Regulation (EC) 606/2009.

(b) Food categories authorised according to Annex IB to Regulation (EC) No 606/2009.

I. Main food categories contributing to exposure to sulfur dioxide–sulfites (E 220–228) from their use as food additives (> 5% to the total mean exposure) and number of surveys in which the food category is contributing in the 'non-brand-loyal refined exposure scenario' considering dataset 1[(a)]

Food category number	Food category description	Specifications	Min–max % of contribution (n surveys)					
			Infants	Toddlers	Other children	Adolescents	Adults	Elderly
04.1.2	Peeled, cut and shredded fruit and vegetables	Only garlic Only onions	10.6–20.8 (3)	5.5–13.1 (2)	8.4 (1)	5.5 (1)	5.6–10.4 (3)	12.4 (1)
04.2.1	Dried fruit	Only dried tomatoes Only dried fruits, excluding dried apples, pears, bananas, apricots, peaches, grapes, prunes and figs Only nuts in shell nuts Only dried apples Only dried pears Only dried bananas Only dried apricots Only dried grapes Only dried prunes Only dried figs	13.1–25.5 (3)	7.8–13.6(4)	6.7 (1)			5.3–10.5 (3)
04.2.6	Processed potato products	Except dehydrated potatoes	8.5–77.8 (5)	7.9–59.6 (8)	7.3–35.4 (11)	5.8–31.7 (10)	5.4–12.1 (6)	8.4–13.7 (4)
		Only dehydrated potatoes products			7.14–7.14 (1)	6.78–6.78 (1)		
07.2	Fine bakery wares	Only dry biscuits	8.7 (1)					

08.2	Meat preparations as defined by Regulation (EC) No 853/2004	Only breakfast sausages; burger meat with a minimum vegetable and/or cereal content of 4% mixed within the meat Only salsicha fresca, longaniza fresca and butifarra fresca	35.9–52.2 (4)	21.3–70 (10)	29.8–84.9 (18)	21.7–85.0 (17)	21.7–84.0 (17)	11.3–65.8 (14)
14.1.4	Flavoured drinks	Only carry-over from concentrates in non-alcoholic flavoured drinks containing fruit juice Only non-alcoholic flavoured drinks containing at least 235 g/L glucose syrup	7.0 (1)	5.3–12.1 (3)	5.0–18.6 (9)	6.0–16.8 (9)	6.2 (1)	
14.1.2	Fruit juices as defined by Directive 2001/112/EC[14] and vegetable juices	Fruit juice, not specified Only orange Only grapefruit Only apple Only pineapple Only lemon Only lime	16.0–24.3 (2)	5.2–44.3 (6)	5.5–49.6 (10)	5.1–29.8 (9)		
14.2.1	Beer and malt beverages						5.4 (1)	
14.2.3	Cider and perry						7.8–17.8 (3)	5.1 (1)
	Wine**					5.1 (1)	11.9–57.4 (16)	15.5-71.6(14)

(a) Dataset 1 includes reported use levels and analytical results (not exceeding the MPLs) for food categories for which direct addition of sulfur dioxide–sulfites (E 220–228) is authorised, according to Annex II to Regulation (EC) No 1333/2008 and Annex IB to Regulation (EC) 606/2009.
(b) Food categories authorised according to Annex IB to Regulation (EC) No 606/2009.

J. Main food categories contributing to exposure to sulfur dioxide–sulfites (E 220–228) from their use as food additives (> 5% to the total mean exposure) and number of surveys in which the food category is contributing in 'the brand-loyal refined exposure scenario' considering dataset 2[(a)]

Food category number	Food category description	Specifications	Min–max % of contribution (n surveys)					
			Infants	Toddlers	Other children	Adolescents	Adults	Elderly
04.1.2	Peeled, cut and shredded fruit and vegetables	Only garlic Only onions	5.1 (1)				5.8 (1)	7.7 (1)
04.2.1	Dried fruit	Only dried tomatoes Only dried fruits, excluding dried apples, pears, bananas, apricots, peaches, grapes, prunes and figs Only nuts in shell nuts Only dried apples Only dried pears Only dried bananas Only dried apricots Only dried grapes Only dried prunes Only dried figs	5.6–47.4 (4)	6.9–18.1 (4)				5–11.1 (4)
04.2.6	Processed potato products	Except dehydrated potatoes	23.4–43.2 (4)	5.4–47.4 (6)	7.3–18.4 (7)	7.3–19.6 (5)	5.9–5.9 (1)	7.6–7.6 (1)
05.2	Other confectionery including breath refreshening microsweets	Only glucose syrup-based confectionery (carry-over from the glucose syrup only)						
07.1	Bread and rolls		9.3–19.7 (4)	7.7–25.6 (8)	5.8–32.2 (16)	5.2–30 (16)	6.3–14.7 (15)	6.9–14 (12)
07.2	Fine bakery wares	Pastries and cakes		7.4–7.4 (1)	5.1–19.1 (10)	5–18.1 (9)	5–8.7 (2)	5.8–6.9 (3)
08	Meat	Only chicken meat	10–56 (4)	8.2–49.9 (7)	5.4–37 (16)	6.9–44.4 (16)	7.7–30.4 (15)	6.2–30.3 (11)

08.2	Meat preparations as defined by Regulation (EC) No 853/2004	Only breakfast sausages; burger meat with a minimum vegetable and/or cereal content of 4% mixed within the meat Only salsicha fresca, longaniza fresca and butifarra fresca	6.7–26.2 (4)	7.5–39.8 (10)	9.9–55.5 (18)	19.7–57.4 (16)	8.4–45.9 (17)	5.3–35.4 (13)
12.5	Soups and broths	Ready to eat soups	24.2 (1)					
14.1.2	Fruit juices as defined by Directive 2001/112/EC[14] and vegetable juices	Fruit juice, not specified Only orange Only grapefruit Only apple Only pineapple Only lemon Only lime	19.7 (1)	5.5–37.7 (4)	44.1 (1)	5.2 (1)		
14.1.4	Flavoured drinks	Only carry-over from concentrates in non-alcoholic flavoured drinks containing fruit juice Only non-alcoholic flavoured drinks containing at least 235 g/L glucose syrup	15.3 (1)	6.7–31.7 (4)	6–33.2 (10)	5–27.1 (11)	5.8–8.7 (2)	
14.2.1	Beer and malt beverages							
14.2.3	Cider and perry						5.2–11.1 (3)	
16	Desserts, excluding products covered in categories 1,3 and 4	Only ices and desserts	6.8 (1)	6.9 (1)				
	Wine[(b)]						13.9–62.5 (16)	14.5-74.7(14)

(a) Dataset 2 includes in addition to dataset 1, the available analytical data for foods categories which may contain sulfur dioxide–sulfites (E 220–228) 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.
(b) Food categories authorised according to Annex IB to Regulation (EC) No 606/2009.

K. Main food categories contributing to exposure to sulfur dioxide–sulfites (E 220–228) from their use as food additives (> 5% to the total mean exposure) and number of surveys in which the food category is contributing in the 'non-brand-loyal refined exposure scenario' considering dataset 2[(a)]

Food category number	Food category description	Specifications	Min–max % of contribution (n surveys)					
			Infants	Toddlers	Other children	Adolescents	Adults	Elderly
04.1	Unprocessed fruit and vegetables	Only cucumbers			5.3 (1)	6.2 (1)		
		Only pumpkins	15 (1)					
04.1.2	Peeled, cut and shredded fruit and vegetables	Only garlic Only onions	5.6–8.1 (3)	5.3 (1)			7.4 (1)	8.3 (1)
04.2.1	Dried fruit	Only dried tomatoes Only dried fruits, excluding dried apples, pears, bananas, apricots, peaches, grapes, prunes and figs Only nuts in shell nuts Only dried apples Only dried pears Only dried bananas Only dried apricots Only dried grapes Only dried prunes only dried figs	9.9–13.4 (3)	5.5–10.2 (4)				7 (1)
04.2.6	Processed potato products	Except dehydrated potatoes	21.6–58.5 (4)	5.1–41.9 (8)	6.1–17.5 (9)	7.2–17.2 (7)	5.4–9.9 (3)	5.6–11.9 (4)
05.2	Other confectionery, including breath refreshening microsweets	Only glucose syrup-based confectionery (carry-over from the glucose syrup only)						

06.3	Breakfast cereals	Only cereal flakes		5.2–5.2 (1)				
07.1	Bread and rolls		7.8–16.3 (4)	7–13.3 (8)	6–13.8 (16)	5.8–14.8 (16)	5.1–9.9 (17)	6.3–10.4 (13)
07.2	Fine bakery wares	Pastries and cakes	5.3–5.4 (2)	5.7–14.5 (7)	5.7–25.4 (15)	6.5–22.1 (14)	6.2–14.2 (11)	6.2–13.4 (10)
08	Meat	Only chicken meat	5.8–35.5 (5)	7.1–25.3 (6)	5.4–17.4 (12)	6.6–22.8 (13)	5.3–16.8 (16)	5.1–16.9 (8)
08.2	Meat preparations as defined by Regulation (EC) No 853/2004	Only breakfast sausages; burger meat with a minimum vegetable and/or cereal content of 4% mixed within the meat Only salsicha fresca, longaniza fresca and butifarra fresca	13.8–38.2 (4)	14.9–51.3 (10)	12.1–57.4 (18)	8.6–57.6 (17)	16.5–56.7 (17)	9–49.2 (14)
12.5	Soups and broths	Ready to eat soups	20.3 (1)	7.3 (1)	5.7–6.5 (2)	5.3 (1)	5.7 (1)	6.9 (1)
12.6	Sauces	Only savoury sauces	5.6 (1)			5.3–5.5 (2)		
14.1.2	Fruit juices as defined by Directive 2001/112/EC[14] and vegetable juices	Fruit juice, not specified Only orange Only grapefruit Only apple Only pineapple Only lemon Only lime	5.9–9.9 (2)	10.3–33.3 (3)	5.9–37.7 (4)	6.1–11.8 (3)		
14.1.4	Flavoured drinks	Only carry-over from concentrates in non-alcoholic flavoured drinks containing fruit juice Only non-alcoholic flavoured drinks containing at least 235 g/L glucose syrup	5.2 (1)	8.9 (1)	5.8–11.5 (5)	5.6–10.2 (7)		
14.2.3	Cider and perry						10–11.3 (2)	

17	Food supplements as defined in Directive 2002/46/EC	5.1–8.8 (2)	6 (1)	7.5–9.5 (2)
	Wine*		8.5–44.6 (16)	8.9–56.9 (14)

(a) Dataset 2 includes in addition to dataset 1, the available analytical data for foods categories which may contain sulfur dioxide–sulfites (E 220–228) 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.

L. Summary of the available *in vitro* and *in vivo* genotoxicity studies

Reliability (validity):

1. reliable without restriction (valid without restriction)
2. reliable with restrictions (valid with restrictions or limited validity)
3. insufficient reliability (insufficient validity)
4. reliability cannot be evaluated (validity cannot be evaluated)
5. reliability not evaluated since the study is not relevant and/or not required for the risk assessment

The reliability criteria are based on Klimisch et al. (1997) as recommended by the Scientific Committee in its scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment (EFSA Scientific Committee, 2011). The relevance of the study result is based on its reliability and on the relevance of the test system (genetic endpoint): high, limited or low.

Sodium sulfite

In vitro studies

Test System	Test Object	Concentration	Result	Reference	Reliability/Comments	Relevance of the test System	Relevance of the Result
Sex-linked recessive lethal mutations	*Drosophila*	0.04 and 0.08 mol/L	Negative	Valencia et al. (1973)	4	Low	Low
Ames test	*Salmonella* Typhimurium TA1535; TA1537; TA92; TA94; TA98; TA100	Preincubation method: Up to 5 mg/plate	Negative	Ishidate et al. (1984)	2 (Not all strains as recommended in OECD 471, results not reported in detail). Purity: 95%	High	Limited
Chromosomal aberration assay	CHL cells	Up to 0.5 mg/ml	Negative	Ishidate et al. (1984)	2 (Tested only in the absence of S9, results not reported in detail). Purity: 95%	High	Limited
Ames test	*S.* Typhimurium TA1535; TA1537; TA98; TA100	Plate incorporation and preincubation method: Up to 5 mg/plate	Negative	BASF (1989a)	2 (Not all strains as recommended in OECD 471). Purity: 96–98%	High	Limited
Gene mutation assay (*gpt* locus)	AS52 cells	5 and 10 mM	Inconclusive	Meng and Zhang (1999)	2 (Purity not reported) Statistically significant increases in mutant frequency were accompanied by cytotoxicity at both concentrations	High	Low
Rec assay	*Bacillus subtilis* strain M45rec$^-$ and wild type strain H17rec$^+$	5 mg/plate	positive	Ueno et al. (2002)	3 (Only a single concentration tested)	Limited	Low

In vivo studies

Test System	Test Object	Route	Dose	Result	Reference	Reliability/Comments	Relevance of the Test System	Relevance of the Result
Micronucleus test	Mouse (bone marrow)	Subcutan	250, 500 and 1000 mg/kg bw	Negative	BASF (2008) (= Schulz and Landsiedel 2008)	1 (Route of administration not justified, otherwise consistent with OECD 474). Marked reduction of PCE/NCE ratio indicated that the bone marrow was exposed. Purity: 98.1%	High	High
Comet assay	Mouse (brain, lung, heart, liver, stomach, spleen, thymus, bone marrow and kidney)	i.p.	125, 250 or 500 mg/kg bw daily for 7 days	Positive	Meng et al. (2004)	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. Source of test substance is reported but the purity is not reported	High	Low

Sodium bisulfite

In vitro studies

Test System	Test Object	Concentration	Result	Reference	Reliability / Comments	Relevance of the Test System	Relevance of the Result
Rec assay	*Bacillus subtilis*	Up to 400 ppm	Negative	Khoudokormoff et al. (1978)	4 (Conference Proceeding Abstract)	Limited	Low
SCE test	CHO cells	Up to 7.3 mM	Inconclusive	MacRae and Stich (1979)	3 (pH and osmotic activity not measured) The positive results described might be due to non-physiological treatment conditions	Low	Low
Gene mutation assay	*E. coli* WP2 (wild-type for DNA repair) and $WP2_s$ (uvrA), WP6 (polA), WP5 (lexA) and WP10 (recA)	Up to 100 mM for 15 min	Negative	Mallon and Rossman (1981)	2 (Deviations from OECD TG 471, i.e. reporting deficiencies, not all strains used as currently recommended; purity ('reagent grade $NaHSO_3$') not numerically reported)	High	Limited
Gene mutation assay (ouabain resistance)	Chinese hamsters V79 cells	10 and 20 mM for 15 min, 1 and 5 mM for 48 h	Negative		2 (Reporting deficiencies, i.e. methods only briefly described, purity ('reagent grade $NaHSO_3$') not numerically reported)	High	Limited
Gene mutation assay	*S.* Typhimurium *his*G46, TA92, TA1950, TA2410, TS24 and GW19 *S.* Typhimurium *his*G46	Preincubation method: up to 2 M (equal to 0.2 mmol/plate) Plate incorporation method: (probably up to 0.2 mmol/plate)	Positive Negative	De Giovanni-Donnelly (1985)	2 (Deviations from OECD 471 with respect to bacterial strains, purity not reported, result obtained with positive control not reported, results obtained with the plate incorporation method were not reported in detail)	High	Limited
Gene mutation assay	*S.* Typhimurium TA88, TA110, TA97, SB2802, TA92;	Preincubation method: up to 0.3 M (probably equal to 0.15 mmol/plate)	Positive	Pagano and Zeiger (1987)	2 (Study focused mainly on mode of action, deviations from OECD 471, e.g. identity of the test substance (sodium bisulfite or	High	Limited

	S. Typhimurium TA1535; TA100; TA90; TA1538; TA98; TA1537; TA1977		Negative		sodium metabisulfite) not fully clear, purity not reported, no positive control)		
Sister chromatid exchange (SCE) test	Hamster fetal cells	Up to 20 mM	Positive	Popescu and DiPaolo (1988)	3 (The effects were observed at a concentration of 20 mM which is above the physiological limits of 10 mM for *in vitro* tests)	Low	Low
Chromosomal aberration assay	Hamster fetal cells	Up to 20 mM	Negative	Popescu and DiPaolo (1988)	2 (Increased frequencies of chromosomal aberrations were observed at a concentration of 20 mM which is above the physiological limits of 10 mM for *in vitro* tests)	High	Limited
Gene mutation assay	Syrian hamster embryo cells	Up to 5 mM	Negative	Tsutsui and Barrett (1990)	2 (Reporting deficiencies, e.g. purity not reported)	High	Limited
Chromosomal aberration assay	Syrian hamster embryo cells	Up to 5 mM	Negative	Tsutsui and Barrett (1990)	2 (Reporting deficiencies, e.g. purity not reported)	High	Limited
SCE test	Syrian hamster embryo cells	Up to 5 mM	Positive	Tsutsui and Barrett (1990)	2 (Reporting deficiencies, e.g. purity not reported), (Effects might be due to cytotoxicity; pH and osmolality were not measured)	Low	Low
Ames test	*S.* Typhimurium TA1535; TA1537; TA98; TA100	Up to 10 µl/plate	Negative	Bayer (1988)	2 (Not all strains as recommended in OECD 471)	High	Limited
Gene mutation assay (*gpt* locus)	AS52 cells	5 and 10 mM	Positive	Meng and Zhang (1999)	3 (Effects were accompanied by cytotoxicity) Source of test substance is reported but the purity is not reported	High	Low
Chromosomal aberration assay	Human lymphocytes	Up to 2 mM	Positive	Meng and Zhang (1992)	3 (Reporting deficiencies and deviations from OECD guideline 473, e.g. source and purity of sodium bisulfite not reported, treatment and sampling times not clearly reported, only 200 metaphases scored instead of 300, tested only in the absence of S9, pH and osmolality not measured)	High	Low

Micronucleus test			Positive		3 (Reporting deficiencies and deviations from OECD guideline 487, e.g. source and purity of sodium bisulfite not reported, treatment and sampling times not clearly reported, tested only in the absence of S9, pH and osmolality not measured)	High	Low
SCE test			Positive		3 (Reporting deficiencies, e.g. source and purity of sodium bisulfite not reported, treatment and sampling times not clearly reported, tested only in the absence of S9, pH and osmolality not measured)	Low	Low

In vivo studies

Test System	**Test Object**	**Route**	**Dose**	**Result**	**Reference**	**Reliability / Comments**	**Relevance of the Test System**	**Relevance of the Result**
Dominant lethal and heritable translocations assay	Male mice (germ cells)	i.p.	300 and 400 mg/kg bw per day 20 times over a period of 26 days	Negative	Generoso et al. (1978)	3 (Reporting deficiencies, source and purity of sodium bisulfite not reported, not clear if target tissue was exposed, no positive control)	High	Low
Dominant lethal assay	Female mice (germ cells)	i.p.	550 mg/kg bw	Negative	Generoso et al. (1978)	3 (Reporting deficiencies, source and purity of sodium bisulfite not reported, not clear if target tissue was exposed, no positive control)	High	Low
Comet assay	Mouse (brain, lung, heart, liver, stomach, spleen, thymus, bone marrow and kidney)	i.p.	125, 250 or 500 mg/kg bw daily for 7 days	Positive	Meng et al. (2004)	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.	High	Low

Sodium and potassium metabisulfite

In vitro studies

Test System	Test Object	Concentration	Result	Reference	Reliability / Comments	Relevance of the Test System	Relevance of the Result
Chromosomal aberration assay	Chinese hamster (Don) cells	0.1, 0.5 and 1 mM	Negative	Abe and Sasaki (1977)	3 (Major deviations from current standards, e.g. tested only in the absence of S9; Cytotoxicity not reported; only 100 cells analysed per concentration)	High	Low
SCE assay	Chinese hamster (Don) cells	0.1, 0.5 and 1 mM	Equivocal	Abe and Sasaki (1977)	3 (Major deviations from current standards, e.g. tested only in the absence of S9; Cytotoxicity not reported; only 20–50 cells analysed per concentration)	Low	Low
Chromosomal aberration assay	Chinese hamster lung (CHL) cells	0.6 mM	Negative	Ishidate and Odashima (1977)	3 (Tested only in the absence of metabolic activation)	High	Low
Ames test	*S.* Typhimurium TA1535; TA1537; TA92; TA94; TA98; TA100	Up to 3 mg/plate	Negative	Ishidate et al. (1984)	2 (Not all strains as recommended in OECD 471, results not reported in detail) Potassium metabisulfite 93% purity	High	Limited
Chromosomal aberration assay	Chinese hamster lung (CHL) cells	Up to 0.06 mg/ml	Negative	Ishidate et al. (1984)	2 (Tested only in the absence of metabolic activation, results not reported in detail) Potassium metabisulfite 93% purity	High	Limited
Ames test	*S.* Typhimurium TA1535; TA1537; TA98; TA100	Plate incorporation and preincubation method: Up to 5 mg/plate	Negative	BASF (1989b), BASF (1989c)	2 (Not all strains as recommended in OECD 471) Purity: 97–98%	High	Limited

Ames test	*S.* Typhimurium TA1535; TA1537; TA1538; TA98; TA100 and *E. coli* WP2	Up to 10 mg/plate	Negative	Prival et al. (1991)	2 (Only the standard plate incorporation method but not the preincubation method applied). This is a relevant limitation since De Giovanni-Donnelly (1985) reported a negative result using the plate incubation method but a positive result using the preincubation method	High	Limited
Chromosomal aberration assay	Human lymphocytes	75, 150 and 300 µg/ml	Positive	Recuzogullari et al. (2001)	1 Cytotoxic at the highest concentration but weakly positive (up to 2.4-fold compared to negative control) also at non-cytotoxic concentrations. Purity was not reported, however, the source was reported and it seems reasonable to assume that the purity was high	High	Limited
SCE assay	Human lymphocytes	75, 150 and 300 µg/ml	Positive		1 Cytotoxic at the highest concentration but weakly positive also at non-cytotoxic concentrations	Low	Low
Chromosomal aberration assay	Human lymphocytes	25, 50, 100 and 200 µg/ml	Positive	Yavuz-Kocaman et al., (2008)	1 Cytotoxic at the two highest concentrations but positive (11.25% aberrant cells vs 2.5% in the negative control) also at the two lower concentrations which are only moderately cytotoxic. Purity of E 224: 99.9%	High	Limited
Micronucleus assay	Human lymphocytes	25, 50, 100 and 200 µg/ml	Positive		1 Moderately cytotoxic at the highest concentration but positive (up to 1.46% micronucleated cells vs 0.55% in the negative control) also at the three lower concentrations which were not cytotoxic. Purity of E 224: 99.9%	High	Limited

SCE assay	Human lymphocytes	25, 50, 100 and 200 µg/ml	Positive		1 Cytotoxic at the highest concentration but positive (11.09 SCE/cell vs 5.83 SCE/cell in the negative control) also at the three lower concentrations which are only weakly cytotoxic. Purity of E 224: 99.9%	Low	Low
Gene mutation assay (HPRT locus, 6TG resistance)	Mouse lymphoma cells	Up to 1902 µg/ml (equal to 10 mM)	Negative	Covance (2010) [Doc. provided to EFSA n. 21]	1	High	High

In vivo studies

Test System	**Test Object**	**Test substance**	**Route**	**Dose**	**Result**	**Reference**	**Reliability / Comments**	**Relevance of the Test System**	**Relevance of the Result**
Chromosomal aberration assay	NMR1 mice (wild type)	Sodium metabisulfite	Twice oral gavage	2 x 660 mg/kg bw	Negative	Renner and Wever (1983)	2 (Not clear if the bone marrow was exposed)	High	Limited
	NMR1 mice (SOX-deficient)		Twice oral gavage	2 x 165 mg/kg bw	Negative				
	Chinese hamsters (wild type)		Twice oral gavage	2 x 660 mg/kg bw	Negative				
	Chinese hamsters (SOX-deficient)		Twice oral gavage	2 x 330 mg/kg bw	Negative				
Micronucleus assay	NMR1 mice (wild type) bone marrow		Twice oral gavage	660 mg/kg bw	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)	High	Low
	NMR1 mice (SOX-deficient) bone marrow		Oral gavage	2 x 165 mg/kg bw	Negative				
	Chinese hamsters (wild type) bone marrow		Twice oral gavage	660 mg/kg bw	Negative				
	Chinese hamsters (SOX-deficient)		Oral gavage	2 x 330 mg/kg bw	Negative				

	bone marrow								
SCE assay	NMR1 mice (wild type)		Single oral gavage	660 mg/kg bw	Negative		2 (Not clear if the bone marrow was exposed)	Low	Low
			12 Subcutaneous injections at 20 min intervals	12 x 50 mg/kg bw	Negative				
	NMR1 mice (SOX-deficient)		Single oral gavage	165 mg/kg bw	Negative				
			8 Subcutaneous injections at 20 min intervals	8 x 50 mg/kg bw	Negative				
	Chinese hamsters (wild type)		Single oral gavage	660 mg/kg bw	Negative				
			12 Subcutaneous injections at 20 min intervals	12 x 50 mg/kg bw	Negative				
	Chinese hamsters (SOX-deficient)		Single oral gavage	330 mg/kg bw	Negative				
			8 Subcutaneous injections at 20 min intervals	8 x 50 mg/kg bw	Negative				
Chromosomal aberration assay	Mouse bone marrow	Sodium metabisulfite	Single i.p.	200, 300 and 400 mg/kg bw	Positive	Pal and Bhunya (1992)	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	High	Low
			Subcutaneous	400 mg/kg bw	Positive				
			Oral	400 mg/kg bw	Negative				
Micronucleus assay	Mouse bone marrow	Sodium metabisulfite	Twice i.p.	200, 300 and 400 mg/kg bw	Negative		3 (Major deviations from OECD TG 474) Purity not reported. The negative result obtained after twice i.p.	High	Low

							administration is not consistent with the positive result obtained in the chromosomal aberration assay after single i.p. administration		
Chromosomal aberration assay	Rat bone marrow	Potassium metabisulfite	Single i.p.	150, 300 and 600 mg/kg bw	Positive	Yavuz-Kocaman et al., (2008)	2/ (Only two animals per sex and dose, historical control data not reported and not tested at 36–42 h after treatment, however, the latter would only be a limitation for negative results) Moderately cytotoxic at the highest dose (MI = 51% and 68% at 12 and 24 h, respectively, compared to negative control) but positive (13.00% and 13.25% aberrant cells at the mid dose at 12 and 24 h, respectively, vs 5.50% in the negative control group) also at the two lower doses which were non-cytotoxic (MI = 67% and 81% at the mid-dose at 12 and 24 h, respectively, compared to negative control). The increased frequencies in chromosomal aberrations were statistically significant at all doses. Purity of E 224: 99.9%	High	Limited
Comet assay	Mouse (liver, blood and bone marrow)	Sodium metabisulfite	Oral gavage	0.5, 1 and 2 g/kg bw	Positive	Carvalho et al. (2011a)	3 (Samples were taken only at 24 h after treatment but not at 3–6 h; additionally,	High	Low

							the genotoxicity was investigated based on a 'damage index' which is uncommon and not validated and 'clouds' and 'halos' were not investigated)		
Micronucleus assay	Mouse bone marrow Mouse peripheral blood	Sodium metabisulfite	Oral gavage	0.5, 1 and 2 g/kg bw	Positive 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)	High	Low
Chromosomal aberration assay Micronucleus assay	*Allium cepa*	Water and sediment samples containing sodium metabisulfite	(Not applicable; plant study)	Sample dilutions of 50%, 25% and 10%	Negative Negative	Carvalho et al. (2011b)	5 (Reliability not evaluated since this study is not required for the risk assessment)	Low	Low

Sulfur dioxide

In vitro studies

Test System	Test Object	Concentration	Result	Reference	Reliability / Comments	Relevance of the Test System	Relevance of the Result
Micronucleus assay	Human lymphocytes	0.1, 0.5 and 1 ppm	Positive	Uren et al. (2014)	3 (Reporting deficiencies, i.e. not clear if 'MN median' is micronuclei per 1,000 cells or % micronucleated cells; tested only in the absence of S9). Cytotoxic at the highest concentration	High	Low
SCE assay			Positive		3 (The positive control did not show a clearly positive result; reporting deficiencies, i.e. not clear if 'SCE median' is SCEs per cell or per 1,000 cells or % SCEs; tested only in the absence of S9)	Low	Low
Cytogenetics	Human lymphocytes	'Single exposure to 100 cc of 5.7 ppm SO_2 in air by bubbling the gas through the culture medium at either 0, 1, 2 or 3 days of incubation'	'Chromosome abnormalities mainly in the form of clumping.' 'Only one chromosome break was observed, this from an air-treated culture.'	Schneider and Calkins (1970)	3 (The effects were not evaluated according to current criteria established for the evaluation of chromosomal aberrations)	Low	Low

In vivo studies

Test System	Test Object	Route	Dose	Result	Reference	Reliability / Comments	Relevance of the Test System	Relevance of the Result
Micronucleus assay	Mouse bone marrow	Inhalation	Up to 84 mg/m^3	Positive	Meng et al. (2002)	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)	High	Limited to Low
Chromosomal aberration assay	Mouse bone marrow	Inhalation	Up to 56 mg/m^3 for 4 h/day for 7 days	Weak positive	Meng and Zhang (2002)	2–3 (No positive control group; the statistical method does not appear to be appropriate; historical control data were not reported)	High	Limited to Low
			14 mg/m^3 for 1, 3, 5 and 7 days	Weak positive				
Comet assay	Mouse (brain, lung, heart, liver, stomach, spleen, thymus, bone marrow and kidney)	Inhalation	Up to 112 mg/m^3 for 6 h/day for 7 days	Positive	Meng et al. (2005)	2–3 (No concurrent positive control; only 50 cells per animal; historical control data were not reported)	High	Limited to Low
Micronucleus assay	Mouse bone marrow	Inhalation	Up to 80 mg/m^3 for 4 h/day for 7 days	Negative	Ziemann et al. (2010)	1–2 (Maximum dose not justified; bone marrow exposure not directly demonstrated, there are only data on oxidative stress indirectly indicating that the bone marrow might have been exposed)	High	High to Limited

Chromosomal aberration assay	Humans (workers exposed to sulfur dioxide)	Inhalation	The SO_2 concentrations in the workplace ranged from 0.34 to 11.97 mg/m^3 air.	Positive	Meng and Zhang (1990b)	3 (Exposure data were only based on a range of SO_2 in air)	High	Low
Micronucleus assay	Humans (workers exposed to sulfur dioxide)	Inhalation	The SO_2 concentrations in the workplace ranged from 0.34 to 11.97 mg/m^3 air.	(To be checked) Positive	Meng and Zhang (1990a)	3 (Exposure data were only based on a range of SO_2 in air)	High	Low
Chromosomal aberration assay	Humans (workers exposed to sulfur dioxide)	Inhalation	Variable concentrations between 0.2 and 3 ppm in air	Negative	Sorsa et al. (1982)	3 (Exposure data were only based on a range of SO_2 in air; only smoking considered as possible confounder)	High	Low
SCE assay				Negative			Low	Low
DNA-protein crosslinks	Mouse (lung, liver, heart)	Inhalation	0, 14, 28 and 56 mg/m^3 for 6 h/day for 7 days	Positive	Xie et al. (2007)	2 (Method not validated, no historical control data reported)	Low	Low
Chromosomal aberration assay	Humans (workers exposed to sulfur dioxide)	Inhalation	Average concentration of 41.7 mg/m^3	Positive	Yadav and Kaushik (1996)	3 (Reporting deficiencies, only 100 metaphases per person analysed)	High	Low
SCE assay				Positive		3 (Reporting deficiencies, only 25 metaphases per person analysed)	Low	Low
Chromosomal aberration assay	Humans (workers exposed to sulfur dioxide)	Inhalation	Not reported	Positive	Nordenson et al. (1980)	3 (No data on exposure to sulfur dioxide reported. 19 workers and 15 controls. Results reported as chromosomal aberrations per cell but not as percent cells with chromosomal aberrations. In most cases, about 100 metaphases per person analysed but in some cases less than 100.	High	Low

M. Other studies

Neurotoxicity

In vitro studies

Human neuroblastoma cells were exposed to concentrations of sodium metabisulfite from 80 to 800 µM for 3 or 20 h (Seravalli and Lear, 1987). All concentrations of sodium metabisulfite tested were found to inhibit colony forming efficiency in a dose-dependent manner as compared to medium-treated controls.

Zhang et al. (2004) investigated the effects of sodium sulfite on rat brain mitochondria and Neuro-2a and PC12 cells and observed that micromolar concentrations of sulfite produced an increase in reactive oxygen species (ROS) in Madin–Darby canine kidney (MDCK) and opossum kidney cells. The sulfite-mediated oxidative stress was accompanied by a depletion of intracellular adenosine triphosphate (ATP), and the authors presented evidence that this was due to an inhibitory action of sulfite on mitochondrial glutamate dehydrogenase.

In a study with mouse neuronal cells (Dani et al., 2007), concentrations of 10 and 100 µM solutions of sodium metabisulfite were found to significantly increase neuronal death as evaluated by measuring the release of lactate dehydrogenase. According to the authors, cysteine S-sulfate, a metabolite of sulfites, and the production of oxygen and sulfur radicals could be involved but the mechanisms of sulfite toxicity remain largely not understood.

Effects on neurons have also been shown in rat dorsal root ganglion neurons *in vitro* (Nie et al., 2009). When neurons were exposed to different concentrations of sodium metabisulfite, the amplitudes of both transient outward potassium currents and delayed rectifier potassium currents increased in a concentration and voltage-dependant manner. The EC_{50} was found to be 28 µM. This result suggests that sodium metabisulfite might adjust pain sensitivity in dorsal root ganglion neurons through modulating potassium currents.

Grings M. et al. (2014) investigated the *in vitro* effects of sulfite and thiosulfate on rat brain mitochondria. Sulfite per se, but not thiosulfate, decreased respiratory control ratio in mitochondria respiring with glutamate plus malate. Sulfite inhibited the activities of glutamate and malate dehydrogenases. Sulfite also induced mitochondrial swelling and reduced mitochondrial membrane potential, $Ca^{(2+)}$ retention capacity. Ruthenium red, cyclosporine A and ADP prevented these alterations, supporting the involvement of mitochondrial permeability transition (MPT). The authors presumed that disturbance of mitochondrial energy homoeostasis and MPT induced by sulfite could be involved in the neuronal damage characteristic of SOX deficiency

Parmeggiani et al. (2015) evaluated the *in vitro* effects of sulfite and thiosulfate on glutamatergic neurotransmission and redox homoeostasis in rat cerebral cortex slices. One hour treatment of cerebral cortex slices with sulfite, but not thiosulfate, significantly decreased glutamate uptake. Thiosulfate inhibited glutamine synthetase (GS) activity, a pronounced trend towards GS inhibition induced by sulfite was also found. Sulfite, at the concentration of 10 µM, increased thiobarbituric acid-reactive substances and decreased glutathione concentrations after 1 h of exposure. In contrast, thiosulfate did not alter these parameters. At 500 µM, sulfite increased sulfhydryl group content in rat cerebral cortex slices and increased GSH levels in a medium containing GSSG and devoid of cortical slices, suggesting that sulfite reacts with disulfide bonds to generate sulfhydryl groups. The authors concluded that sulfite may impair glutamatergic neurotransmission and redox homoeostasis in cerebral cortex.

Takenami et al. (2015) reported that when examining the effects of sodium bisulfite with and without procaine on axonal transport in cultured mouse dorsal root ganglion neurons, sodium bisulfite resulted in a dose-dependent damage to the cell membrane and axonal transport. Sodium bisulfite at more than

1 mM caused cell membrane damage and complete inhibition of axonal transport, whereas 0.1 mM sodium bisulfite maintained axonal transport at 40% and 60% of control with intact cell membrane.

Animal studies

Rats

The effect of sulfite on the *N*-methyl-D-aspartate (NMDA) receptor in hippocampus of normal and sulfite oxidase (SOX)-deficient (SOXD) rats was studied by Öztürk et al. (2006). A total of 40 adult male Wistar albino rats were divided into two groups: SOX-competent (SOXC) and SOXD. The latter group was made deficient by administration of a low-molybdenum diet with concurrent addition of 200 ppm tungsten to their drinking water for at least 3 weeks in advance of sulfite dosing. Within each of the two groups, a further two groups of 10 animals each were formed: control and sodium metabisulfite-treated with 25 mg/kg bw per day in drinking water for 6 weeks. Expressed as SO_2 this would be equivalent to 17 mg SO_2/kg bw per day. No abnormal clinical signs of toxicity were seen in any of the experimental groups. A decrease of the expression of two NMDA receptor subunits by 80–90% as compared to control level from SOXC animals ($P < 0.0001$) was found. The SOXD control group showed a similar decrease.

Kucukatay et al. (2007) investigated the effects of ingested sulfite on hippocampus superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities in SOXC and SOXD rats. Hippocampus SOD, CAT and GPx activities were found to be significantly increased by sulfite treatment in SOXC groups. On the other hand, exposure to sulfite had no effect on antioxidant status in hippocampus of SOXD rats. The authors concluded that these results suggest that sulfite treatment may cause oxidative stress and SOXC animals can cope with this stress by elevating the level of antioxidant enzyme activity, whereas SOXD rats, which is an exaggerated model for the human situation, cannot handle the sulfite-dependant oxidative stress, the mechanism of which remains to be explained.

Kucukatay et al. (2008) investigated the possible effects of sodium metabisulfite treatment on spinal reflexes in anesthetised SOXC and SOXD male albino rats. The rats were divided into four groups: control group, sulfite group, SOXD group and SOXD + sulfite group. Rats in SOXD groups were made deficient in SOX by the administration of low-molybdenum diet with concurrent addition of 200 ppm tungsten to their drinking water. Sodium metabisulfite, 70 mg/kg bw was given orally by adding to drinking water for 6 weeks to the sulfite and SOXD + sulfite groups. Monosynaptic reflex potentials were recorded from the ipsilateral L5 ventral root. SOXD rats had an approximately 15-fold decrease in hepatic SOX activity compared with normal rats. This makes SOX activity of SOXD group rats in the range of human SOX activity. Sulfite treatment statistically significantly ($P < 0.05$) increased the amplitude of the monosynaptic reflex response in both sulfite and SOXD + sulfite groups with respect to their respective control groups (control and SOXD groups). SOXD rats also had enhanced spinal reflexes when compared with control rats. The authors concluded that sulfite has increasing effects on the excitability of spinal reflexes and they speculated that sulfite may exhibit its effects on nervous system by affecting sodium channels.

Overall, several studies clearly 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 are needed before a clear conclusion on the possible neurotoxic effects of sulfites used as food additives can be reached.

Anti-vitamin B_1 effect of sulfites

It has been reported that thiamine is cleaved by sulfites into its inactive constituent compounds, pyrimidine and thiazole. Treatment of foods with sulfites reduced their thiamine content (Morgan et

al., 1935, as referred to by JECFA, 1987; Williams et al., 1935, as referred to by JECFA, 1987), and it has been suggested that the ingestion of SO_2 in a beverage may effectively reduce the level of thiamine in the rest of the diet (Hötzel, 1962, as referred to by JECFA, 1987). Bhagat and Lockett (1964) reported that 6 g metabisulfite/kg food (~3.4 g SO_2/kg food) destroyed the thiamine content in the diet to the extent that the diet cannot support the thiamine nutrition of animals.

As reported by JECFA (1987), six rats were given a diet providing 40 mg thiamine daily, and at weekly intervals, an additional 160 mg thiamine was given and the urinary excretion of thiamine measured on the following 2 days. When the response, in terms of urinary output of thiamine, appeared to be constant, 160 mg thiamine was given together with 120 mg potassium metabisulfite. It was found that the addition of SO_2 greatly reduced the urinary output of thiamine, especially on the day when both were given together (Causeret et al., 1965, as referred to by JECFA, 1987).

When sulfite preserved meat is fed alone or at the same time as a thiamine source (for example, commercial pet food or brewer's yeast), the thiamine in all the food is cleaved and a thiamine-deficient state can result. The extent of thiamine destruction increases linearly with the amount of sulfur dioxide in the meat. A level of 400 mg of sulfur dioxide/kg depletes thiamine by 55% and 1,000 mg/kg depletes it by 95%. Deactivation can also occur in the stomach and the majority of thiamine cleavage occurs within the first hour (Studdert and Labuc, 1991).

As reported by JECFA (1987), in wine containing 0.04% SO_2, 50% of the thiamine was destroyed in 1 week. However, no loss of thiamine was observed in 48 h. The authors concluded that the small amounts of SO_2 resulting from the recommended levels of usage in wine are not likely to inactivate the thiamine in the diet during the relatively short period of digestion (Jaulmes, 1965, as referred to by JECFA, 1987).

As also reported by JECFA (1987), in a series of studies, Hötzel et al. (1969, as referred to by JECFA, 1987) gave 400 mg/sulfite person per day to a group of subjects who were fed on a thiamine-deficient diet. The diet produced signs of vitamin deficiency in 50 days and the sulfite, dissolved in wine or grape juice was given between days 15 and 40. No effect on thiamine status was detected by measurement of blood thiamine levels, urinary thiamine excretion and by determination of thiamine-dependent enzyme activity. Clinical, neurophysiological and biochemical investigations produced no indication of adverse effects from sulfite.

The panel noted that there is a great variability between animal species in the sensitivity to the anti-vitamin B1 effect of sulfites; cats and dogs being highly sensitive. The only study in humans available to the Panel was conducted with doses of sulfites equivalent to 3.5 mg/kg bw per day (5 times the ADI) administered for 25 days to the subjects.

Nephrotoxicity

In vitro

Vincent et al (2004) reported an immediate increase in ROS in MDCK, type II, and opossum kidney cells that had been previously exposed to 5–500 µM/L sulfite. This was accompanied by a depletion of intracellular ATP, which according to the authors could be explained by the inhibitory effect of sulfite on mitochondrial glutamate dehydrogenase.

Animal studies

Akanji et al. (1993) studied the effect of chronic consumption of metabisulfite on the integrity of the rat kidney cellular system. Feeding of metabisulfite (5 mg/kg bw) to rats resulted in loss of alkaline phosphatase activities from the kidney. This was accompanied by a reduction in lactate dehydrogenase activity, which was noticed as a secondary reaction, taking place after five daily doses. The authors also reported an increase in alkaline phosphatase and a decrease in lactate dehydrogenase activities in the serum, as well as an increased urinary excretion of protein and alkaline phosphatase activity. The

authors concluded that the reported effects indicated that there is cellular damage to rat kidney as a result of chronic consumption of metabisulfite, and that the damage was primarily on the plasma membrane.

Human studies

Kajiyama et al (2000) reported that sera from patients with CRF contain significantly higher amounts of sulfite than those from healthy subjects. Mean ± SD of serum sulfite in healthy subjects (n = 20) was 1.55 ± 0.54 μM, whereas those in patients under maintenance haemodialysis (n = 44) and CRF patients before introducing dialysis therapy (n = 33) were 3.23 ± 1.02 μM($p < 0.01$) and 3.80 ± 3.32 μM($p < 0.01$), respectively. Multiple regression analysis revealed serum creatinine as the sole independent predictor of serum sulfite levels. Each haemodialysis treatment was associated with approximately 27% reduction in serum sulfite levels, suggesting the presence of a dialysable form in serum. The authors concluded that these results indicated that reduced glomerular filtration is a factor that determines serum sulfite levels, and that the chronic elevation in serum sulfite levels might contribute to tissue or organ dysfunction in patients with CRF.

Hepatotoxicity

SOX-inactivated rat hepatocytes were found to be highly susceptible to sulfites (Niknahad and O'Brien, 2008). Cultured hepatocytes were isolated from male Sprague–Dawley rats, which had been previously depleted in SOX-inactivated by putting them on a low-molybdenum diet and supplying them drinking water with 200 mg/L sodium tungstate for 2 weeks before isolation of hepatocytes. The cells were subsequently exposed to 0–10 mM sodium sulfite alone or in combination with different enzyme inhibitors, such as cyanide or azide. Sulfite was not toxic towards isolated rat hepatocytes even with concentrations as high as 30 mM, however, it was toxic in a dose- and incubation time-dependent manner towards hepatocytes treated with a non-toxic concentration of cyanide, an inhibitor of cytochrome a/a_3 which results in inactivation of sulfite oxidase. According to the authors, cytotoxicity of sulfite was mediated by free radicals as ROS formation increases by sulfite and antioxidants prevent its toxicity. Reaction of sulfite or its free radical metabolite with disulfide bonds of GSSG and GSH resulted in the compromise of GSH/GSSG antioxidant system leaving the cell susceptible to oxidative stress.

To examine the response of the p53 signalling pathway to stimulation with different concentrations of sulfite, a time course study of p53, Mdm2 and Bcl-2 expression was conducted in an immortalised hepatic cell line, HL-7702 (Bai et al., 2013). Although sulfite has been reported as an important risk factor for the initiation and progression of liver diseases due to oxidative damage, the expression of p53 and p-p53 (Ser15) remained unchanged. In addition, no obvious alterations in Mdm2 and Bcl-2 expression were observed in HL-7702 cells that had been stimulated with various concentrations of sulfite. The expression levels of caspase-3 and proliferating cell nuclear antigen (PCNA) were unchanged, but RIP1 expression was increased significantly after 24 h of exposure. Accordingly, the authors suggested that sulfite is cytotoxic to hepatocytes, but this cytotoxicity is not achieved by direct interruption of the p53 signalling pathway, and that an alternative necrotic process underlies hepatocellular death following sulfite exposure.

Potential roles of SH_2

The Panel noted that hydrogen sulfide (H_2S) and sulfites have close interactions and can be produced from each other.

According to Mitsuhashi et al. (2005) oxidative stress-dependent conversion of hydrogen sulfide to sulfite might occur *in vitro* and *in vivo*. Sulfite production from activated neutrophils stimulated with *N*-formyl-methionyl-leucyl-phenylalanine gradually increased with an increased concentration of sodium hydrosulfide in the medium. The production of sulfite was markedly suppressed with an NADPH oxidase inhibitor, diphenyleneiodonium. Serum concentrations of sulfite and sulfide were

investigated in an *in vivo* model of neutrophil activation induced by systemic injection of lipopolysaccharide (LPS) into rats. There was a significant increase in serum sulfite and sulfide after LPS injection. Co-administration of ascorbic acid with LPS further increased serum sulfide but suppressed sulfite levels.

Sulfate-reducing bacteria can produce hydrogen sulfide from ingested sulfites, and this depends on the type of bacteria present in the gastrointestinal tract, mainly in the colon. Hydrogen sulfide may be responsible for inflammation in the colon and toxicity to colonic epithelial cells. Colonic bacteria types are variable from one species to another, and sulfide generation in the colon is probably driven by dietary components, such as sulfur-containing amino acids and inorganic sulfur (e.g. sulfite) (Magee et al., 2000). The Panel noted that in a study on patients with ulcerative colitis, the same authors finally concluded that 'the evidence for hydrogen sulfide as a metabolic toxin in ulcerative colitis remain circonstential' (Pitcher et al. 2000).

The Panel also noted that recent publications reported that hydrogen sulfide may have a protective effect for Caco-2 cells against TNF α– and IFN γ-induced injury (Chen et al., 2015). The authors suggested that the suppression of MLCK-P-MLC signalling mediated by NF-kB P65 might be one of the mechanisms underlying the protective effect of hydrogen sulfide.

Hepatotoxicity

Norris et al (2013) suggested that the current understanding of the role of sulfide in the hepatic microcirculation is incomplete. Rather, a more complex role is likely in which sulfide acts as a vasodilatory in the presinusoidal resistance vessels and exerts a constrictor effect in the hepatic sinusoids, which may contribute to hepatic microcirculatory dysfunction during sepsis.

Overall, the Panel noted that the reported effects of hydrogen sulfide suggested that this compound might have various physiologic roles, which deserve consideration in the evaluation of sulfites, however, further research on the relationship between hydrogen sulfide and the use of sulfites as food additives are needed before a conclusion can be drawn on their beneficial or detrimental roles in modulating hydrogen sulfide activities.

Obesity and metabolic syndrome

In vitro

Ciardi et al (2012) investigated a potential influence of food additives on the release of leptin, IL-6 and nitrite in the presence of LPS in murine adipocytes. Leptin, IL-6 and nitrite concentrations were analysed in the supernatants of murine 3T3-L1 adipocytes after co-incubation with LPS and sodium sulfite for 24 h. In addition, the kinetics of leptin secretion was analysed. Sodium sulfite decreased leptin concentrations after 24 h of treatment, and increased, LPS-stimulated secretion of IL-6. Nitrite production was not influenced. According to the authors, decreased leptin release during the consumption of nutrition-derived food additives could decrease the amount of circulating leptin to which the central nervous system is exposed and may therefore contribute to an obesogenic environment. From the data obtained in the present *in vitro* study, however, it was unclear how food additives interfere in a complex system, such as the human organism, with regard to leptin metabolism. Therefore, the authors concluded that it is unclear to what extent any conclusion from the present *in vitro* study can be extrapolated to the *in vivo* situation, and clearly more studies are needed to investigate the potential contribution of diet-derived agents in a complex organism and a possible influence on the development of obesity.

Animal studies

Chassaing et al. (2014) reported that promotion of metabolic syndrome and local inflammation were not seen upon exposure of mice for 12 weeks to sodium sulfite (1% in drinking water, equivalent to 1,500 mg sodium sulfite/kg bw per day or approximately 500 mg equivalent SO2/kg bw per day, that

is more than 700 times the ADI).

The Panel noted that the effects reported in this study were not consistent with the effects reported *in vitro* by Ciardi et al (2012) and do not support, at least in mice, the hypothesis of an inflammatory effect of hydrogen sulfide derived from sulfite consumption in ulcerative colitis (Pitcher and Cummings, 1996).

ABBREVIATIONS

AAF	European Starch Industry Association
AAOC	Association of Official Agricultural Chemists
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
ANS Panel	EFSA Panel on Food Additives and Nutrient Sources added to Food
ANSES	French Agency for Food, Environmental and Occupational Health and Safety
ATP	adenosine triphosphate
ATSDR	Agency for Toxic Substances and Disease Registry
BMPA	British Meat Processors Association
BHR	bronchial hyperresponsiveness
bw	body weight
CAS	Chemical Abstract Service
CAT	catalase
CHL	Chinese hamster lung
CHO	Chinese hamster ovary
CONTAM	Scientific EFSA Panel on Contaminants in Food Chain
CRF	chronic renal failure
DSH	3-deoxy-4-sulfohexosulose
EC	European Commission
ECHA	European Chemicals Agency
EINECS	European Inventory of Existing Commercial chemical Substances
FAO	Food and Agriculture Organization of the United Nations
FCS	food categorisation system
FDA	Food and Drug Administration
FDE	FoodDrinkEurope
FDRL	Food and Drug Research Laboratories
FIA	flow injection analysis
FSANZ	Food Standards Australian New Zealand
FEF	forced expiratory flow
FEV	forced expiratory volume
GD	gestation day
GME	Gelatine Manufacturers of Europe
GPx	glutathione peroxidase
GS	glutamine synthetase
GSH	reduced glutathione

GSSG	oxidised glutathione
HFC	hamster fetal cells
HILIC	hydrophilic interaction liquid chromatography
HPRT	hypoxanthine-guanine phosphoribosyl transferase
HPLC	high-performance liquid chromatography
IgE	Immunoglobulin E
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC-MS/MS	liquid chromatography-mass spectrometry/ mass spectrometry
LD_{50}	median lethal dose
LOD	limit of detection
LOQ	limit of quantification
LPS	lipopolysaccharide
MB	medium-bound
MDCK	Madin–Darby canine kidney
MNNG	*N*-methyl-*N*'-nitro-*N*-nitrosoguanidine
MPL	maximum permitted level
MPT	mitochondrial permeability transition
MRM	multiple reaction monitoring
MTD	maximum tolerated dose
NADPH	micotinamide adenine dinucleotide phosphate
NCE	normochromatic erythrocytes
NDA Panel	EFSA Panel on Dietetic Products, Nutrition and Allergies
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
PCE	polychromatic erythrocytes
PCR	polymerase chain reaction
PCNA	proliferating cell nuclear antigen
PEF	peak expiratory flow
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RET	reticulocytes
ROS	reactive oxygen species
SCCNFP	Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers
SCE	sister chromatid exchange

SCF	Scientific Committee on Food
SHE	Syrian hamster embryo
SOD	superoxide dismutase
SOX	sulfite oxidase
SOXC	sulfite oxidase-competent
SOXD	sulfite oxidase-deficient
SRB	sulfate-reducing bacteria
TBARS	thiobarbituric acid reactive substances
TemaNord	Nordic Working Group on Food Toxicology and Risk Assessment
TNO	Netherlands Organization for Applied Scientific Research
UNESDA	Union of European Soft Drinks Associations
WHO	World Health Organization