Bundesministerium
Klimaschutz, Umwelt,
Energie, Mobilität,
Innovation und Technologie

SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Sodium dithionite

EC No 231-890-0

CAS No 7775-14-6

Evaluating Member State: Austria

Dated: December 2020

Evaluating Member State Competent Authority

Austrian MSCA:
Austrian Federal Ministry of Climate Action, Environment, Energy, Mobility, Innovation and Technology
Radetzkystraße 2,
1030 Vienna, Austria

The evaluation was performed by:
Umweltbundesamt GmbH
Spittelauer Lände 5
1090 Vienna, Austria
on behalf of the Austrian MSCA.

Contact:
Dr. Maximilian Kinzl
Umweltbundesamt GmbH
Department for Chemicals and Biocides
Spittelauer Lände 5
1090 Vienna, Austria
Tel:+43-(0)1-313-04/5655
Email: Stoffbewertung@umweltbundesamt.at

Year of evaluation in CoRAP: 2016

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

Sodium dithionite was originally selected for substance evaluation in order to clarify concerns about:

- Suspected carcinogenic
- Suspected sensitiser
- Exposure of workers
- Consumer use
- Wide dispersive use
- High (aggregated) tonnage

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

Table 1: Completed or ongoing processes

OTHER PROCESSES	MAN. SCREENING	RMOA	EVALUATION			AUTHORISATION		RESTRICTION	HARM C&L	PROCESS UNDER OTHER EU LEGISLATION		PREVIOUS LEGISLATION		STOCKHOLM CONVENTION
Identity			CCH	TPE	SEV	Candidate List	Annex XIV	Annex XVII	Annex VI (CLP)	PPP	BPR	NONS	RAR	POPs
Sodium dithionite EC 231-890-0	AT (2014)	-	2015, closed	-	eMSCA AT 2016-2019	-	-	-	-	-	-	-	-	-

A new CCH is proposed by eMSCA AT based on the outcome of SEV (2016-2019).

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State (eMSCA) to the following conclusions, as summarised in the table below.

Table 2:

CONCLUSION OF SUBSTANCE EVALUATION	
Conclusions	**Tick box**
Need for follow-up regulatory action at EU level	(X)
Harmonised Classification and Labelling	(X)
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	X

Based on the evaluation of available data e.g. registration dossier(s) and comments provided by the Registrant(s), concerns (e.g. on sensitisation, mutagenicity) could not be clarified fully by the eMSCA. A SEV draft decision containing data requests for clarifying the identified concerns had been submitted to the Registrants. Based on the comments and information received by the Registrant(s), eMSCA's understanding of data to be needed for conclusion has changed. The identified concerns are currently expected to be covered and clarified, (1) as soon as the discussion on harmonised classification for sulphur dioxide is finished including an agreement on Respiratory Sensitisation category 1 and (2) a compliance check (CCH) is performed for the endpoints related to the concerns and REACH-compliance is achieved for them.

No need for regulatory follow-up action at EU level is expected at this stage as long as these new data are not available for conclusion. Nevertheless, regulatory follow-up actions might be necessary as a next step based on the provided data and outcome.

SEV has been terminated, as this process is not expected to be required for requesting further testing after a CCH performed leading to REACH-compliance of the dossiers and probably the clarification of the identified concerns.

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

4.1.1. Harmonised Classification and Labelling

A CLH dossier for sodium dithionite might be proposed as follow-up regulatory action as soon as the data requested in CCH are available and the harmonised classification of sulphur dioxide has been published as RAC opinion.

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

Not applicable at this stage.

4.1.3. Restriction

Not applicable at this stage.

4.1.4. Other EU-wide regulatory risk management measures

Not applicable at this stage.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

Table 3:

REASON FOR REMOVED CONCERN The concern could be removed because	Tick box
Clarification of hazard properties/exposure with reservation of a CCH performed	X
Actions by the registrants to ensure safety, as reflected in the registration dossiers(e.g. change in supported uses, applied risk management measures, etc.)	

The identified concerns could not be clarified fully by the eMSCA during SEV. Nevertheless, they are considered to be clarified sufficiently for conclusion, as soon as (1) the CLH discussion for a read-across substance is finished including an agreement on Resp Sens category 1 and (2) the relevant endpoints are evaluated under CCH and data are requested for achieving REACH-compliance (see section 3). No regulatory follow-up foreseen at EU level is indicated at this stage. Further regulatory follow-up actions might be necessary in the future based on the data and outcome to be provided by the CCH performed as well the results of the harmonised classification and labelling of sulphur dioxide.

5.2. Other actions

Not applicable at this stage.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

A stepwise approach is proposed: waiting for the results of the CCH on the following endpoints: Skin sentisation and mutagenicity. Based on the results of the CCH and the outcome of the discussion on the harmonised classification of sulphur dioxide (read across substance), a harmonised classification might be proposed.

Table 4:

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
CCH	-	ECHA
CLH	-	Not agreed yet

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

Sodium dithionite was originally selected for substance evaluation in order to clarify concerns about:

- Suspected carcinogenic
- Suspected sensitiser
- Exposure of workers
- Consumer use
- Wide dispersive use
- High (aggregated) tonnage

Table 5: Evaluated endpoints HH and ENV

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Acute toxicity: oral	Classifies as acute toxic category 4, H302
Acute toxicity: inhalation	No classification
Acute toxicity: dermal	No classification
Narcotic effect	No classification
Skin irritation	No classification
Eye irritation	Classification as Eye Irrit 2 warranted*
Resp. tract irritation	Classification as STOT SE 3 warranted*
Skin Sensitisation	The available information is not conclusive for a final decision on the sensitising property of the substance. CCH to be initiated.
Respiratory sensitisation	The available information is not conclusive, wait for the outcome of CLH discussion on Sulphur dioxide, classification as Resp Sens is under discussion.
Repeated dose toxicity	Reevaluation after EFSA call
Mutagenicity	CCH to be initiated
Carcinogenicity	wait for the outcome of CCH on mutagenicity
Reproductive toxicity	No concern
Aquatic toxicity	No classification, wait for the outcome of CCH

* Note: not part of the present harmonised classification.

7.2 Procedure

Evaluation of sodium dithionite was launched in March 2016. The Registrant(s) of sodium dithionite were contacted before start of evaluation and asked to support the evaluation by providing the original studies used for the individual registrations. The Registrant(s) provided the studies. In a first step, the performed evaluation of sodium dithionite was not targeted and covered all sections of the chemical safety assessment. In a second step, the main focus of evaluation was on the areas of concern- identified prior and during evaluation. Studies provided by the Registrant(s), publicy available studies/data, studies for reference substances, QSARs and exposure modelling tools were used by the eMSCA for assessment and conclusion.

A SEV draft decision containing data requests for clarifying the concerns had been submitted to the Registrants in 2017. Based on the comments and information received by the Registrant(s), eMSCA's understanding of data to be needed for conclusion has changed. The identified concerns are currently expected to be probably covered sufficiently and clarified, if a CCH is performed for the endpoints related to the concerns and REACH-compliance is achieved for them. Therefore, a CCH is proposed as follow-up.

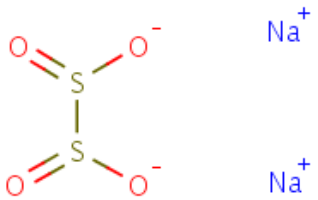
7.3. Identity of the substance

Table 6: Substance ID (ECHA dissemination site)

SUBSTANCE IDENTITY	
Public name:	Sodium dithionite
EC number:	231-890-0
CAS number:	7775-14-6
Index number in Annex VI of the CLP Regulation:	016-028-00-1
Molecular formula:	$H_2O_4S_2$ 2Na
Molecular weight range:	174.108
Synonyms:	*Disodium dithionate, Disodium dithionite, Dithionous acid, disodium salt, Sodium dithionate, Sodium hydrosulfite, Disodium hydrosulfite, Dithionous acid, Sodium sulfoxylate, Hydrosulfite R Conc, Blankit, V-Brite B, Vatrolite*

Type of substance ☒ Mono-constituent ☐ Multi-constituent ☐ UVCB

Structural formula:



7.4. Physico-chemical

Table 7: Data available on ECHA's dissemination web site based on registration data

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Solid Sodium dithionite is a white or grayish-white, crystalline powder with a slight characteristic odour.
Vapour pressure	Nonvolatile solid inorganic compound
Water solubility	Sodium dithionite has a water solubility of 18.61 g/100 mL solution (approximately 186.1 g/L) at 20.0 °C.
Partition coefficient n-octanol/water (Log Kow)	*Data waiving: Study does not to be conducted as the substance is inorganic*
Flammability	not highly flammable solid
Explosive properties	The test item has no explosive properties according to EC 440/2008, Part a, A.14.
Oxidising properties	This test item does not contain a surplus of oxygen or any structural groups known to be correlated with a tendency to react exothermally with combustible material It can be concluded that no oxygen is released. Therefore the substance is designated as non-oxidising substance.
Granulometry	The median particle size L50 of the test items deduced from the particle size distributions is 107.1 µm. The particle size L10 of the test items deduced from the particle size distributions is 13.9 µm. The particle size L90 of the test items deduced from the particle size distributions is 314.4 µm.
Stability in organic solvents and identity of relevant degradation products	*Data waiving: Study does not to be conducted as the substance is inorganic*

7.5. Manufacture and uses

7.5.1. Quantities

This substance is manufactured and/or imported in the European Economic Area in 100 000 - 1 000 000 tonnes per year.

Table 8: Aggregated tonnage (per year)

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☐ 10 – 100 t	☐ 100 – 1000 t	☐ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☒ 100,000 – 1,000,000 t			☐ Confidential

7.5.2. Overview of uses

The provided overview of uses is a summary of the information given on ECHA's dissemination web site (date: 14th July, 2020).

Referring to the latest and current information on registered uses, please follow the corresponding links of ECHA's dissemination website given in following table.

The substance is used in the following industries: food, mining/metal, paper/pulp for bleaching, pharmacy, photography, rubber/plastic and textile/leather. Furthermore, the substance is used for water and surface treatment. The substance is used industrially and professionally in these areas.

Following consumer uses have been registered: consumer use of textile cleaning products; consumer use of ink eraser containing sodium dithionite

Examples of technical function of the substance are:
reducing agent, food/feedstuff additive, bleaching agent, processing aid, plating agent and metal surface treating agent, pH-regulating agent

The substance is used for the manufacture of following articles: rubber articles, plastic articles, fabrics, textiles and apparel, leather articles, paper articles.

Table 9: Uses according ECHA dissemination site

USES	Use(s)
Uses as intermediate	---
Formulation	http://echa.europa.eu/de/registration-dossier/-/registered-dossier/15288/3/1/3
Uses at industrial sites	http://echa.europa.eu/de/registration-dossier/-/registered-dossier/15288/3/1/4
Uses by professional workers	http://echa.europa.eu/de/registration-dossier/-/registered-dossier/15288/3/1/5
Consumer Uses	http://echa.europa.eu/de/registration-dossier/-/registered-dossier/15288/3/1/6
Article service life	http://echa.europa.eu/de/registration-dossier/-/registered-dossier/15288/3/1/7

7.6. Classification and Labelling

Please find information on classification in C&L Inventory database on ECHA web site. The inventory includes both harmonised classification when available and the notified self-classifications.

http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database.

7.6.1. Harmonised Classification (Annex VI of CLP)

Table 10: Harmonized classification of sodium dithionite

HARMONISED CLASSIFICATION ACCORDING TO ANNEX VI OF CLP REGULATION (REGULATION (EC) 1272/2008)							
Index No	International Chemical Identification	EC No	CAS No	Classification		Spec. Conc. Limits, M-factors	Notes
				Hazard Class and Category Code(s)	Hazard statement code(s)		
016-028-00-1	Sodium dithionite	231-890-0	7775-14-6	Self-heat. 1 Acute Tox. 4*	H251 H302		

7.6.2. Self-classification

- In the registration(s)*: Not self-classified*
- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory*:*

Skin Irrit. 2, H315
Eye Irrit. 2, H319
Aquatic Chronic 3, H412

7.7. Environmental fate properties

7.7.1. Degradation

The substance is inorganic: Therefore no biodegradation can be expected. Nevertheless, the substance can undergo different reactions.

Sodium dithionite decomposes and disproportionates rapidly in aqueous media (especially under acidic conditions and under oxygen consumption). It has strongly reducing properties.
According to Hofmann and Rüdorff (1969) and Holleman and Wiberg (1995), this process can roughly be described by the following equations:

$2\ Na_2S_2O_4 + H_2O \rightarrow Na_2S_2O_3 + 2\ NaHSO_3$ (anaerobic conditions) (1)
$Na_2S_2O_4 + O_2 + H2O \rightarrow NaHSO_4 + NaHSO_3$ (aerobic conditions) (2)
Under aerobic conditions and with low concentrations, reaction (2) is favoured.

According to the literature overview of Münchow (1992) the following principal decomposition patterns can be described for dithionite in relation to pH ranges at temperatures between 0°C and 32°C for 0.0025 molar solutions:

- strongly alkaline medium: $3\ Na_2S_2O_4 + 6\ NaOH \rightarrow 5\ Na_2SO_3 + Na_2S + H_2O$
- weakly alkaline/acidic medium: $2\ Na_2S_2O_4 + H_2O \rightarrow 2\ NaHSO_3 + Na_2S_2O_3$
- acidic medium: $2\ H_2S_2O_4 \rightarrow 3\ SO_2 + S + 2\ H_2O$
- strongly acidic medium: $3\ H_2S_2O_4 \rightarrow 5\ SO_2 + H_2S + 2\ H_2O$

Referring to equations 1 and 2 (dissolution of sodium dithionite in weakly alkaline/acidic medium), the formation of hydrogen sulfite (HSO_3^-) and hydrogen sulfate (HSO_4^-) lowers the pH of the media and accelerates the process of decomposition strongly. Referring to work practice, to keep solutions of dithionite stable for several days, they need to be cooled, kept in an alkaline state by excess of NaOH and oxygen has to be excluded.
Sulfite and hydrogen sulfite anions are both in a pH-dependent equilibrium with gaseous SO_2:
$SO_2 + H_2O <> H+ + HSO_3^-$ $HSO_3^- + OH^- <> H_2O + SO_3^{2-}$

In the presence of oxygen, the sulfite anion may be further oxidized to sulfate:
$2\ Na_2SO_3 + O_2 \rightarrow 2\ Na_2SO_4$

Photolysis
Photodegradation of sodium dithionite in water is considered to be not relevant because it dissociates quickly and decomposes in water.

Summary: Sodium dithionite is hydrolytically unstable. The reaction is fast depending on temperature and pH. Depending on the pH-value, sulfur dioxide, sodium hydrogen sulfite, sodium sulphite, sodium thiosulfate and sodium sulfide are present in aqueous media. No quantitative information on the formed species is available.

7.7.2. Environmental distribution

Due to the high water solubility of the compounds involved as well as due to the polar nature of the hydrolysis products, the main compartment of dithionite and its conversion products is the hydrosphere.
Adsorption is expected to be low due to the low estimated Koc value of 1.7 according to a PCKOCWIN estimation (see unpublished study report, 2011) for sodium dithionite, although caution is necessary as estimated values for inorganic and ionic substances are quite uncertain using this model for prediction. Nevertheless, very small Koc values can be also expected for the hydrolysis products.
The substance or its decomposition products are not expected to be volatile – By an estimation of the HENRY constant (the substance is inorganic and outside the estimation domain) a value of 15.28 according HENRYWIN (v.3.2) bond estimation method is gained.

7.7.3. Bioaccumulation

No log Kow can be derived for inorganic substances like sodium dithionite. Nevertheless, due to its inherent physico-chemical properties like rapid hydrolysis, bioaccumulation is not expected.

No potential for secondary poisoning is identified.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

7.8.1.1. Fish

Based on data from an acute fish study according to DIN 38412, Part 15 (Draft January 1979) with *Leuciscus idus* an 96h-LC_{50}-value of 62.3 mg/L was identified for sodium dithionite (Unpublished study report, 1982). No data on analytical monitoring are available. The oxygen content in the beginning of the test was partly very low and inversely related to the rising test concentration. In a pre-test in which the fish were placed into the aquaria 1 h after preparation of the test solution the initial oxygen consumption was compensated by the continuous aeration and the concentration of 100 mg/L did not cause any mortalities or symptoms.

Taking into account the available evidence, it is concluded that the mortality seen in the main test could be due to serious oxygen depletion and not due to inherent toxicity.

An early life stage test with *Danio rerio* with the hydrolysis product sodium sulphite (Unpublished study report, 2010b) was provided without proper read across justification giving information that the read across substance is representative for sodium dithionite and its hydrolysis products. For the tested substance sodium sulphite no effects were seen at the highest tested concentration (316 mg/L). No analytical measurements of the test concentrations were performed, leading to a hampered validity of the test.

ECHA considered in its compliance check decision on the substance from 3. May 2016 (ECHA, 2016) that the read-across justification provided did not constitute a reliable basis for making a prediction of the properties of the registered substance, as sodium sulfite may only be one of the breakdown products of sodium dithionite besides sulphate and thiosulphate and no justification is provided why sulphite can be used to predict the properties of the parent substance. Moreover, ECHA concluded that the available results show that the proposed analogue substance has different properties from the registered substance. Hence, the Registrant was requested via the compliance check decision to submit a Fish, early-life stage (FELS) toxicity test (test method: OECD 210) until 10. May 2017. Up to finalisation of the SEV report this test was not available.

For assessment of potential endocrine effects in fish no data are available.

7.8.1.2. Aquatic invertebrates

In an acute *Daphnia magna* test according to Directive 79/831/EEC a 48h-EC_{50} value of 98.3mg/L (nominal) was observed (Unpublished study report, 1989a).

In a chronic static renewal test according to EEC Guidelnie XI/681/86, Draft 4, with *Daphnia magna* a nominal NOEC of > 10 mg/L (nominal) was obtained (Unpublished study report, 1994). No analytical monitoring was performed.

Although there are some deficiencies in the provided studies, it is conluded that there is no acute or chronic hazard for aquatic invertebrates.

7.8.1.3. Algae and aquatic plants

In an algae study with Scenedesmus subspicatus a 72h-EC50 value of 206 mg/L (nominal) for the growth rate, a NOEC of 62.5 mg/L (nominal) were obtained according to OECD SIDS, 2004 which is referring to reports from 1989b and 2004b.

It is concluded, that there is no notable acute or chronic hazard for algae and aquatic plants.

7.8.1.4. Sediment organisms

No data are presented in the registration dossier regarding sediment organisms.

7.8.1.5. Other aquatic organisms

No data on other aquatic organisms are available.

7.8.2. Terrestrial compartment

No data on terrestrial soil macroorganisms, terrestrial arthropods, terrestrial plants or soil microorganisms are available.

7.8.3. Microbiological activity in sewage treatment systems

In a recent OECD 209 test a 3h EC_{10} value of 89.8 mg/L was observed (Unpublished study report, 2010a). Additionally a growth inhibition test with *Pseudomonas putida* resulted in an EC_{50} value of 106.5 mg/L after 17h (Unpublished study report, 1988)

7.8.4. PNEC derivation and other hazard conclusions

The atmospheric compartment is under potential risk, as substantial SO_2 emissions might be the consequence of the use of sodium dithionite. The atmospheric compartment is not considered in the registration dossier.

Table 11:

PNEC DERIVATION AND OTHER HAZARD CONCLUSIONS		
Hazard assessment conclusion for the environment compartment	**Hazard conclusion**	**Remarks/Justification**
Freshwater	PNEC aqua (freshwater): 0.1 mg/L	Assessment factor: 100 as one long-term result with daphnia resulting in a NOEC > 10 mg/L and one long-term result with algae is available. Nevertheless these trophic levels do not cover the potentially most sensitive species from acute tests, which is fish. Caution is necessary as the NOECs are based on nominal values.
Marine water	PNEC aqua (marine water): 0.01 mg/L	Assessment factor: 1000 as one long-term result with daphnia resulting in a NOEC > 10 mg/L and one long-term result with algae is available. Nevertheless these trophic levels do not cover the potentially most sensitive species from acute tests, which is fish. Caution is necessary as the NOECs are based on nominal values.
Intermittent releases to water	PNEC aqua (intermittent releases): 0.983 mg/L	Assessment factor: 100 on the lowest (valid) EC_{50} value, which is 98.3 mg/L)
Sediments (freshwater)	PNEC sediment (freshwater): 0.38 mg/kg sediment dw	Equilibrium partioning using a Koc value of 1.7 (although caution is necessary as the estimated value is referring to an inorganic substance and aquatic PNEC has uncertainties)
Sediments (marine water)	PNEC sediment (marine water): 0.038 mg/kg sediment dw	Equilibrium partioning using a Koc value of 1.7 (although caution is necessary as the estimated value is referring to an inorganic substance and aquatic PNEC has uncertainties)
Sewage treatment plant	PNEC STP: 8.98 mg/L	Assessment factor: 10 (on the achieved EC_{10} value of 89.8 mg/L in an OECD 209 test)
Soil	PNEC soil: 0.017 mg/kg soil dw	Equilibrium partioning using a Koc value of 1.7 and a HENRY constant of 15.28 (although caution is necessary as the estimated values are referring to an inorganic substance and aquatic PNEC has uncertainties)
Air	Regarding air no assessment is included in the registration dossier. Potentially the formation of SO_2 might lead to hazards for the environment	

Secondary poisoning	The bioaccumulation potential is expected to be negligible	

It is noted that the aquatic PNECs (ECHA Website visited on 24.3.2020) are lower compared to the PNECs provided by the Registrant(s): The chronic fish study for a break down product was included into the assessment although ECHA concluded in their compliance check decision that the justification for use of this fish test is not sufficient and requested a new fish test, which has not been submitted at the timepoint of finalistion of this assessment.

No PNECs have been derived for soil, sediment and air by the Registran(s) as no hazard has been identified.

7.8.5. Conclusions for classification and labelling

No classification is currently warranted for aquatic toxicity according to CLP Regulation (EC) No. 1272/2008 for sodium dithionite. No data from a long-term fish test are available, although requested by a compliance check decision in 2016.

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

7.9.1.1. Read-across concept for sulfites, hydrogensulfites, metabisulfites, dithionites and thiosulfates

Sodium dithionite is not stable under physiological conditions, with the rate of decomposition increasing with increasing acidity. Upon contact with moisture, it oxidizes to hydrogen sulfite and hydrogen sulphate.

A read across concept has been provided by the registrants for sulfites, hydrogensulfites and metabisulfites, based on the pH-dependant equilibrium in aqueous solutions and on the assumption that the nature of the cation (i.e., sodium, potassium, ammonium…) is not assumed to contribute substantially to differences in toxicity and solubility.

In principle the eMSCA can support this conclusion. It is further strengthened by previous assessments performed by WHO, OECD SIDS, 2004 and a recent EFSA assessment on sulphites (EFSA 2016). Although sodium dithionite was not evaluated itself, the read across substances sulfur dioxide (CAS 7446-09-5), sodium sulphite (CAS 7757-83-7), sodium bisulfite (CAS 7631-90-5), sodium metabisulfite (CAS 7681-57-4) and potassium metabisulfite (CAS 16731-55-8) were evaluated for their use as food additives. The EFSA Panel considered that once ingested, based on their capacity to form sulfite ions, read across between the different sulfite sources is possible. According to the eMSCA this applies also for sodium dithionite.

The registrant further points out that the read across concept does not fully cover thiosulfate; it is restricted to appropriate physiological conditions i.e. oral administration where the gastric passage with the strongly acidic conditions will facilitate the chemical disproportionation.

Table 12: Overview on studies on absorption, metabolism, distribution and elimination

Method,	Results	Remarks	Reference
In vivo studies			
rabbit (New Zealand White)	under physiological	Key study	Gunnison, A.

inhalation Exposure regime: Rabbits 1 and 2: 14 hours + >200 hours post exposure Rabbits 3 and 4: 62 hours + >200 hours post exposure Doses/conc.: 23.5 ppm The hydrate of sulfur dioxide in mammalian plasma was investigated. The longevity of sulfite in contact with mammalian plasma and known components of blood was investigated. Free sulfite present in the plasma was determined as cyanolytic sulfite.	conditions sulphite reacts reversibly with disulphide bonds present in the plasma result in in formation of S-sulfonates (sulfitolysis). No free sulphite was detected in rabbits immediately following exposure, but there was evidence for substantial elevation of plasma and serum S- sulfonate content.	Klimisch 2 Test material: sulphite	F. & Benton, A.W. unpublished study report (1971)
mouse (Kunming albino mice) male inhalation: SO_2 was administered for 4h/d for 7 days. Doses/conc.: 14.00 +/- 1.25 mg/m^3, 28.00 +/- 1.98 mg/m^3, and 56.00 +/- 3.11 mg/m^3 of SO_2 The levels of sulfite in brains, hearts and lungs from male mice exposed to SO2 were determined by high-performance liquid chromatograph with fluorescence detection (HPLC-FD).	<u>distribution</u>: A significant increase of the contents of sulfite in brain, heart, and lung tissues was caused by exposure to SO2 ($p < 0.05$) compared with the control groups, and the sulfite content was increased in a dose-dependent manner ($r > .92$). The sulfite contents in lung tissues were the highest, the contents in the brain tissues were the lowest, and heart tissues were between them. No data on metabolites	key study Klimisch 2 Test material: sulfur dioxide	Meng et al. (2005)
rabbit (New Zealand White) male intravenous Exposure regime: Rabbits No. 3, 4, and 5: Rabbits were given only 1 dose level per day with a minimum of 1 day between experiments. rabbits received different doses/conc.: 0.58, 0.56, 0.15, 0.30, and 0.60, 0.56, 0.62 nmol sulfite/kg one rabbit received a priming dose of 0.61 nmol sulfite/kg and a postinjection iv infusion: 37.1 µmol/min	<u>excretion:</u> Sulfite is cleared predominantly by oxidation to sulfate. A mathematical model has been established which describes sulphite removal mechanisms, metabolic and physical in an intact mammalian organism. The results indicate that the efficiency of this enzyme decreases as sulphite dose increases	key study Test material disodium disulfite	Gunnison, A.F. and Palms, E.D. (1976)
monkey (Rhesus monkey) female intravenous Doses/conc.: Experiment performed while animal physically restrained: 0.27 nmol sulfite/kg Experiment performed while animal sedated: 0.30 nmol sulfite/kg	<u>excretion:</u> pattern of sulphite distribution and elimination is similar to rabbits but the kinetics of removal mechanisms are different,	key study Test material: disodium disulfite	Gunnison, A.F. and Palms, E.D. (1976)

rat (Crj: CD(SD)) male inhalation Exposure regime: 6 hours/day (weekends and holidays were excluded); there were 99 actual exposure days over the 21-week treatment period. Doses/conc.: 1. Systemic exposure Groups in the systemic exposure series breathed clean room air. 2. inhalation exposure: Nominal sulfur dioxide concentrations: 0, 10, and 30 ppm of SO2 Actual concentrations: 10.1 +/- 0.3 and 29.9 +/- 1.2 (mean +/- SD of daily means) for groups 10 SO2 and 30 SO2, respectively. Daily standard deviations averaged approximately 11% of the mean concentration. Objective: compare the distribution, metabolism, and toxicity of endogenously generated sulfite in sulfite oxidase deficient rats with that of inhaled sulfur dioxide in sulfite oxidase-competent rats. The focus was on the respiratory tracts of these two animal models.	Endogenously generated sulfite and S-sulfonate compounds (a class of sulfite metabolite) accumulated in the respiratory tract tissues and in the plasma of sulphite oxidase deficient rats in inverse proportion to hepatic sulfite oxidase activity. In contrast to this systemic mode of exposure, sulfite exposure of normal, sulfite oxidase-competent rats via inhaled SO2 was restricted to the airways. Minor pathological changes consisting of epithelial hyperplasia, mucoid degeneration, and desquamation of epithelium were observed only in the tracheas and bronchi of the rats inhaling SO2, even though the concentration of sulfite plus S-sulfonates in the tracheas and bronchi of these rats was considerably lower than that in the endogenously exposed rats. The authors attributed this histological damage to hydrogen ions stemming from inhaled SO2, not to the sulfite/ bisulfite ions that are also a product of inhaled SO2. In addition to the lungs and trachea, all other tissues examined, except the testes, appeared to be refractory to high concentrations of endogenously generated sulfite. The testes of grossly sulphite oxidase-deficient rats were severely atrophied and devoid of spermatogenic cells.	key study Test material: sulfur dioxide Restriction: only 2 animals used	Gunnison A.F et al 1987
rat (albino) oral: feed Exposure regime: 24, 48 hours, 1 week and 2 weeks Doses/conc.: 10 mg SO2/kg 50 mg SO2/kg (giving 3.37 x 10^7 count/minute) The fate of ingested sulfite was investigated in rats using dose levels of 10 and 50 mg SO2/kg administered as NaHSO3 mixed with Na2[35]SO3. Additionally, it was tried to define what level of ingested sulfite could be tolerated by rats before they would start to excrete unaltered sulfite in their urine.	absorption: Most (70-95%) of the ingested [35S]sulfite was absorbed from the intestine and voided in the urine within 24 hours. excretion: Most of the remaining [35]S was eliminated in the faeces. Residual [35]S in the animal carcasses after 1 week accounted for 2% or less of the administered dose in all cases. No free sulfite was detected in rat urine even after administration of a single oral dose as high as 400 mg/ SO2/kg.	key study Test material: sodium sulfite	Gibson and Strong, (1973)

	no data on metabolites		
mouse (albino) male/female oral: feed Exposure regime: 24, 48 hours, 1 week and 2 weeks Doses/conc.: 50 mg SO2/kg (giving 3.37 x 10^7 count/minute) The fate of ingested sulfite was investigated in mice using dose levels of 50 mg SO2/kg administered as NaHSO3 mixed with Na2[35]SO3.	Main ADME results: absorption: Most (70-95%) of the ingested [35S]sulfite was absorbed from the intestine and voided in the urine within 24 hours. excretion: Most of the remaining [35]S was eliminated in the faeces. Residual [35]S in the animal carcasses after 1 week accounted for 2% or less of the administered dose. no data on metabolites	key study Test material sodium sulfite	Gibson and Strong, (1973)
monkey (Rhesus) male/female oral: gavage Exposure regime: 5 days Doses/conc.: 50 mg SO2/kg The fate of ingested sulfite was investigated in monkeys using dose levels of 50 mg SO2/kg administered as NaHSO3 mixed with Na2[35]SO3.	Main ADME results: absorption: Most (70-95%) of the ingested [35S]sulfite was absorbed from the intestine and voided in the urine within 24 hours. excretion: All of the [35]S was eliminated from the body in 3 days. An efficient voidance of the dose via the urinary tract was demonstrated in the first 24 hours. excretion: The greatest cumulative amount found in the faeces of the 6 monkeys was 6%, the remainder being recovered in the urine. Metabolites identified: no Details on metabolites: no data	key study Test material: sodium sulfite	Gibson and Strong, (1973)
rat (Wistar (male) and rhesus monkey (female)) intravenous Exposure regime: Please refer to "Details on exposure" above Doses/conc.: Rats: Rat No. 1: 1.17 mmol SO3^2-/kg (4 x injection was given) Rat No. 2: 0.82 mmol SO3^2-/kg (3 x injection was given) Rat No. 3: 0.50, 0.80, and 1.18 mmol SO3^2-/kg Rat No. 4: 0.60, 0.80, and 0.99 mmol SO3^2-/kg Rat No. 5: 0.52, 0.78, and 1.03 mmol SO3^2-/kg Rhesus monkey: Rhesus monkey No.1: 0.14, 0.30, and 0.42 Method Results Remarks Reference mmol SO3^2-/kg Rhesus monkey No.2: 0.13, 0.26, and 0.35 mmol SO3^2-/kg (also 0.26 mmol SO3^2-/kg when animal was sedated)	<u>metabolism</u>: The logarithm of clearance plotted as a function of injected dose demonstrates an inverse relationship in both species. It was demonstrated that sulfite clearance in the rat is several times more rapid than in the rhesus monkey. Assuming that man is probably more similar to the rhesus monkey than to the rat with respect to sulfite clearance, the authors assumed that the latter species is a poor experimental animal to use for toxicity evaluation for human health.	supporting study Test material: sulphite Klimisch 2	Gunnison, A.F. et al. (1977) Gunnison, A.F. and Palms, E.D. (1976)

The clearance of injected sulfite, which is due principally to its metabolism to sulfate, was measured in rats and rhesus monkeys for the purpose of estimating the in vivo function of sulfite oxidase.			
In vitro studies			
vitro study human human polymorphonuclear leukocytes Exposure regime: Cell culture used: Human polymorphonuclear leukocytes were isolated from buffy coat (obtained from the Blood Donor center of Sabbatsbergs Hospital, Stockholm, Sweden). In the present study, the oxidation of sulfite in human polymorphonuclear leukocytes was investigated and the sulfite-oxidase catalyzed reaction and the non-enzymatic pathway were compared.	Main ADME results: metabolism: Two different oxidation routes of sulfite to sulfate have been identified in the human polymorphonuclear leukocytes. 1. Via sulfite oxidase and 2. via an one electron oxidation step with an intermediate formation of sulfur trioxide radicals. Metabolites identified: yes Details on metabolites: Addition of sulfite to polymorphonuclear leukocytes significantly stimulated the uptake of oxygen. The oxygen consumption varied substantially between cells from different donors and were divided in those with low (0 -200 nmol O2/ml/min.) and high (>200 nmol O2/ml/min.) capacity. The interindividual difference in oxygen uptake was also reflected in the rates of sulfite disappearance and sulfate formation, the correlation between these two parameters being fairly good. The correlation was not affected by varying the concentration of sulfite added to the leukocytes. It is assumed that the variation in oxygen consumption mainly reflects the cells capacity to oxidize sulfite direct to sulfate, thus the activity of sulfite oxidase. Only 30 % to 40% of the sulfite added to cells with low sulfite oxidase activity was oxidized to sulfate after 30 min. incubation whereas on average about 60% was oxidized in cells with high activity. In the presence of sulfite, addition of phorbol myristate acetate to cells with low sulfite oxidase activity	key study read-across from supporting substance (structural analogue or surrogate) Test material (EC name): sodium sulfite (See endpoint summary for justification of read-across)	Constantin, D. et al. (1994)

	increased the O2 consumption substantially (up to 600 nmol/ml/ min.) In cells with high enzyme activity an inhibitory effect of phorbol myristate acetate on oxygen consumption was observed. The effect of phorbol myristate acetate can also be seen on the oxidation of sulfite to sulfate. In cells with low sulfite oxidase activity the addition of phorbol myriastate acetate increases the rate of sulfate formation whereas in cells with high activity phorbol myriastate acetate has an inhibitory effect. The EPR spectrum shows signals consistent with the presence of sulfur trioxide radicals formed during autoxidation of sulfite. A similar spectrum is observed after addition of sulfite to non-phorbol myristate acetate stimulated human polymorphonuclear leukocytes. When phorbol myristate was added to polymorphonuclear leukocytes and sulfite, an EPR spectrum compatible with the presence of sulfur trioxide radicals as well as hydroxyl adducts with DMPO was observed.		
in vitro study rabbit (New Zealand White) not applicable Exposure regime: continuously until measurement (after 20 minutes) Doses/conc.: 1.7, 3.6 and 5.2 nanomols of sulfite The hydrate of sulfur dioxide in mammalian plasma and serum was investigated. The longevity of sulfite in contact with mammalian plasma and known components of blood was investigated. The reactivity of sulfite added to serum or plasma in vitro was followed by monitoring the concentration of the sulfite in the reaction mixture.	Main ADME results: disappearance from serum or plasma: Sulfite which enters the bloodstream during exposure of a mammal to atmospheric SO2 forms S-sulfonate groups with constituents of the plasma, probably exclusively by sulfitolysis of disulfide groups. Metabolites identified: no Details on metabolites: No details are given.	key study read-across from supporting substance (structural analogue or surrogate) Test material (Common name): sulfite (See endpoint summary for justification of read-across)	Gunnison, A. F. & Benton, A.W. (1971)

in vitro study human not applicable Exposure regime: continuously until measurement (after 20 minutes) Doses/conc.: 1.7, 3.6 and 5.2 nanomols of sulfite The hydrate of sulfur dioxide in mammalian plasma and serum was investigated. The longevity of sulfite in contact with mammalian plasma and known components of blood was investigated. The reactivity of sulfite added to serum or plasma in vitro was followed by monitoring the concentration of the sulfite in the reaction mixture.	Main ADME results: disappearance from serum or plasma: Sulfite which enters the bloodstream during exposure of a mammal to atmospheric SO_2 forms S-sulfonate groups with constituents of the plasma, probably exclusively by sulfitolysis of disulfide groups. Metabolites identified: no Details on metabolites: No details are given.	key study read-across from supporting substance (structural analogue or surrogate) Test material (Common name): sulfite (See endpoint summary for justification of read-across)	Gunnison, A. F. & Benton, A.W. (1971)

Table 12 gives an overiew on toxicokinetics studies.

The registrant concludes on the ADME section: *Sulfites, hydrogensulfites and metabisulfites are present in dissociated form in aqueous solutions, depending on solution pH. Dithionites disproportionate in water to form hydrogen sulfites and thiosulfates. Thiosulfates also disproportionate in acidic aqueous solution to form polythionic acids and SO2 (HSO3-). For these reasons, extensive read-across between these substances is considered justified.*

Inhalation absorption: based on particle size dependant deposition modelling (MPPDl), an inhalation absorption factor of 55.2% was derived for sodium dithionite.

Dermal absorption: in the absence of measured data on dermal absorption, dermal absorption factors of 1% (exposure to liquid media) and 0.1% (exposure to dry solids/dust) are assumed (HERAG).

Oral absorption: according to animal toxicokinetic data (70-95% absorption), an oral absorption factor of 100% was derived.

Metabolism: inorganic substances such as sulfites are not subject to metabolism as such; however, these substances are known to undergo oxidative transformation under physiological circumstances.

Distribution and elimination: upon systemic uptake, sulfites are distributed widely between tissues because of their high solubility/bioavailability, and are cleared almost exclusively by oxidation to sulfate with subsequent renal excretion.

The eMSCA can agree with the registrant's conclusion concerning the justification of the read across. Depending on the solution pH, sulfites, hydrogensulfites and metabisulfites are present in dissociated form in aqueous solutions. Dithionites disproportionate in water to form hydrogen sulfites and thiosulfates. Thiosulfates also disproportionate in acidic aqueous solution to form polythionic acids and SO2 (HSO3-).

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 (H2S). 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 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.

There are only few and rather old in vivo studies on toxicokinetics available. It was demonstrated that sulfite clearance in the rat is several times more rapid than in the rhesus monkey, which indicates that the rat is not the adequate organism to assess sulphite toxicity in humans.

7.9.1.1.1. **Inhalation absorption:**

According to the WHO air quality guideline absorption of sulfur dioxide in the mucous membranes of the nose and upper respiratory tract occur as a result of its solubility in aqueous media: 1 volume of water dissolves 45 volumes of sulfur dioxide at 15°C. Absorption is concentration-dependent, with 85% absorption in the nose at 4–6 µg/m3 and about 99% at 46 µg/m^3 (WHO, 2000). The pH of the surface fluid in the respiratory tract is 6.5–7.5, and thus appreciable amounts of both the bisulfite and the sulfite will be present. Absorbed (bi)sulfite is converted to sulfate by molybdenumdependent sulfite oxidase. The highest concentrations of this enzyme occur in the liver and kidney, while lower levels are found in the lung (WHO, 2000).

Meng et al., 2005a have demonstrated that inhaled SO_2 is metabolised into sulphites and dose-dependent significant increases in lung, heart and brain can be measured. Together with detection of oxidative damage and DNA damage in multiple organs it demonstrates systemic toxicity of SO2.

The assumption is that the most material will be cleared rapidly either by expulsion or by translocation to the gastrointestinal tract. A small fraction will be subjected to more prolonged retention, which can result in direct local adsorption. An uptake of 100 % is assumed for the material deposited in the pulmonary region and 100 % is assumed for gastrointestinal uptake.

An absorption factor of 55.20% for Dithionite following inhalation has been calculated by the registrant.

The eMSCA can agree with the registrant's conclusion.

7.9.1.1.2. **Dermal absorption:**

In the absence of measured data on dermal absorption the registrant followed the methodology proposed by the HERAG fact sheet for metals and their inorganic substances. The HERAG fact sheet "Assessment of dermal absorption for metals and inorganic metal compounds" was published in 2007 by the consortium of the registrant. It proposes default absorption factors for metals deviating from those defined in the respective TGD of 10% or 100%. The TGD referenced (2003, Part I, Chapter 2, Appendix IV B) states, in the case of lack of data, assigns default dermal absorption rates of 100 % or 10 % depending on the properties of a chemical substance, with an argumentation developed by de Heer (1999). Without relevant experimental data 10 % dermal absorption is used when the molecular weight (MW) of the substance is > 500 and the log Pow is smaller than -1 or higher than 4, otherwise 100 % dermal absorption is used. Also, the more recent and relevant REACH guidance document, chapter R.7c: Endpoint specific guidance refers to default factors of 10% or 100% respectively (ECHA, 2017).

The proposal to adapt the default factor is supported by information on dermal absorption from previous risk assessments and with information on the nature of metal cations adhering to human skin. In 2017, the eMSCA was informed that the fact sheet was updated and was provided with a draft of the updated fact sheet (working draft, status January 2017). The updated draft contains more recent data on dermal absorption of metal compounds and new proprietary dermal absorption studies conducted for REACH purposes and dermal absorption data available in published scientific literature (2010-2016). However, there are several limitations in respect to the fact sheet. First, it is still a draft, and some studies are still under evaluation. Second, several studies have been conducted with nanoparticles and are of minor relevance for the substance evaluation on sodium dithionite. Third, a study using calcium chloride assuming that magnesium, calcium, nitrate and sulfate ions comes to the conclusion human skin is permeable to magnesium, calcium, nitrate and sulfate ions under the tested conditions. This study was not evaluated further as it was not following an OECD or similar guideline.

The proposed default dermal absorption factors for metals and inorganic metal compounds are 1.0% from exposure to liquid/wet media and 0.1% from dry (dust) exposure. A guidance fact sheet (HERAG, 2007) was used by the Registrant(s) which has recently been updated. This guidance document is stated to provide reasoning to deviate from the default factors as depicted in the REACH guidance document R7c, endpoint specific guidance of 100 and 10%. It is proposed using instead a default dermal absorption value of 1% from liquid aqueous media and 0.1% for dry powders, substances and materials. The HERAG fact sheet was not part of the registration dossier, but delivered by registrants in February 2017 and providing data from registration dossiers of metals and several nano applications. One publication concluding that human skin is permeable to magnesium, calcium, nitrate and sulfate ions was considered as not reliable and not further used (Laudanska et al., 2002).

As no substance specific dermal adsorption data are available and the literature data base for dermal absorption factors of anions is not extensive it is recommended by the eMSCA to use a default dermal absorption rate of 10% for risk assessment.

7.9.1.1.3. **Oral absorption:**

Soluble sulphite substances are considered to be readily absorbed by the gastrointestinal tract and therefore the registrant assumed 100% absorption of sulphite substances after ingestion.

The eMSCA can agree with the registrant’s conclusion.

7.9.1.2. Metabolism, distribution and elimination:

Sufite is converted to sulfate, primarily in the liver by the enzyme sulphite oxidase (SO_X). The activity of this enzyme is 10-20 times lower in the human liver compared to the rat. Some studies demonstrate an alternative pathway of the metabolism with intermediates the formation of sulfur trioxide radicals. The EFSA panel noted in its scientific opinion that it was not possible to ascertain the relative contribution of the differing pathways of sulphite metabolism at realistic levels (EFSA, 2016). Further, the contribution of the different pathways is expected to vary substantially due to the great interindividual variation in sulfit oxidase activity. In individuals with low sulfite oxidase activity the contribution of the trioxide radical pathway is expected to be high. The identified radical mechanism may promote the activation of certain carcinogens and may also increase the risk of amino acid destruction, degradation of DNA and lipid peroxidation.

It has further been described that bisulfite radicals can be formed in the stomach through the reaction between nitrite and sulphite with the formation of NO (Takahama and Hirota, 2012).

The effects of sulfite in foods are conventionally related to the amount and concentration of free sulfur dioxide, and to the speed at which it is released from serum proteins to which it becomes bound upon systemic uptake. Sulfite may also react reversibly with disulfide linkages in proteins.

7.9.2. Acute toxicity and Corrosion/Irritation

7.9.2.1. Acute Toxicity: oral

Table 13: Study on acute oral toxicity

Method	Results	Remarks	Reference
rat male/female oral: gavage equivalent or similar to OECD Guideline 401 (Acute Oral Toxicity)	LD50: ca. 2500 mg/kg bw (male/female)	key study Klimisch 2 Test material: sodium dithionite	Unpublished study report (1971), Unpublished study report (2003b)

7.9.2.2. Acute toxicity: inhalation

Table 14: Studies on acute inhalation toxicity

Method	Results	Remarks	Reference
rat (Sprague-Dawley) male/female inhalation: dust/aerosol test (nose/head only) equivalent or similar to OECD Guideline 403 (Acute Inhalation Toxicity)	LC50 (4 h): > 5.5 mg/L air (male) LC50 (4 h): > 5.5 mg/L air (female) LC50 (4 h): > 5.5 mg/L air (male/female). Statistical certainty: 99.9% LC50 (1 h): > 22 mg/L air (male/female) (Recalculated by using the Haber-Rule C X t = k.)	1 (reliable) key study read-across Test materia:l sodium sulfite	Klimisch, H.-J. (1982)
Guinea pigs Exposure: head only, 1h to 474, 669 and 972 µg/m^3 Sodium sulphite aerosol to assess irritant properties Another group of guinea pigs was exposed whole body for 1 hr to the same aerosol at 0, 204, 395, and 1152 micrograms SO3(2-)/m3. Immediately after the exposures, lung volume, diffusion capacity for carbon monoxide (DLCO), and wet lung weight were evaluated in anesthetized, tracheotomized animals.	Dose related increases in resistance (59% resistance) and decreases in compliance (19%) decrease at highest dose) were observed. The results were used to assess the irritant potency of sodium sulfite aerosol. As compared to controls, total lung capacity, vital capacity, functional residual capacity, residual volume, and DLCO were all decreased with increasing concentrations of sodium sulfite. Dose-related increases in wet lung weights were also observed.	1 (reliable) Additional study(eMSCA) Test material: Sodium sulfite	Chen et al. (1987)
Guinea pigs, Exposure: head only, 1h to 50, 250, 450 mg/m3 of an ammonium sulphite/ammonium sulphate aerosol	No deaths were observed in guinea pigs exposed for 6 h to 400 mg/m3 of ammonium sulfite, LC 50: >400 mg/m3	Additional study(eMSCA) Test material: Ammonium sulfite	Rothenberg et al. (1986)
Beagle dogs (five female, three male) Exposure: nose only, 1 h to 1 mg/m^3 of aerozolized ammonium sulphite mixed with sulfat	No significant differences between pre-exposure and post-exposure tracheal mucous clearance rates	2 Additional study (eMSCA) Test material: ammonium sulphite/ammonium sulfate aerosol	Rothenberg et al. (1986)

7.9.2.3. Acute toxicity: dermal

Table 15: Studies on acute dermal toxicity

Method	Results	Remarks	Reference
rat (Wistar) male/female similar to OECD Guideline 402 (Acute Dermal Toxicity) semiocclusive	LD50: > 2000 mg/kg bw (male) LD50: > 2000 mg/kg bw (female) LD50: > 2000 mg/kg bw (male/female)	key study Test material: sodium sulfite	Unpublished study report (2009)

7.9.2.4. Irritation

Table 16: Studies on skin irritation

Method	Results	Remarks	Reference
rabbit (Vienna White) Coverage: occlusive (shaved) Vehicle: water equivalent or similar to OECD Guideline 404 (Acute Dermal Irritation / Corrosion) (adopted 1981-05-12)	not irritating Erythema score: 0.33 of max. 4 (4 of 4 animals) (Time point: 24,48 hours) (fully reversible within: 48 hours) Edema score: 0 of max. 4 (4 of 4 animals)	2 (reliable with restrictions) key study read-across Test material sodium sulfite	Unpublished study report (1981)
rabbit (Vienna White) Coverage: semiocclusive (shaved) Vehicle: water Equivalent or similar to OECD Guideline 404 (Acute Dermal Irritation / Corrosion) (adopted 1981-05-12)	not irritating Erythema score: 0 of max. 4 (4 of 4 animals) Time point: 24, 48, and 72 hours Edema score: 0 of max. 4 (4 of 4 animals)	2 (reliable with restrictions) key study Test material potassium sulfite Form: solution	Unpublished study report (1989a)
rabbit (New Zealand White) Coverage: occlusive (shaved) Vehicle: unchanged (no vehicle) equivalent or similar to OECD Guideline 404 (Acute Dermal Irritation / Corrosion) (adopted 2002-04-24)	not irritating Erythema score: 0 of max. 4 (3 of 3 animals) Time point: 24, 48, and 72 hours Edema score: 0 of max. 4 (3 of 3 animals)	1 (reliable without restriction) supporting study Test material ammonium hydrogensulphite Form: solution	Unpublished study report (2004a)
rabbit (New Zealand White) Coverage: occlusive (shaved) Vehicle: unchanged (no vehicle) equivalent or similar to OECD Guideline 404 (Acute Dermal Irritation / Corrosion) (adopted 2002-04-24)	not irritating Erythema score: 0 of max. 4 (3 of 3 animals) (Time point: 24, 48, and 72 hours) Edema score: 0 of max. 4 (3 of 3 animals) (Time point: 24, 48, and 72 hours)	1 (reliable without restriction) supporting study Test material: sodium hydrogensulfite	Unpublished study report (2004b)
rabbit (New Zealand White) Coverage: occlusive (shaved) Vehicle: unchanged (no vehicle) equivalent or similar to OECD Guideline 404 (Acute Dermal Irritation / Corrosion) (adopted 2002-04-24)	not irritating Erythema score: 0 of max. 4 (3 of 3 animals) (Time point: 24, 48, and 72 hours) Edema score: 0 of max. 4 (3 of 3 animals) (Time point: 24, 48, and 72 hours)	1 (reliable without restriction) supporting study Test material: potassium hydrogen sulfite	Unpublished study report (2004c)

rabbit (New Zealand White) Coverage: occlusive (shaved) Vehicle: unchanged (no vehicle) equivalent or similar to OECD Guideline 404 (Acute Dermal Irritation / Corrosion) (, adopted 1981-05-12)	No classification of the test substance can be carried out, since an aqueous solution was used in the study and not the pure test substance. Hence, the real applied amount was below the required dose. Erythema score: 0 of max. 4 (6 of 6 animals) (Time point: 24, 48, and 72 hours) Edema score: 0 of max. 4 (6 of 6 animals) (Time point: 24, 48, and 72 hours)	3 (not reliable) Test material: ammonium thiosulfate	Unpublished study report (1988)

7.9.2.5. Eye irritation

Table 17: Studies on eye irritation

Method	Results	Remarks	Reference
Rabbit (New Zealand White) OECD Guideline 405 (Acute Eye Irritation / Corrosion) (, adopted 2002-04-24)	Irritating Cornea score: 0 of max. 4 (3 of 3 animials) (Time point: 24, 48, and 72 hours) Iris score: 0 of max. 2 (3 of 3 animals) Time point: 24, 48, and 72 hours) Conjunctivae score: 2, 2.67,2 of max. 3 (3 animals) (Time point: 24, 48, and 72 hours) (fully reversible within: 7 days) Chemosis score: 1,0 and 0.67 of max. 4 (3 animals) (Time point: 24, 48, and 72 hours) (fully reversible within: 7 days)	1 (reliable without restriction) weight of evidence experimental result Test material (EC name): sodium dithionite	Unpublished study report (2006)
rabbit (New Zealand White) OECD Guideline 405 (Acute Eye Irritation / Corrosion)	irritating Cornea score: 0 of max. 4 (3 of 3 animals) (Time point: 24, 48, and 72 hours) Iris score: 0 of max. 2 (3 of 3 animals) (Time point: 24, 48, and 72 hours) point: 24, 48, and 72 hours) Conjunctivae score: 2.67, 2.67, 3 of max. 3 (3 animals) (Time point: 24, 48, and 72 hours) (fully reversible within: 7 days) 3 of max. 3 (mean (animal #3)) (Time point: 24, 48, and 72 hours) (fully reversible within: 7,7,14 days) Chemosis score: 1, 0.33, 1 of max. 4 (3 animals) (Time point: 24, 48, and 72 hours) (fully reversible within: 72 hours, 4hours, 7 days	1 (reliable without restriction) Test material sodium dithionite	Unpublished study report (2003b)

7.9.2.6. Irritation to the respiratory tract

Table 18: Studies on respiratory irritation

Method	Results	Remarks	Reference
Guinea pigs Exposure: head only, 1h to 474, 669 and 972 µg/m^3 Sodium sulphite aerosol to assess irritant properties Another group of guinea pigs was exposed whole body for 1 hr to the same aerosol at 0, 204, 395, and 1152 micrograms SO3(2-)/m3. Immediately after the exposures, lung volume, diffusion capacity for carbon monoxide (DLCO), and wet lung weight were evaluated in anesthetized, tracheotomized animals.	Dose related increases in resistance (59% resistance) and decreases in compliance (19%) at highest dose) were observed. The results were used to assess the irritant potency of sodium sulfite aerosol. As compared to controls, total lung capacity, vital capacity, functional residual capacity, residual volume, and DLCO were all decreased with increasing concentrations of sodium sulfite. Dose-related increases in wet lung weights were also observed.	1 (reliable) Additional study(eMSCA) Test material: Sodium sulfite	Chen et al. (1987)
Dog (Beagle) chronic (inhalation: aerosol) (whole body) 1mg Na2S2O5 /m3 (nominal conc.in air) Exposure: 320 days exposure to clean air (control period) followed by 290 days exposure to Na2S2O5 aerosol Eight Beagle dogs Selected parameters of lung clearance, biochemistry, cytology, and morphology were determined during both periods to find sensitive parameters for early changes of lung function. Only changes of lung clearance parameters are described in this paper.	Three dogs showed significant changes in clearance rate of moderately soluble particles during the sulphite exposure compared to the clearance rate during clean air exposure. The results were confirmed by in vitro clearance measurements in alveolar macrophages.	2 (reliable with restrictions) supporting study Test material disodium disulfite	Ferron, G.A.; et al. (1990)
dog (Beagle) subchronic (inhalation: aerosol) (whole body) 1 mg Na2S2O5 /m3 (nominal conc.in air) (equivalent to 0.6 mg/m3 as SO2) exposure period: 290 days The aerosol was generated from an aqueous 3.5% solution of Na2S2O5 adjusted to pH 7. Three dogs served as control. After sacrifice the nasal cavity was prepared for histological examination.	Morphologic changes were observed in nasal cavities of beagle dogs after long-term exposure to a respirable sulfur(W) aerosol. The changes were characterized by a thickened epithelial layer resulting from epithelial proliferation, by a loss of secretory material, and by moderate mononuclear cell infiltration. Key study	2 (reliable with restrictions) supporting study Test material: disodium disulfite	Takenaka, S.; et al. (1994)

Repeated exposure to sulphite aerosols caused adverse effects on the extrapulmonary airways of beagle dogs at 1 mg/m3. Hyperplastic foci were observed in the respiratory region of the posterior nasal cavity in exposed dogs. Changes included a thickened epithelial layer due to epithelial proliferation, loss of secretory material, and moderate mononuclear cell infiltration. Laryngeal changes characterized by a focal loss of cilia and slight subepithelial mononuclear cell infiltration were observed in the exposed dogs. Focal disappearance of ciliated cells in the transitional region between cartilaginous and membranous trachea was observed in exposed and control dogs.
However, incidences of changes in lung capacity parameters, mild pulmonary edema and change of the tracheal epithelium were noted after using fine aerosol containing fine respirable particles in the inhalation toxicity studies**.**

Summary on Acute toxicity and Corrosion/Irritation:

One animal study for sodium dithionite on acute oral exposure is available; the study was conducted in 1971 and reevaluated in 2003. The determined LD50 value was ca. 2500 mg/kg bw.

The substance has a harmonised classification according to EC regulation No. 1272/2008 as acute toxic category 4, H302.

One animal study, equivalent to OECD 403 for inhalation exposure was performed with sodium sulphite. The determined LC50 value was greater than 5,5 mg/l.

One animal study, equivalent to OECD 402 for acute dermal exposure was performed with sodium sulphite. The determined LD50 value was greater than ca. 2000 mg/kg bw.

Acute inhalation toxicity studies in Guinea pigs, Beagle dogs and Sprague-Dawley rats at relatively low concentration levels of sodium sulphite and human observations of the respiratory tract in relation to the irritation effect of disodium disulphite (itching, rhinitis, nasal congestion) the application of classification category STOT SE 3 for respiratory tract irritation to disodium disulphite seems to be necessary.

Conclusion on eye irritation:
The registrant proposes on the basis of two reliable eye irritation studies for sodium dithionite that a classification as eye irritanat Category 2 is warranted. The eMSCA supports this proposal.

Conclusion on acute toxicity and irritation:

The eMSCA supports the classification as acute toxic category 4, H302.

The eMSCA further recommends classification as eye irritant Category 2 and STOT SE 3 covering respiratory irritation.

7.9.3. Sensitisation

Animal studies

7.9.3.1. Skin sensitisation

Table 19: Animal studies on the sensitizing property of sodium dithionite

A Local Lymph Node Assay with the substance sodium dithionide is documented in the

Method	Results	Remarks	Reference
LLNA – non standard test protocol (LNCC) NMRI/Crl:NMRI mice, f 6 animals/group Concentration: 10%, 25%, 50% (w/w) Solvent: aqua ad iniectabilia	Not sensitising (using cut-off values 1.4 (cell count) or 1.1 (ear weight)) 10 % w/w: SI: 1.154(cell count); SI: 1.220 (lymph node weight); SI: 1.020 (ear weight) 25 % w/w: SI: 0.761 (cell count); SI: 1.195(lymph node weight); SI: 1.020 (ear weight) 50 % w/w: SI: 1.034 (cell count); SI: 1.244 (lymph node weight); SI: 1.048 (ear weight)	2 (reliable with restriction) GLP Key study Test material: sodium dithionite	Unpublished study report (2010c)

registration data (unpublished study report, 2010c). The test was performed according to OECD TG 429 but with alternative endpoints according to Ehling (2005). Instead of radioactive labelling to measure cell proliferation lymph node weight and lymph node cell count (LNCC) were used to assess proliferation. In addition acute inflammatory skin reaction were measured by ear weight and ear thickness measurement. This alternative method is documented by Ehling (2005 a and b). Further deviations from the test protocol are the use of different mice (NMRI mice) than recommended and the excision of lymph nodes at day 4 instead of day 6 after first application. No justification is provided. The test substance was dissolved in aqua ad iniectabilia despite the recommendation in the guideline (OECD, 2002) that aqueous vehicles are to be avoided. The revised guideline TG429 (OECD, July 2010) further recommends the use of a solubiliser: *"Particular care should be taken to ensure that hydrophilic substances are incorporated into a vehicle system, which wets the skin and does not immediately run off, by incorporation of appropriate solubilisers (e.g. 1% Pluronic® L92). Thus, wholly aqueous vehicles are to be avoided."* This guideline has not been taken into acount for the provided LLNA as it was conducted in June, 2010. However the vehicle may strongly affect the final result and therefore this is regarded as a very sensitive parameter (Rovida, 2011).

Testing under these modified conditions resulted in cell count stimulation indices (SI) of 1.154, 0.761 and 1.034 for 10%, 25% and 50% respectively. Based on a threshold level of 1.4 (determined by Ehling, 2005b) the substance was classified as not sensitising by the registrant.

According to the TG 429 (OECD, 2002) other endpoints for assessment of proliferation may be employed provided there is justification and appropriate scientific support, including full citations and description of the methodology. Ehling (2005a) showed that LNCC is sensitive and robust. But it was also stated there that the reaction pattern of BALB/c mice were more pronounced than that of NMRI outbred mice. Therefore threshold levels were set at 1.55 and "around" 1.4 for BALB/c and NMRI respectively. An evaluation by ECVAM (Basketter, 2008) showed that further experience need to be gained with this approach, in a setting where there are no other deviations from the standard LLNA, and formal comparison of the same endpoints in the same animals are performed. Further experience in the interpretation of ear thickness measurements is also required, so that criteria can be set for judging when a positive ear swelling response can reverse the conclusion of sensitisation, based on proliferation of cells (numbers) in the LLNA.

The adequacy of the lymph node cell count (LNCC) as alternative endpoint has been evaluated several time since the recommendation from ECVAM. For these tests the recommended mice strain (CBA) has been used and the time schedule of TG 429 has been followed. Basketter, 2012 reported an accuracy of 78% for the LNCC and 83% for the LLNA. The overall agreement between LLNA and LNCC was 95% (21/22 substances). In a second approach 180 materials were assessed by both methods resulting in an overall agreement of 86% between LLNA and LNCC. Combining both datasets (n=202) yields a concordance of 87% (Kolle, 2012; Kolle, 2013).

One limitation of the LLNA is false negative predictions with certain metals (metal cations). The reason why some metals (esp. nickel) interfere with the test is still a matter of discussion (Rovida, 2011) but due to the ionic nature of sodium dithionite this has to be kept in mind.

Due to the structure of the upper layer of the skin the dermal absorption for this hydrophil substance is assumed to be very low. In addition ionized species in general do not penetrate the skin very well (WHO, 2006) but an *in vitro* study by Laudanska (2002) found that human skin is permeable to sulphate anions (SO_4^{2-}). However, for skin sensitisation the critical information is an understanding of the dose that becomes available in the viable epidermis (via the stratum corneum) to bind covalently with skin proteins to form the immunogenic complex. Therefore a distinction between penetration through the skin and a penetration to gain access to the relevant skin compartment (viable epidermis) is necessary.

Because of the amended testing protocol, major doubts on adequate contact of the test substance with the skin and the ionic nature of the test substance no final conclusion on the sensitizing property of the substance based on the provided LLNA can be drawn.

Read-across data

For the evaluation of the sensitizing property studies from related substances have been provided. A read-across concept has been presented by the registrants based on the instability in water and the resulting anions sulphites, hydrogen sulfites and metabisulfites. Sodium dithionite dissolves in water and forms sodium hydrogen sulphite, sodium hydrogen sulphate and sodium thiosulfate. Depending on the pH-value, sulphur dioxide, sodium hydrogen sulfite, sodium sulfite and sodium sulfide are present in aqueous solution. Due to the rapid conversion into various related sulphite species under physiological conditions a read across to sodium sulphite, sodium hydrogen sulphite and sodium metabisulfite is justified (OECD, 2004).

EFSA (2016) concluded that the predominant species in aqueous fluids will be bisulfite and sulphite ions and therefore, based on their capacity to form sulfite ions, read across between the different sulphite sources is possible.

In the registration data LLNAs (LNCC method) with ammonium thiosulfate, sodium sulphite and sodium metabisulfite (read-across) with the same adaptations and shortcomings as discussed above for sodium dithionite are presented. All gave negative results.

Human studies

No information on sodium dithionite itself is available but due to the broad read-across possibilities, a huge amount of human evidence from related substances is available.

Registrants list a lot of scientific literature on the sensitizing potential of sulfites. Sulfites are used in cosmetics, therefore several case reports are available showing sensitizing effects. 14 cases are presented by the registrants showing symptoms like dermatitis and eczema after the use of cosmetic products. The patients were all tested positive (patch test) for sodium metabisulfite and/or sodium sulfite or sodium bisulfite or ammonium bisulfite (Malik, 2007; Seitz, 2006; Harrison, 2002; Köhler, 2000; Tucker, 1999; Lodi,

1993; Visser-Croughs, 1988; Ikehata, 1996; Kim, 2002; Pambor, 1996 – as cited in registration data). Sulphites (as preservatives) in medical products (anesthetic solutions) can provoke allergic reactions in patients (oedema, urticaria, asthma). In 9 documented cases 7 patients were tested (Patch test, Prick test) positive for sodium metabisulfite or sodium bisulfite (Dooms-Goossens, 1989; Schwartz, 1989; Schwartz, 1985; Fisher, 1989; Meisel, 1992; Yoshikawa, 1990; Campbell, 2001; Levanti; 1996 – as cited in registration data). Skin reactions after exposure to sulfite in food are described in open literature (e.g. DeLuque, 2013; Yao, 2007). Allergic reactions to sodium metabisulfite also have been seen in a child (Vitaliti, 2015) and a newborn (Huston, 2009). Allergic contact dermatitis due to occupational exposure to sodium metabisulfite is documented e.g. by Madan (2007) or Kaaman (2010). Recent cases after occupational exposure to potassium metabisulfite are described by Stingeni (2009) and Garcia-Ortiz (2014).

In total a review by Garcia-Gavin (2012) lists 22 cases of allergic contact dermatitis due to occupational exposure to sodium metabisulfite, 14 cases due to non-occupational exposure to sodium metabisulfite, 8 cases due to non-occupational exposure to sodium sulphite, 7 cases due to exposure to other sulphites.

The relevance of sulfite contact allergy has been investigated in eight retrospective studies via patch testing. An overview is given in the table below. In summary it can be concluded that allergic contact dermatitis caused by sulfites is seen and often relevant. However there are still a significant number of cases where the relevance is unestablished or speculative (Madan, 2007).

Table 20: Retrospective studies on sulphite allergy

Method	Substance	Results	Reference
Patch test	Sodium metabisulfite (2%, 1%)	Total n=2763 4,5% pos. (n=124) →occupational n=13 →relevant in 80 cases	Garcia-Gavin, J (2012)
Patch test	Sodium metabisulfite (1%)	Total n=396 13.1% pos (n=26)	Polanska A. (2011)
Patch test	Sodium metabisulfite (2%)	Total n=1518 3.4% pos (n=51) →relevant in 3 cases (5.9%)	Kaaman A.C. (2010)
Patch test	Sodium metabisulfite (1%) Sodium sulphite (1%)	Total n=183 Sodium metabisulfite: 5,5% pos (n=10) →relevant in 8 cases (80%) Sodium sulphite 3.8% pos (n=7) →relevant in 3 cases (42.9%)	Oliphant T (2012)
Patch test	Sodium metabisulfite (1%)	Total n=1751 4.1% pos (n=71)	Madan V. (2007)

		→relevant in 47 cases →occupational n=5	
Patch test	Sodium metabisulfite	Total n=117 6.8% pos (n=8) →relevant in 4 cases (59%)	Malik M.M. (2007)
Patch test	Sodium metabisulfite (1%)	Total n=980 1.4% pos (n=14)	Angelini G. (1997)
Patch test	Sodium metabisulfite (1%)	Total n=2894 1.7% pos (n=50) →occupational n=7 →relevant in 12 cases (24%)	Vena G. A. (1994)
Patch test	Sodium sulfite	Total n=1762 1.4% pos (n=25) →relevant in 3 cases	Petersen C. S. (1992)

In a cross-sectional study Febriana (2012) investigated workers at two Indonesian tanneries. From 472 workers included occupational contact dermatitis was suspected in 77 cases. Patch tests were performed in 63 of theses workers. 13 had a positive patch test reaction to one or more tannery allergens. Two were tested positive for sodium metabisulfite.

Mode of action:

Roberts (2012) concluded that the read across substance sodium metabisulfite is an unusual but not infrequent contact allergen whose chemistry suggests a previously unrecognized protein modification mechanism: nucleophilic attack by the allergen on electrophilic S-S linkages (cystine) in skin proteins. In water sodium metabisulfite (as well as sodium dithionite) exists as a mixture of sulfite di-anions, bisulfite anions, sulfurous acid, and sulfur dioxide, in proportions depending on the pH. The pKa of bisulfite is 7.2, so, at physiological pH, a significant proportion will exist as the sulfite di-anion. The sulfite di-anion is a reactive borderline soft nucleophile, and it is considered that this is the basis of the sensitization potency of sodium metabisulfite. However, it is likely that the reaction with S-S units is rapidly reversible. Then the sensitization potency will be influenced by the extent to which the equilibrium favours the reaction product and whether the reversibly formed reaction product can react further to give a stable reaction product (Roberts, 2012).

The scientific literature gives no clear picture but some skin reactions to sulfites may be IgE-mediated. While involvement of IgE was shown e.g. by Yao (2007) or Sokol (1990) or Yang (1986) others failed to do so. Boxer (1988) demonstrated that there is evidence of IgE antibody by passive transfer for one patient studied, but no evidence of IgE antibody by ELISA to a metabisulfites-albumin conjugate in any of the four patients. This study illustrates the complexities involved in the evaluation and mechanism of sulfit-induced sensitivity.

A sensitising rather then an irritating effect is also indicated by the finding that patch test reactions (to sodium metabisulfite or potassium metabisulfite) were more dominant on day 4 than on day 2 by Madan (2007), Garcia-Gavin (2012) and Garcia-Ortiz (2014).

For the final conclusion on the skin sensitizing proterties of sodium dithionite registrants refer to the interpretation of scientific bodies:

OECD (2004) concluded that literature on allergic dermatitis after exposure to sulfites is rare (only one case study is cited there developing hand dermatitis presumably due to regular preparation of sodium sulfite – Rudzki, 1980) and therefore sodium dithionite is not considered to possess a significant skin sensitizing potential. No evaluation of read-across data has been done there. This evaluation is considered no to be relevant for this substance evaluation.

The Cosmetic Ingredient Review Expert Panel (published by Nair, 2003) stated in there evaluation of sulfites in cosmetics that skin penetration would be low due to the highly charged nature of sulfites. Their overall conclusion, based only on few studies, was that sodium sulfite, potassium sulfite, ammonium sulfite, sodium bisulfite, ammonium bisulfite, sodium metabisulfite, and potassium metabisulfite are safe as used in cosmetic formulations. Only the remark that sodium and potassium salts produced positive reactions in dermatologic patients under patch test has been done but an indepth discussion of sensitisation is missing. Therefore this evaluation was not considered to be relevant for this assessment.

The Scientific Committee for Food (1997) concluded that delayed eczematous and immediate urticarial reactions due to sulfites in foods have been demonstrated but altogether sulphites represent a rare cause of urticaria. Also clinical cases of contact allergy due to sulphites are mentioned but no final evaluation of the sensitising property of sulfites has been done by this committee.

The German MAK Commission (2014) published a MAK value documentation for sulfites. The document is based on old literature (up to 1996). The final conclusion was that many cases of contact allergies and patch test reactions to sodium metabisulfite and occassionally also to potassium metabisulfite are described. But in view of the widespread use of sodium metabisulfite, and therefore the numerous possibilities for contact in everyday life and the occupational field, the number of persons epidermally sensitized is, however, very small.

Altogether, these reviews are rather old, do not reflect the actual developments and therefore cannot be used exclusively for a scientific based evaluation of the sensitising property of sodium dithionite. Additional literature that became available since then should be reflected in the evaluation.

EFSA (2015) concluded that "there are numerous reports of sensitivity/intolerance reactions in humans exposed to sulfited solid foods and beverages" and recommend "studies on the origin and mechanisms (forms of sulfites involved) of the reactions of individuals who are sensitive or intolerant to sulphites". No final conclusion on the sensitizing property of sulphites has been done.

7.9.3.2. Respiratory sensitisation

Respiratory sensitisation is not a standard requirement under REACH but available data and information should be taken into account to evaluate this endpoint.

Animal data

No relevant data are available.

Human data

Registrants present a great amount of studies with read across substances. As the pH of the surface fluid in the respiratory tract is 6.5–7.5 appreciable amounts of both the bisulfite and the sulfite will be present (WHO, 2000a) and read across justified.

Respiratory sensitisation has been seen in a child (Vitaliti, 2015) with symptoms like bronchial asthma, allergic rhinitis and dermatitis and documented by a positive patch test for sodium metabisulfite and serum specific IgE for sulphite. Frick (1991) describes the case of oral sulfit sensitivity in a two-year old asthmatic child. Prevalence in asthmatic children may be as high as 66% (Towns, 1984); is seems to be a frequent problem in children.

Sulphites are also described as causing agent for occupational asthma. Malo (1995) reports the case of an agricultural worker, Valero (1993) the case of a patient who experienced episodes of bronchospasm after handling sodium bisulphite at work. Metabisulphite-induced occupational asthma has also been reported in a photographic technician (Jacobs, 1995) and a radiographer (Merget, 2005). Three cases of occupational asthma related to metabisulphite exposure were reported in France (Agard, 1998). Asthma cases in fishing industry are described by Madsen (2004), Steiner (2008) or Pougnet (2010). All cases showed a fall in FEV_1 during challenge. However the immunological evidence is missing in most of the cases. In a questionaire based study on the incidence of asthma in sulphite workers it was concluded that repeated events of peak exposure to an irritant gas, such as SO_2, causes a three-fold increased incidence of asthma (Andersson, 2006).

The prevalence of sulfite sensitivity in the general population is unknown but prevalence of sulfite sensitivity in asthmatics has been investigated several times indicating prevalences between 3-10% (see table below). Steroid dependent asthmatics appear to be at greater risk. There are some indications that respiratory sensitivity to sulphites may be more common amongst women and children (Vally, 2012).

Table 21: Prevalence of sulphite sensitivity in asthmatics

Investigated asthmatics	Challenge	Results	Additional information	Reference
203 adults • 120 non-steroid dependent • 83 steroid dependent	Combined: capsule and solution	pos n=21 • nonsteroid dependent (n=5) • steroid dependent (n=16)	8.4% of steroid-dependent asthmatics are sulfite sensitive prevalence of 0.8% for the 120 non-steroid-dependent asthmatics studied	Bush R. K. (1986)
adults n=134	Capsule challenge with potassium metabisulfite	pos n=50 (37%)	minimum 4,6% prevalence	Buckley C.E. (1985)
adults n=61	Capsule challenge with metabisulfite, following metabisulfite solution	pos n=5	8.2%	Simon R. A. (1982)

adults n=15	Capsule challenge with metabisulfite	pos n=1	Prevalence 7%	Koepke (1982) – as cited in Bush R. K., (1986)
children n=29	5/15/50mg metabisulfite in 0.5% citric acid	pos n=19	66% incidence No reaction to metabisulfite in capsules	Towns S. J. (1984)
children n=65	Potassium bisulfite in capsules or in citric acid solution	capsules: pos n=4 solution n=2	All neg in skin prick test Children only had mild chronic asthma	Boner A. L. (1990)
children n=51	metabisulfite in acid solution	pos n=18 (35.3%)	-	Friedman M. E. (2009) Abstract only

Asthma-related mortality among sulfit mill workers has been seen probably as a consequence of repeated exposures to peak concentrations of SO_2. Andersson (1998) showed an increased mortality from asthma and chronic obstructive pulmonary disease with an odds ratio of 1.6 (90% CI 0.9-3.0). When the analysis was restricted to asthma only the risk increased (OR 2.8, 90% CI 1.1-6.8). The exposure assessment is a limitation of this study as it was based upon job titles, and no information on co-exposure to other substances then sulphur dioxide is given. Data from a pervious study on pulp and paper mill workers also show an increased risk for asthma (OR 1.9, 95% CI 0.95-4.0) (Wingren, 1991 as cited in Andersson 1998).

The German MAK commission (2014) concluded that ingested or inhaled sodium metabisulfite can lead in predisposed persons to urticaria and bronchospasms. An immunological pathogenesis has not been proven for these reactions and is assumed—if at all—only for a small minority of affected persons. Sodium metabisulfite is, therefore, not regarded as a respiratory allergen by the MAK commission.

In 2016 EFSA did a reevaluation of sulphites used as food additives (sulphur dioxide, sodium sulphite, sodium bisulfite, sodium metabisulfite, potassium metabisulfite, calcium sulphite, calcium bisulfite and potassium bisulfite). It was concluded that most sulphite sensitivities are not true allergic reactions and the mechanism of sulphite 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 or may stimulate the parasympathetic system and provoke cholinergic-dependent bronchoconstriction. The EFSA NDA panel 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 also recommended "*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".*

Mode of action

Immunological mechanisms for respiratory sensitisation do not have to be clarified for a possible classification (CLP guidance, Chapter 3.4.2.1.3.) but in the literature several

attempts can be found. Cosmetic Ingredient Review Expert Panel (published by Nair, 2003) stated that many asthmatics with bisulfite sensitivity have negative allergy skin tests suggesting a non-atopic nature. The lack of an IgE mechanism is suggested.

From the current state of knowledge it is unlikely that a single mechanism can explain the reaction to sulfites. Potential mechanisms are (Vally, 2012):

- Stimulation of the parasympathetic system (bronchoconstriction mediated by a cholinergic pathway)
- release of calcitonin gene-related peptide (CGRP) from capsaicin-sensitive sensory nerves
- Sulphite oxidase deficiency (oxidation of sulfite to form the inactive sulfate)
- Sulfite mediated release of histamine of other mediators as a consequence of mast cell degranulation through IgE or non-IgE mediated mechanisms.
- Role of prostaglandins or leukotriens

Asthmatic reactions to sulfit-containing foods are known and it is postulated that sulphur dioxide, generated from ingested sulfites may cause respiratory symptoms. Allen (1985) tried to clarify the mechanism by oral challenge with sulphite in citric acid solution, liberating significant amounts of sulphur dioxide. Dosing in capsules showed that in the stomach they liberate sufficient quantities of sulphur dioxide to produce asthma by eructation and subsequent inhalation. Challenge with mouthwash demonstrated that also inhalation of sulphur dioxide during ingestion is a possible mechanism (Wright, 1990; Taylor, 1986; Allen, 1985). However, sulphur dioxide is not classified for sensitizing properties or respiratory tract irritation up to now.

Asthmatics are general more sensitive to inhaled sulphur dioxide than non-asthmatic normal subjects, while steroid dependent asthmathics may be at greater risk. According to ATSDR (1998) sensitive asthmatics may respond to concentrations of sulphur dioxides as low as 0.1 ppm. Healthy non-asthmatics respond to higher concentrations of sulfur dioxide (≥1.0 ppm). Factors that can exacerbate the respiratory effects of sulfur dioxide include exercise and breathing of dry or cold air.

Skin sensitisation

7.9.3.3. Conclusion of skin and respiratory sensitisation:

The provided LLNAs are negative with sodium dithionite and further read-across substances. A final conclusion on the validity of the study cannot be drawn due to uncertainty concerning adequate skin contact and amendments in the test protocol. Available tests with read-across substances were conducted under equal test conditions; therefore, these results cannot be used to substantiate the adequacy of the modified LLNA with sodium dithionite.

According to regulation (EU) 2016/1688 amending REACH regulation (EC) 1907/2006 *in vitro* methods are recommended for the testing of chemicals on their sensitising properties. An *in vivo* study shall be conducted only if in vitro/in chemico test methods described under point 8.3.1 are not applicable, or the results obtained from those studies are not adequate for classification and risk assessment according to point 8.3".

Based on discussion with registrants the following conclusions for *in vitro* testing can be drawn:

- The DRPA assay is outside the applicability as the substance is a reducing agent (rapid reaction of sulfite anions with cysteine-containing proteins, see also Roberts, 2012) and a metal-like compound. The result can be predicted to be false positive.

- The KeratinoSens™ test method is predicted to be negative. Based on the chemical structure of sodium dithionite a reaction with the Keap1-protein is not expected.
- h-CLAT method seems to be possible for sodium dithionite, however, based on this result no sound decision on the sensitizing propterty of the substance can be built.

Most of the available *in vitro/in chemico* test methods are not applicable for the substance and the results obtained from those studies will not be adequate for classification and risk assessment based on a weight of evidence approach.

In case the available *in vitro/in chemico* test methods are not applicable for the substance an *in vivo* study shall be conducted. The local lymph node assay (LLNA, OECD 426) shall be applied as first-choice method for new *in vivo* testing (ECHA-Guidance on information requirements and chemical safety assessment chapter R.7.3). To ensure that the hydrophilic substance sodium dithionite is incorporated into a vehicle system which wets the skin and does not immediately run off, an aqueous solution with an appropriate solubiliser (e.g. 1% Pluronic® L92) is recommended.

As a follow up of this evaluation the registrants were asked to conduct a test on surface tension/wettability with sodium dithionite in water and sodium dithionite in water plus Pluronic L92 (as recommended in OECD 429, 2010). Based on these results a decision on further testing shall be drawn.

Respiratory sensitisation

For the evaluation of this endpoint no animal studies are available but human evidence with read-across substances show some indications. Occupational asthma, asthmatic reactions to sulfit-containing foods as well as sulphite sensitivity in asthmatics is documented in literature. There are indications of possible modes of actions, however, immunological mechanisms for respiratory sensitisation are not fully clarified to prove respiratory sensitisation.

As no standard testing procedure is available for this endpoint the available data has to be used in a weight of evidence approach. Currently a proposal for harmonised classification for sulphur dioxide, considering also respiratory sensitisation, has been submitted and will be discussed in RAC. For a final conclusion on sodium dithionite this discussion has to be awaited.

7.9.4. Repeated dose toxicity

7.9.4.1. Repeated dose toxicity: oral

Table 22: Overview of experimental studies on repeated dose toxicity: oral

Method	Results	Remarks	Reference
rat (Wistar) male/female combined repeated dose and reproduction toxicity (oral: feed) 0.125 % 0.25, 0.5, 1, 2% nominal in the diet Corresponding to 50, 110, 220, 460, 960 mg/kg bw (nominal in diet) Exposure regime: Exposure period: 104 weeks (F0 and F1 generation) and 30 weeks (F2 generation) Premating exposure period males and females: 21 weeks Duration of test: until the weaning of the F3 animals	NOAEL for local effects: 0.25% or 0.215% due to the loss of disodium disulfite ~ 108 mg/kg bw/day or 72 mg/kg SO2/day (nominal) (male/female). Based on the occurrence of occult blood in the faeces and changes in gastric morphology at dose levels of 0.5% (220 mg/kg bw/d or 72 mg SO2/kg bw/day)	2 (reliable with restrictions) key study Test material: disodium disulfite	Til, H.P., et al. (1972a)
three-generation feeding study, 20 male and 20 female Wistar rats dose levels: 0, 0.125, 0.25, 0.5, 1.0 and 2.0% sodium metabisulfite, i.e. 49, 108, 220, 460, and 955 mg/kg bw/d as actual dose in a thiamine-containing diet over periods of 2 years.	NOAEL (systemic effects): > 955 mg/kg bw/day (nominal) (male/female) based on: test mat. (No signs of systemic toxicity were observed and the NOAEL can be expected above the highest dose of 2% metabisulfite corresponding to 955 mg/kg bw/d Na2S2O5 (or 640 mg/kg bw/d as SO2 equivalents).)	Test material: disodium disulfite (sodium metabisulfite)	Unpublished study report (2001)
rat (Wistar) male/female chronic (oral: in the diet short term (10- 56 days): 1.0, 2.0 % Long term: for and 8, 12 and 24 months 0.125, 0.25, 0.5, 1.0, 2.0 % for 8, 12 and 24 months Pathological and microscopic examinations of the stomach of the rats were performed after treatment periods of 10, 28 and 56 days (short-term) and after 8, 12 and 24 months.	NOAEL (local toxicity): 108 mg/kg bw/day (nominal) (male/female):_ based on the **induction of hyperplastic and inflammatory changes in the forestomach at dietary levels of 0.5%** and higher, NOAEL: 0.25% (corrected to 0.215% based on analytical verifications) ~ 108 mg/kg bw/d Na2S2O5 (~ 72 mg/kg bw/d as SO2 equivalents).)	2 (reliable with restrictions) supporting study Test material: disodium disulfite	Feron, V.J.; Wensvoort, P.(2001)

rat (Osborne-Mendel) male/female chronic (oral: feed) Exp. 1: 0.5, 1.0 and 2.0% NaHSO3 Exp. 2: 0, 0.1, 0.25, 1.0 and 2.0% NaHSO3 Exp. 3: 0.0125, 0.025 and 0.05 NaHSO3 Exposure: - Experiment 1: 1 year - Experiment 2: 1.5 year - Experiment 3: 2 years Objectives: to detect toxic effects of sodium hydrogensulfite and effects of thiamine supplementation.	NOAEL: 0.05 % based on: NOAEL: 0.05% NaHSO3 (corresponding to 25 mg/kg bw/d or 16 mg/kg bw/d as SO2)	2 (reliable with restrictions) supporting study Test material: sodium hydrogensulfite	Fitzhugh, G.O.; et al. (1946)
rat (Sprague-Dawley) female subacute (oral: drinking water) Six groups of rats (3 groups of normal animals and 3 sulfite oxidase-deficient groups) received Na2S2O5 in the drinking water. metabisulfite doses equivalent to 7 and 70 mg/kg bw/d SO2 were administered for 8 weeks and a metabisulfite dose equivalent of 350 mg/kg bw/day SO2 for 3 weeks followed by 175 mg/kg bw/d SO2 (metabisulfite equivalent) for 5 weeks The diet was fortified with 50 ppm thiamine. Haematological and urinary assays were performed and urine samples were analyzed for sulfite and thiosulfate. Body weights, consumption of food and water were measured, and at the end of the dosing period, all animals were killed, autopsied and histopathological examinations were performed Sulfite oxidase activity and thiamine levels were determined in liver samples.	NOAEL (local toxicity): 70 mg/kg bw/day (actual dose received) (female) based on: SO2 equivalent to Na2S2O5 (104 mg/kg bw/d) (At the highest dose level 350/175 mg SO2/kg bw/d, gastric lesions of the forestomach were noted histologically in both normal and enzyme-deficient rats.)	2 (reliable with restrictions) supporting study Test material: disodium disulfite	Hui, J.Y.; et al. (1989) Unpublished study report (1999)

Three generation study rat (uniform strain bred for cancer research) male/female combined repeated dose and reproduction 375 and 750 ppm SO2 as sodium metabisulfite (nominal in water) Exposure: Up to 2.5 years (over 3 generations) before and throughout mating and during pregnancy and lactation Generation I consisted of three groups: group 1 (tap water controls), group 2 (750 ppm as SO2) and group 3 (375 ppm as SO2). The metabisulfite drinking water groups of generation II were produced from matings of the sulfite drinking water groups of generation I. Generation III was derived similarly from generation II. Observations on growth, feed consumption, fluid intake, faecal output, reproduction, lactation and the incidence of tumours were recorded. Detailed post-mortem and histological examinations were made.	no NOAEL identified: > 53 mg/kg bw/day (nominal) (male/female) conclusion of authors: no evidence of systemic toxicity	2 (reliable with restrictions) Test material: disodium disulfite	Lockett, M.F.; Natoff, I.L.(1960)

Virgin female Wistar rats combined repeated dose and reproduction / developmental screening experiment 1: sulfite-oxidase-deficient rats 9 weeks oral; in drinking water: 25, 50 mM Na2S2O5 Experiment 2: Normal rats: 3 weeks nominal in diet: 1 % powdered Na2S2O5) 2 % powdered Na2S2O5 6 % powdered Na2S2O5 50 ppm thiamine was added to the metabisulfite diets Toxicity resulting from exposure to sulfite originating both endogenously and exogenously was investigated. parameters measured: haemoglobin, red blood cell glutathione, prothrombin time, haematocrit (Hc), hepatic thiamine and tissue S-sulphonates (RS-SO3-).	4/149 sulfite-oxidase activity deficient rats developed mammary tumours in the age <5 months compared to 0/143 rats with normal sulphite oxidase. Result not statistically significant but seems due to the rarity of spontaneous tumours in these rats treatment related. Further it was concluded that anaemia und thiamine deficiency result from high concentrations of sulphite in the diet and in the gut and not from systemic sulphite exposure.	2 (reliable with restrictions) supporting study Test material disodium disulfite	Gunnison (1981)
Chronic toxicity rat (Wistar) male/female 40 and 80 mg/kg bw/d sodium bisulfite (actual ingested) 80 mg/kg bw/d sodium bisulfite + 40 mg/kg bw/d benzoic acid (actual ingested) (50 males and females) Exposure: 18 months (daily) in a paste prior to feed Food and water consumption, weight gain and the effects of stress factors were recorded. In addition, haematology and blood chemistry and kidney function were studied. Poisoning with carbon tetrachloride was analyzed, too.	Cold stress, centrifugation and kidney loading wieh K2HPO4 had most effect in rats given the benzoic acid/sodium bisulphite combination. Also weight gain was less in these animals. Effects of tetrachloride: 20% of the animals treated with benzoic acid/sodium bisulphite combination showed signs of haemorrhagic keratoconjunctivitis, which developed 2 months after the beginning of the study. The toxic effects of bencoic acid and sodium bisulfite when administered in combination appeared to be synergistic or additive to some extent.	2 (reliable with restrictions) supporting study Test material: sodium hydrogensulfite	Shtenberg, A.J.; Ignatev, A.D. (1970)

Subchronic, oral study 48 weeks pig (Dutch Landrace) male/female groups of 40 pigs 0.06, 0.16, 0.35, 0.83, 1.72% Na2S2O5 (in diet, 2x/day)) in the first 3 weeks Na2SO3 7H2O was used administered in a thiamine-supplemented diet (50 mg/kg diet). After 15 weeks of treatment, 14 males and 14 females from of each group were killed. The remaining pigs were kept on the same diets for up to 48 weeks. Food consumption was measured during the whole period. Haematological studies and determination of occult blood in faeces and of thiamine levels were performed. Examination was made for macroscopic and microscopic abnormalities in all pigs of the 0, 0.5 and 2.0% groups killed at 15 weeks and in pigs of all groups slaughtered after week 48.	NOAEL: 0.35 % (male/female) based on: Inflammatory and hyperplastic changes of the gastric mucosa were observed in several animals fed 0.83 or 1.72% Na2S2O5 in the diet.	2 (reliable with restrictions) supporting study Test material: disodium disulfite	Til, H.P.; et al. (1972)
mouse (cross-bred white mice) male/female subchronic 90 days (oral: gavage) 160 mg/kg bw/d sodium bisulfite, 80 mg/kg bw/d benzoic acid (actual ingested) 160 mg/kg bw/d sodium bisulfite + 80 mg/kg bw/d benzoic acid (actual ingested) Survival, food consumption and weight gain were recorded daily. The effects of dietary restriction, physical stress and poisoning with carbon tetrachloride (0.1 ml per mouse by oral intubation) were analyzed and compared with untreated controls. At the end, a co-carcinogenicity test with Ehrlich ascites carcinoma (EAC) was performed (transplantation of EAC intraperitoneally	The highest mortality rates were in the benzoic acid/sodium bisulphite combination This effect was more pronounced in the food restriction group: (90% food restriction after 3 month treatment: mortality 83.3% versus 56.3% in the control group Also the effect of carbon tetrachloride on the mortality was more intense in the sodium bisulphite group (45% versus 28.6% in the control group) Cocarcinogenicity assay: After the feeding of preservative diets for 17 month 8/100 mice in the benzoic acid/ sodium bisulphite group of the first generation and 1/8 in the third generation of the same group whereas no tumours were found in the control group Ehrlich ascites carcinoma test: the tumour growth was greatest in mice that received sodium bisulphite	2 (reliable with restrictions) supporting study Test material: sodium hydrogensulfite	Shtenberg, A.J.; Ignatev, A.D. (1970)

Chronic study: 17 month mouse (cross-bred white mice) male/female groups of 25 male and 25 females 80 mg/kg bw/d sodium bisulfite (actual ingested) 40 mg/kg bw/d benzoic acid (actual ingested) 80 mg/kg bw/d sodium bisulfite + 40 mg/kg bw/d benzoic acid (actual ingested) in a paste prior to feed Exposure: 17 months (daily) The same experiment (series II) was repeated with slightly heavier mice. Survival, food consumption and weight gain were recorded daily. The effects of starvation, physical stress and poisoning with carbon tetrachloride (0.1 ml per mouse) were analyzed and compared with untreated controls. After 8 months on the combination or control diets some animals were mated and effects on body weight gain of offspring were studied for 3.5 months over four generations.	Adverse effects on growth, survival and susceptibility to stress were reported. The highest mortality rates were in the benzoic acid/sodium bisulphite combination This effect was more pronounced in the food restriction group: (100% food restriction after 17 month treatment: mortality 51.5% versus 12.5 in the control group) The authors recommended that the use of benzoate and sulphite as preservatives should be further restricted.	2 (reliable with restrictions) supporting study Test material: sodium hydrogensulfite	Shtenberg, A.J.; Ignatev, A.D. (1970)

7.9.4.2. Repeated dose toxicity: inhalation

Table 23: Overview of experimental studies on repeated dose toxicity: inhalation

Method	Results	Remarks	Reference
rat (Sprague-Dawley) male combined repeated dose and carcinogenicity (inhalation: gas) (whole body) BaP treatment: 15 consecutive weekly intratracheal instillations Some of the rats were treated with 0, 10 and 30 ppm SO2 (nominal conc. in air) Exposure: 21 weeks (101 exposure days) (6 hr per day, 5 days per weeks) Or exposed to sulphite/bisulfite anions that accumulated systemically from endogenous generation in rats with induced sulphite oxidase deficiency by means of high tungsten to molybdenum ratio in the diet. Thereafter, the rats were observed for the development of tumours in the respiratory tract for 737 days. Complete necropsy was performed on all animals with particular attention given to the respiratory tract.	BaP treated rats began to die with squamous cell carcinoma(SQCA) of the respiratory tract at approximately 200 days after the first treatment, 2 years after the first treatment almost all rats in the BaP treated rats died, most with SQCA. The authors concluded that neither inhalation exposure to SO2 nor systemic exposure to sulphite/bisulfite anions affected the induction of SQCA of the lung by intrachtracheally instilled BaP.	2 (reliable with restrictions) supporting study Test material sulfur dioxide	Gunnison, A.F.; et al. (1988)
Dog (Beagle) chronic (inhalation: aerosol) (whole body) 1mg Na2S2O5 /m3 (nominal conc.in air) Exposure: 320 days exposure to clean air (control period) followed by 290 days exposure to Na2S2O5 aerosol Eight Beagle dogs Selected parameters of lung clearance, biochemistry, cytology, and morphology were determined during both periods to find sensitive parameters for early changes of lung function. Only changes of lung clearance parameters are described in this paper.	Three dogs showed significant changes in clearance rate of moderately soluble particles during the sulphite exposure compared to the clearance rate during clean air exposure. The results were confirmed by in vitro clearance measurements in alveolar macrophages.	2 (reliable with restrictions) supporting study Test material disodium disulfite	Ferron, G.A.; et al. (1990)

dog (Beagle) subchronic (inhalation: aerosol) (whole body) 1 mg Na2S2O5 /m3 (nominal conc.in air) (equivalent to 0.6 mg/m3 as SO2) exposure period: 290 days The aerosol was generated from an aqueous 3.5% solution of Na2S2O5 adjusted to pH 7. Three dogs served as control. After sacrifice the nasal cavity was prepared for histological examination.	Morphologic changes were observed in nasal cavities of beagle dogs after long-term exposure to a respirable sulfur(W) aerosol. The changes were characterized by a thickened epithelial layer resulting from epithelial proliferation, by a loss of secretory material, and by moderate mononuclear cell infiltration. Key study	2 (reliable with restrictions) supporting study Test material: disodium disulfite	Takenaka, S.; et al. (1994)

Results of the evaluation of the EFSA Contam-Panel

The EFASA Contam Panel has re-evaluated the oral toxicity of sulphur dioxide-sulfites (sulphur dioxide, sodium sulphite, sodium bisulfite, sodium metabisulfite, potassium metabisulfite, calcium sulfite calcium bisulfite, potassium bisulfite) in 2016. They confirm that 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 a possible tumour promoting activity of sulphites in the pylorus of the glandular stomach which was reported in two initiation-promotion studies in rats, which may be related to hyperplasia of the fundic glands induced by sodium metabisulfite.

The panel addressed various uncertainties from the toxicological data base: 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.

Summary on Repeated dose toxicity

The NOAEL identified in repeated dose toxicity experiments with the read across compounds was based on the induction of hyperplastic and inflammatory changes in the forestomach of rats at dietary levels of 0.5% and higher and was calculated with 108 mg/kg body weight/day or 72 mg/kg bw/day respectively. In pigs mucosal folds in the stomach and black colouration of the caecal mucosa was observed with a diet containing 0.83 and 1.72% sodium metabisulfite, hyperplasia of the mucosal glands, intraepithelial microabscesses, epithel hyperplasia and accumulations of neutrphilic leucocytes in papillae tips were observed in the pars oesophaga.

As depicted above animal experiments with sulfite oxidase (SOX) deficient rats have shown that SOX deficient animals are more susceptible against sulphite exposure than normal rats, these findings include enhanced mortality after sulphite exposure under stress and restriction of diets (Gunnison et al. 1977) as well as development of mammary tumours or damage of testes (Gunnison et al. 1987). Another aspect is that SOX was also found to be lower in the liver of young versus mature rats, in 1- day old rats the SOX activity was approximately 1/10 the level of adult rats (Cohen et al., 1974). Also patients with Chronic Renal failure suffer from elevated levels of serum sulphite.

It has to be pointed out that several uncertainties regarding repeated dose toxicity appear. Many data were obtained from toxicity studies with possible confounding factors, diet with thiamine supplementation, which may induce formation of complexes with sulphites and a resulting modification of their biological effects; or sulphites administered in solution in water, which might modify their stability and /or reactivity (EFSA, 2016).

Further, numerous publications, from non-regulatory studies, have reported biological effects of SO2, sulfites and bisulfites in various cell models and in vivo, which may indicate the possibility of adverse effects. The panel concluded that 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.

Conclusion

There are no animal studies with sodium dithionite available, neither for oral, nor inhalation or dermal exposure route.

The registrants use data waiving according to regulation (EC) 1907/2006, Annex XI. The eMSCA concludes that due to animal welfare reasons it is not justified to initiate animal testing at this stage and concludes the available information for repeated exposure is acceptable and sufficient for evaluation under this framework.

The requirements for STOT-RE classification criteria according to regulation (EC) 1272/2008 as specific target organ toxicant (STOT) – repeated exposure, oral are not met, and no classification as specific target organ toxicant is required.

It is further concluded that no classification for specific target organ toxicant (STOT) – repeated exposure, dermal according to regulation (EC) 1272/2008 is required for this substance.

It is further concluded that no classification for specific target organ toxicant (STOT) – repeated exposure, after inhalation exposure according to regulation (EC) 1272/2008 is required for this substance.

7.9.5. Mutagenicity

Very recently a CLH report for sulphur dioxide (SO2) has been published (BAuA, 2020). As the substance evalutation of sodium dithionit relies on the read across concept proposed by the registrants, with SO2 as one of the transformation products, this report needs to be taken ino consideration.

The authors conclude that that the available data indicate a genotoxic potential of SO2.They propose a classification of Muta.2 for SO2 based on positive evidence obtained from experiments in mammals supported by some in vitro findings. In addistion, there is some indication for genotocxicity in lymphocytes of exposed workers. Also strand-breaking activity in testes in an in vivo comet assay and genotoxic effects in occupational studies (BAuA, 2020). They also refer to the California EPA which indicated that there was considerable evidence that air pollution with SO2 induces DNA damage in human sperm as well as other cell types. The data from animal studies are also indicative of oxidative damage, including DNA damage in the testes caused by exposure to SO2 (California EPA, 2011).

Overview of experimental *in vitro* genotoxicity studies is given in the table below.

7.9.5.1. In vitro data

Table 24: Overview of experimental *in vitro* genotoxicity studies

Method	Description of results	outcome +/-	Remarks	Reference
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) OECD Guideline 471 S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) Test concentrations: 0, 20, 100, 500, 2500, 5000 µg/plate Positive control substance(s): dissolved in DMSO: 2- Aminoanthracene; 10 µg; N-Methyl-N'-nitro-N-nitrosoguanidine; 5 µg; 4-Nitro-o-phenylendiamine; 10 µg; 9-Aminoacridine chloride monohydrate; 100 µg;	Evaluation of results: negative Test results: negative for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100(all strains/cell types tested) ; met. act.: with and without ; cytotoxicity: no vehicle controls valid: yes; positive controls valid: yes	-	2 (reliable with restrictions) key study Test material: sodium dithionite	Unpublished study report (1989a)
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) equivalent or similar to OECD Guideline 471 S. typhimurium TA 1535, TA 1537, TA 98 and TA 100; TA1538 (met. act.: with and without) E. coli WP2 uvr A (met. act.: with and without) Test concentrations: 0.3, 3.3, 33.3, 100, 333.3, 1000, 3333.3 and 10000 µg/plate Positive control substance(s): sodium azide (1 µg) , 9-aminoacridine (100 µg), 2-nitrofluorene (10 µg), substance(s): 2-anthramine; 2.5 µg 2-anthramine; 10 and 2.5 µg	Test results: negative for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100, TA 1538 (all strains/cell types tested) ; met. act.: with and without ; cytotoxicity: yes (Toxicity was observed on TA 1535: -S9, the toxic ranged between 100 and 10000 µg/plate; +S9, the test substance was toxic at 10000 µg/plate. On TA 100, toxicity was observed -S9 only at the highest doses tested.) ; vehicle controls valid: yes; positive controls valid: yes	-	2 (reliable with restrictions) key study Test material disodium disulfite	Simmon, V.F.; Eckford, S.L. (1978)

bacterial reverse mutation assay (e.g. Ames test) (gene mutation) OECD Guideline 471 S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) E. coli WP2 uvr A (met. act.: with and without) Test concentrations: 75, 200, 600, 1800 and 5000 µg/plate Positive control substance(s): 2-aminoanthracene; 1.0 and 10 µg/plate , 2-nitrofluorene (1.0 µg/plate), sodium azide (1.0 µg/plate, 9-aminoacridine (75 µg/plate), methylmethanesulfonate (1000 µg/plate)	Test results: negative (No positive responses were observed with any of the tester strains at any tested dose level.) for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100(all strains/cell types tested) ; met. act.: with and without ; cytotoxicity: no vehicle controls valid: yes; negative controls valid: yes; positive controls valid: yes	–	1 (reliable without restriction) key study Test material (CAS number): 7783-18-8 ammonium thiosulfate	Wagner, V.O.; Klug, M.L. (2001)
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) equivalent or similar to OECD Guideline 471 S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) E. coli WP2 uvr A (met. act.: with and without) Test concentrations: at least six doses: 33.3, 100, 333.3, 1000, 3333.3, 10000 µg/plate Positive control substance(s): sodium azide (1.0 µg/plate), 2-nitrofluorene (5.0 µg/plate) , 9-aminoacridine (50.0 µg/plate) , AF2; 0.1 µg/plate , 2-anthramine; 1.0, 2.5 or 10.0 µg/plate, 2-anthramine; 1.0, 2.5 or 10.0 µg/plate	Test results: negative (No mutagenicity was observed on compound F76-020 in any of the assays performed.) for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100(all strains/cell types tested) ; met. act.: with and without ; cytotoxicity: no vehicle controls valid: yes; negative controls valid, positive controls valid: yes	–	2 (reliable with restrictions) key study Test materia:l disodium trioxidosulfidosulfate(2-), pentahydrate	Mortelmans, K.E.; Tanaka, W. (1979)
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) S. typhimurium TA 1538 (met. act.: with and without) Dose levels tested were selected based on the results of the dose rangefinding study. Main tests: 333, 667, 1000, 3330, 6670 and 10000 µg/plate. Positive control substance(s): 2-aminoanthracene; 2.5 µg/plate, 2-nitrofluorene (1.0 µg/plate), sodium azide (2.0 µg/plate), ICR-191; 2.0 µg/plate EPA OPP 84-2	Test results: negative (All data were acceptable and no positive increase in the number of histidine revertants per plate was observed with any of the tester strains.) for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100(all strains/cell types tested) ; met. act.: with and without ; cytotoxicity: no ; vehicle controls valid: yes; negative controls valid: yes; positive controls valid: yes	–	3 (not reliable) supporting study Test material ammonium thiosulfate	Lawlor, T. E.; Valentine, D.C. (1989)

OECD Guideline 471 Bacterial Reverse Mutation Assay: (e.g. Ames test) (gene mutation) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) S. typhimurium, other: TA 1538 (met. act.: with and without) E. coli WP2 uvr A (met. act.with and without) Test concentrations: 0.3, 3.3, 33.3, 100, 333.3, 1000, 3333.3 and 10000 µg/plate Positive control substance(s): sodium azide (1 µg), 9-aminoacridine (100 µg), 2-nitrofluorene (10 µg), 2-anthramine; 2.5 µg, 2-anthramine; 10 and 2.5 µg	Test results: negative No mutagenic activity was observed in the assays performed with sodium metabisulfite. for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100(all strains/cell types tested) ; met. act.: with and without ; cytotoxicity: yes at highest dose levels	–	1 (reliable without restriction) Test material disodium disulfite	Simmon, V.F.; Eckford, S.L. (1978)
OECD Guideline 471 (Bacterial Reverse Mutation Assay) bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) Test concentrations: 0, 20, 100, 500, 2500, 5000 ug/plate Positive control substance(s) (dissolved in DMSO) : N-methyl-N'-nitro-N-nitrosoguanidine, 4-nitro-o-phenylendiamine; 10 µg, 9-aminoacridine chloride monohydrate; 100 µg, 2-aminoanthracene; 10 µg	Test results: negative (No bacteriotoxic effect was observed.) for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100(all strains/cell types tested (An increase in the number of his+ revertants was not observed both in the standard plate test and in the preincubation test either without S9- mix or after the addition of a metabolising system.)) ; met. act.: with and without ; cytotoxicity: no ; vehicle controls valid: yes; negative controls valid: not examined; positive controls valid: yes	–	1 (reliable without restriction) Test material sodium sulfite	Unpublished study report (1989b)
OECD Guideline 471 (Bacterial Reverse Mutation Assay) (e.g. Ames test) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) Test concentrations: 20, 100, 500, 2500, 5000 µ/plate Positive control substance(s): (dissolved in DMSO) 2-aminoanthracene; 10 µg, N-methyl-N-nitro-N-nitroso-guanidine (MNNG); 5 µg, 4-nitro-o-phenylendiamine; 10 µ 9-aminoacridine chloride monohydrate; 100 µg	Test results: negative (No increase in the number of his+ revertants was observed.) for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100(all strains/cell types tested) ; met. act.: with and without ; cytotoxicity: yes (A slight decrease in the number of his+ revertants was only observed in the standard plate test without S9-mix at 5000 µg/plate with TA 100 .) ; vehicle controls valid: yes; negative controls valid: positive controls valid: yes	–	1 (reliable without restriction) Test material potassium sulfite	Unpublished study report (1989c)

OECD Guideline 471 (Bacterial Reverse Mutation Assay) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) Test concentrations: 0, 20, 100, 500, 2500, 5000 µg/plate Positive control substance(s): dissolved in DMSO: 2-Aminoanthracene; 10 µg, N-Methyl-N'-nitro-N-nitrosoguanidine; 5 µg, 4-Nitro-o-phenylendiamine; 10 µg; 9-Aminoacridine chloride monohydrate; 100 µg;	Evaluation of results: negative Test results: negative (No increase in the number of his+ revertants was observed.) for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100(all strains/cell types tested) ; met. act.: with and without ; cytotoxicity: no (No bacteriotoxic effect (reduced his- background growth) was observed.) ; vehicle controls valid: yes; negative controls valid: not examined; positive controls valid: yes	–	1 (reliable without restriction) Test material sodium dithionite	Unpublished study report (1989d)
OECD Guideline 471 (Bacterial Reverse Mutation Assay) bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) Test concentrations: 0, 20, 100, 500, 2500 and 5000 µg/plate Positive control substance(s): (dissolved in DMSO): 2-aminoanthracene; 10 µg, N-methyl-N-nitro-N-nitrosoguanidine; 5 µg, 4-nitro-o-phenylendiamine; 10 µg, 9-aminoacridine (100 µg	Test results: negative (No increase in the number of his+ revertants.) for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100(all strains/cell types tested) met. act.: with and without cytotoxicity: no (No bacteriotoxic effect (reduced his- background growth) was observed.) ; vehicle controls valid: yes; negative controls valid: not examined; positive controls valid: yes	–	1 (reliable without restriction) Test material Disodium disulfite	Unpublished study report (1989e) OECD (2004)
OECD Guideline 471 (Bacterial Reverse Mutation Assay)(e.g. Ames test) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) Test concentrations: 0, 20, 100, 500, 2500 and 5000 µg/plate Positive control substance(s) (dissolved in DMSO): 2-aminoanthracene; 10 µg, N-methyl-N-nitro-N-nitrosoguanidine (MNNG); 5 µg, 4-nitro-o-phenylendiamine; 10 µg, 9-aminoacridine; 100 µg	Test results: negative (No increase in the number of his+ revertants.) for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100(all strains/cell types tested (Standard plate test and pre-incubation test with Salmonella typhimurium TA 1535, TA 100, TA 1537 and TA 98)) ; met. act.: with and without ; cytotoxicity: yes (A weakly bacteriotoxic effect (slight decrease in the number of his revertant) was observed only using TA 100 at doses >2500µg/plate.) ; vehicle controls valid: yes; negative controls valid: not examined; positive controls valid: yes	–	1 (reliable without restriction) Test material dipotassium disulfite	Unpublished study report (1989f)

bacterial reverse mutation assay (e.g. Ames test) (gene mutation) according to the method of Ames, McCann & Yamasaki (1975) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) S. typhimurium, other: TA 92 and TA 94 (met. act.: with and without) Test concentrations: six test concentrations, up to 50 mg/plate (highest non-cytotoxic dose)	Test results: negative for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100(all strains/cell types tested) ; met. act.: with and without negative for S. typhimurium, other: TA 92 and TA 94(all strains/cell types tested) ; met. act.: with and without	–	2 (reliable with restrictions) Test material Disodium disulfite	Ishidate, M. et al. (1984)
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) S. typhimurium, other: TA 92 and TA 94 (met. act.: with and without) Test concentrations: six concentrations, up to 3.0 mg/plate (highest non-cytotoxic dose) Reverse mutation assays using Salmonella typhimurium strains TA92, TA1535, TA100, TA1537, TA94 and TA98 were carried out according to the method of Ames, McCann & Yamasaki (1975)*. *Reference: - Ames B.N., mcCann J. & Yamasaki E. (1975).	Test results: negative for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100(all strains/cell types tested) ; met. act.: with and without negative for S. typhimurium, other: TA 92 and TA 94(all strains/cell types tested) ; met. act.: with and without	–	2 (reliable with restrictions) Test material dipotassium disulphite	Ishidate, M.et al. (1984)
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) S. typhimurium, other: TA 92 and TA 94 (met. act.: with and without) Test concentrations: six concentrations, up to 5.0 mg/plate (highest non-cytotoxic dose) Reverse mutation assays using Salmonella typhimurium strains TA92, TA1535, TA100, TA1537, TA94 and TA98 were carried out according to the method of Ames, McCann & Yamasaki (1975)*.	Test results: negative for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100(all strains/cell types tested); met. act.: with and without negative for S. typhimurium, other: TA 92 and TA 94(all strains/cell types tested) ; met. act.: with and without	–	2 (reliable with restrictions) Test material sodium sulphite	Ishidate, M.et al. (1984)

equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) E. coli WP2 uvr A (met. act.: with and without) Test concentrations: at least six doses: 33.3, 100, 333.3, 1000, 3333.3, 10000 µg/plate Positive control substance(s): sodium azide (1.0 µg/plate), 2-nitrofluorene (5.0 µg/plate), 9-aminoacridine (50.0 µg/plate) AF2; 0.1 µg/plate, 2-anthramine; 1.0, 2.5 or 10.0 µg/plate 2-anthramine; 1.0, 2.5 or 10.0 µg/plate	Test results: negative (No mutagenicity was observed on compound F76-020 in any of the assays performed.) cytotoxicity: no vehicle controls valid: yes; negative controls valid: positive controls valid: yes	_	2 (reliable with restrictions) Test material sodium thiosulfate pentahydrate	Mortelmans, K.E.; Tanaka, W. (1979)
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium, other: TA 1535, TA 100, TA 1538 and TA 98 (met. act.: without) Test concentrations: 1 M aqueous sodium sulfite solution	No results; bacteriocidal effect at the culture conditions.	/	3 (not reliable) Test material sodium hydrogensulfite	Münzer, R. (1980
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium TA 97 Test concentrations: 0.01, 0.02, 0.04, 0.08, and 0.16 M The mutagenic potential of disodium disulfite was assessed in S. typhimurium TA 97. preincubation protocol to 80mM sodium bisulfite at different pH and temperatures. In addition various buffer additives were added to assess whether any mutation frequency is increased or reduced.	Test results: negative for S. typhimurium TA 97(all strains/cell types tested)	_	3 (not reliable) Test material disodium disulphite	Pagano, D.A. et al. (1990)
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium, other: G46, TA92, TA1535, TA100, SB2802, SB2061, TR3243, TA88, TA110, TA90, TA97, D3052, TA1538, TA98, C3076, TA1537, and TA1977 Test concentrations: 0 - 0.64 M pH values of 5.0 - 8.0. The strains were preincubated for 30 minutes at 37°C, plated and incubated for 48 hrs at 37°C.	Evaluation of results: positive Test results: positive for S. typhimurium, other: G46, TA92, TA1535, TA100, SB2802, SB2061, TR3243, TA88, TA110, TA90, TA97, D3052, TA1538, TA98, C3076, TA1537, and TA1977(strain/cell type: G46 and TR3243) ; cytotoxicity: yes	+	3 (not reliable) experimental result Test material disodium disulphite	Pagano, D.A. & Zeiger, E. (1987)

mammalian cell gene mutation assay (gene mutation) mouse lymphoma L5178Y cells (met. act.: with and without) Test concentrations: Range-Finder: - with and without S9-mix: 59.44, 118.9, 237.8, 475.5, 951 and 1902 µg/mL. Concentrations selected for the Mutation Experiments were based on the results of this cytotoxicity Range finder Experiment Cultures selected for mutation assessment: Experiment I: - with and without S9-mix: 200, 300, 400, 600, 800, 1200, 1600 and 1902 µg/mL, Experiment II: - with and without S9-mix: 100, 300, 600, 900, 1200, 1500 and 1902 µg/mL, Experiment III: - with S9-mix: 200, 800, 1000, 1400, 1600, 1700, 1800 and 1902 µg/mL. Positive control substance(s): 4-nitroquinoline-1-oxide; 0.1 and 0.15 µg/mL (dissolved in DMSO) Positive control substance(s): benzo(a)pyrene OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)	Test results: ambiguous (Statistically significant increases in mutant frequency were observed at the **highest two concentrations** (1600 and 1902 µg/mL). **no significant linear trend** for mouse lymphoma L5178Y cells (all; met. act.: with ; without) cytotoxicity: no ; vehicle controls valid: yes; negative controls valid: positive controls valid: yes	**+/~**	1 (reliable without restriction) key study Test material disodium disulfite	Unpublished study report (2010)
In vitro Mammalian Cell Gene Mutation Test mouse lymphoma L5178Y cells (met. act.: with and without) OECD Guideline 476 Concentrations selected were based on the results of a cytotoxicity Range-Finder Experiment. Cultures selected for mutation assessment: Experiment I: - with and without S9-mix: 200, 400, 600, 800, 1000, 1200, 1350 and 1482 µg/mL Experiment II: - with and without S9-mix: 300, 600, 900, 1100, 1300 and 1482 µg/mL Positive control substance(s): 4-nitroquinoline-1-oxide; 0.1 and 0.15 µg/mL, benzo(a)pyrene (2 and 3 µg/mL (dissolved in DMSO)	Test results: negative (No statistically significant increases in mutant frequency were observed following treatment with ammonium thiosulfate at any concentration tested and there were no significant linear trends.) for mouse lymphoma L5178Y cells(all strains/cell types tested) ; met. act.: with and without ; cytotoxicity: no ; vehicle controls valid: yes; negative controls valid: not examined; positive controls valid: yes	–	1 (reliable without restriction) key study Test material ammonium thiosulfate	Unpublished study report (2010)

OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test) mouse lymphoma L5178Y cells (met. act.: with and without) Concentrations selected for the Mutation Experiments were based on the results of this cytotoxicity Range-Finder Experiment. Experiment I: with and without S9-mix: 200, 300, 400, 600, 800, 1200, 1600 and 1902 µg/mL, Experiment II: - with and without S9-mix: 100, 300, 600, 900, 1200, 1500 and 1902 µg/mL, Experiment III: - with S9-mix: 200, 400, 800, 1000, 1200, 1400, 1600, 1700, 1800 and 1902 µg/mL. Cultures selected for mutation assessment: Experiment I: - with and without S9-mix: 200, 300, 400, 600, 800, 1200, 1600 and 1902 µg/mL, Experiment II: - with and without S9-mix: 300, 600, 900, 1200, 1500 and 1902 µg/mL, Experiment III: - with S9-mix: 200, 800, 1000, 1400, 1600, 1700, 1800 and 1902 µg/mL. Positive control substance(s): 4-nitroquinoline-1-oxide; 0.1 and 0.15 µg/mL (dissolved in DMSO), benzo(a)pyrene	Test results: ambiguous (Statistically significant increases in mutant frequency were observed **at the highest two concentrations** (1600 and 1902 µg/mL). no significant linear trend.) for mouse lymphoma L5178Y cells(all strains/cell types tested (Experiment I)) ; met. act.: with ; cytotoxicity: no ; vehicle controls valid: yes; negative controls valid: not examined; positive controls valid: yes negative (No significant increases in mutant frequency were observed following treatment with sodium metabisulfite at any concentration tested and there were no significant linear trends.) for mouse lymphoma L5178Y cells(all strains/cell types tested (Experiment I and II)) ; met. act.: without ; cytotoxicity: no ; vehicle controls valid: yes; negative controls valid: not examined; positive controls valid: yes negative (No significant increases in mutant frequency were observed following treatment with sodium metabisulfite at any concentration tested and there were no significant linear trends.)	**+/~**	1 (reliable without restriction) Test material disodium disulfite	Unpublished study report. (2010)
mammalian cell gene mutation assay (gene mutation) Chinese hamster lung fibroblasts (V79) Test concentrations: Ouabain^r marker: 15 minute exposure: 10 and 20 mM 48 hour exposure: 1 and 5 mM Thioguanine^r marker: 15 minute exposure: 10 mM Positive control substance(s): UV irradiation (GE germicidal lamp G1578)	Test results: negative for Chinese hamster lung fibroblasts (V79)(all strains/cell types tested) ; cytotoxicity: yes	_	3 (not reliable) Test material sodium hydrogensulfite	Mallon, R.G. & Rossman, T.G. (1981)

mammalian cell gene mutation assay (gene mutation) Chinese hamster Ovary (CHO) Test concentrations: 5 and 10 mM 4 hours Positive control substance(s): ethylmethanesulphonate In an in vitro gene mutation test in AS52 (CHO derivative), the induction of point mutations in the XPRT gene was assessed. Cells were exposed for 4 hours in triplicate and sub-cultured for the expression for 7 days. Cytotoxicity was expressed as percent survival relative (RS) to those from similarly plated untreated control.	Test results: ambiguous cytotoxicity: yes	+/-	3 (not reliable) Test material sodium hydrogensulfite Form: aqueous solution	Meng, Z. & Zhang, B. (1999)

equivalent or similar to OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test Chinese hamster Ovary (CHO) (met. act.: with and without) Test concentrations: 185, 370, 740 and 1480 µg/mL Positive control substance(s): mitomycin C (stock concentration of 1 and 2 µg/mL (dissolved in distilled water)), cyclophosphamide (stock concentration of 100 and 200 µg/mL (dissolved in distilled water))	Test results: negative (cytotoxicity: absence of substantial toxicity: Toxicity in CHO cells was 5% at 1480 µg/mL. The mitotic index at the highest dose level evaluated was 4% reduced relative to the solvent control. ; vehicle controls valid: yes; negative controls valid: not examined; positive controls valid: yes negative (The percentage of cells with aberrations was significantly increased at dose level 1480 µg/mL. However, the percentage was within the historical control range; therefore it is not considered to be biologically significant.) for Chinese hamster Ovary (CHO)(strain/cell type: ; 20 hours treatment) ; met. act.: without cytotoxicity: absence of substantial toxicity: Toxicity in CHO cells was 7% at 1480 µg/mL. The mitotic index at the highest dose level evaluated was 33% reduced relative to the solvent control. ; vehicle controls valid: yes; negative controls valid: not examined; positive controls valid: yes negative (The percentage of cells with structural and numeric aberrations in the test item treated groups was not significantly increased above that of the solvent control.) for Chinese hamster Ovary (CHO)(all strains/cell types tested (; 4 hours treatment)) ; met. act.: with ; cytotoxicity: no (No toxicity was observed when treated for 4 hours) ; vehicle controls valid: yes; negative controls valid: not examined; positive controls valid: yes	+/-	1 (reliable without restriction) key study Test material (CAS number): 7783-18-8 Ammonium thiosulfate	Gudi, R.; Brown, C. (2001)

in vitro mammalian chromosome aberration test (chromosome aberration) Chinese hamster Ovary (CHO) (met. act.: with and without) Test concentrations were established within a Rangefinding assay Chromosomal aberration assay (without S9-mix): 0, 1270, 2550, 3820 and 5100 µg/mL; Chromosomal aberration assay (with S9-mix): 0, 1260, 2510, 3770 and 5020 µg/mL. Positive control substance(s): mitomycin C (0.04 µg/mL and 0.08 µg/mL), cyclophosphamide (25 and 50 µg/mL) EPA OPP 84-2	Test results: negative (No significant increase in cells with chromosomal aberrations was observed at the concentrations analysed.) for Chinese hamster Ovary (CHO)(all strains/cell types tested) ; met. act.: with and without ; cytotoxicity: no ; vehicle controls valid: yes; negative controls valid: yes; positive controls valid: yes	–	3 (not reliable) supporting study Test material (EC name): ammonium thiosulfate	Murli, H. (1989)
in vitro mammalian chromosome aberration test (chromosome aberration) lymphocytes: human blood Test concentrations: 0, 0.05, 0.1, 0.5, 1, 2 mM Cells from 4 different donors were incubated for 48 hours at concentrations of 0, 0.05, 0.1, 0.5, 1, 2 mM. Colcemid was added 4 hours prior harvest. Cells were Giemsa stained after hypotonic treatment and drying. 200 cells per group were scored blindly for the presence of isochromatid and chromatid breaks.	Test results: positive for lymphocytes: human blood(all strains/cell types tested) ; cytotoxicity: yes	+	2 Test material sodium hydrogensulfite Form: aqueous solution	Meng, Z. & Zhang, L. (1992)
in vitro mammalian chromosome aberration test (chromosome aberration) mammalian cell line, other: Chinese hamster fibroblast cell line (CHL) (met. act.: without) Test concentrations: three different doses, up to 0.125 mg/mL (highest non-cytotoxic dose) Chinese hamster fibroblast cells (CHL) were tested for the induction of structural aberrations. Cells were exposed towards sodium bisulfite, anhydrous (95.0% purity) at max. 0.125 mg/mL (3 doses, concentrations not reported) for a period of 24 and 48 hours without metabolic activation. Colcemid was added 2 hours before cell harvest and100 well-spread Giemsa stained metaphases were checked for incidence of polyploid cells as well as of cells with structural chromosomal aberrations such as chromatid or chromosome gaps, breaks, exchanges, ring formations, fragmentations.	Evaluation of results: negative without metabolic activation Test results: negative for mammalian cell line, other: Chinese hamster fibroblast cell line (CHL)(all strains/cell types tested) ; met. act.: without	–	3 (not reliable) Test material disodium disulphite	Ishidate, M.et al. (1984)

in vitro mammalian chromosome aberration test (chromosome aberration) mammalian cell line, other: Chinese hamster fibroblast cell line (CHL) (met. act.: without) Test concentrations: three different doses, up to 0.06 mg/mL (highest non-cytotoxic dose) Cells were exposed towards potassium metabisulphite (93.0% purity) at max. 0.06 mg/mL (3 doses, concentrations not reported) for a period of 24 and 48 hours without metabolic activation. Colcemid was added 2 hours before cell harvest and100 well-spread Giemsa stained metaphases were checked for incidence of polyploid cells as well as of cells with structural chromosomal aberrations such as chromatid or chromosome gaps, breaks, exchanges, ring formations, fragmentations.	results: negative without metabolic activation Test results: negative for mammalian cell line, other: Chinese hamster fibroblast cell line (CHL)(all strains/cell types tested) ; met. act.: without	_	3 (not reliable) Test material dipotassium disulphite	Ishidate, M.et al. (1984),
in vitro mammalian chromosome aberration test (chromosome aberration) mammalian cell line, other: Syrian hamster foetal cells (HFC) Test concentrations: 10, 20, and 40 mM In this study the clastogenic effect of sodium bisulfite was tested in Syrian hamster foetal cells (HFC) at concentrations of 10, 20 and 40 mM for an exposure duration of 15 minutes. Chromosomes were prepared 6 and 24 hours after exposure by adding Colcemid 4 hours before cells harvest and Giemsa staining. 200 metaphases were analysed for chromatid or chromosome aberrations (gaps, breaks and exchanges).	Test results: negative for mammalian cell line, other: Syrian hamster foetal cells (HFC)(all strains/cell types tested)	_	3 (not reliable) Test material sodium hydrogensulfite	Popescu, N.C. &DiPaolo, J.A. (1988)

in vitro mammalian chromosome aberration test (chromosome aberration) lymphocytes: human peripheral blood Test concentrations: 25, 50, 100, 200 µg/mL Positive control substance(s): ethylmethanesulphonate The induction of chromosomal aberrations by potassium disulfite was tested in human peripheral blood lymphocytes. Cells from 4 healthy donors (2 females, 2 males) were used and incubated for 72 hours prior to exposure. The exposure concentrations were 25, 50, 100 and 200 µg/mL (0.13, 0.26, 0.56, 1.05 mM) for a duration of 24 and 48 hours. Colchicine was added 2 hours before harvesting; ethyl methanesulfonate was added as positive control. Cells were harvested, fixed and prepared slides were stained (5% Giemsa); 100 metaphases per donor were scored for structural and/or numerical aberrations (excluding gaps).	Test results: positive for lymphocytes: human peripheral blood(all strains/cell types tested) ; cytotoxicity: yes	+	3 (not reliable) Test material dipotassium disulphite Form: powder	Yavuz-Kocaman, A. et al. (2008a)
in vitro mammalian cell micronucleus test (chromosome aberration) lymphocytes: human peripheral blood Test concentrations: 25, 50, 100, 200 µg/mL Positive control substance(s): ethylmethanesulphonate The induction of micronuclei formation by potassium disulfite was tested in human peripheral blood lymphocytes. Cells from 4 healthy donors (2 females, 2 males) were used and incubated for 72 hours prior to exposure. The exposure concentrations were 25, 50, 100 and 200 µg/mL (0.13, 0.26, 0.56, 1.05 mM) for a duration of 24 and 48 hours. Cytochalasin B was added at 44 hours of incubation to block cytokinesis. After additional 24 hours incubation at 37°C, cells were harvested , fixed and stained for micronucleus analysis. 2000 binucleated lymphocytes were scored from each donor (8000 binucleated cells were scored per concentration).	Test results: positive for lymphocytes: human peripheral blood(all strains/cell types tested) ; cytotoxicity: yes	+	3 (not reliable) Test material dipotassium disulphite Form: powder	Yavuz-Kocaman, A. et al. (2008a)

in vitro mammalian chromosome aberration test (chromosome aberration) lymphocytes: human peripheral lymphocytes Test concentrations: 0.0004 M For the conduct of an in vitro chromosome aberration test peripheral blood lymphocytes taken from two different non-smoking, healthy individuals (sex not stated) were used. The cells were cultured for 72 hours and exposed to the test substance for 48 hours at a concentration of 0.4 mM sodium hydrogensulfite. Chromosome aberrations were scored in 400 cells.	Evaluation of results: positive Test results: positive for lymphocytes: human peripheral lymphocytes(all strains/cell types tested) ; cytotoxicity: yes	+	3 (not reliable) Test material (EC name): sodium hydrogensulfite	Beckman, L. & Nordenson, I. (1986) carval
in vitro mammalian chromosome aberration test (chromosome aberration) mammalian cell line, other: pseudodipoid Chinese hamster cell line (Don) Test concentrations: 0.1, 0.5, and 1 mM Potassium disulfite was tested in a Chinese hamster cell line for the induction of chromosome aberrations at doses of 0.1, 0.5 and 1 mM. For a given dose at least one culture was made. HBSS was used as vehicle and solvent control. Cells were incubated for 26 hours at 37°C in the dark (two cell cycles). 0.25 µg colchicine/ml was added 2 hours prior harvest. Fixed and stained by the fluorescence or Giemsa staining technique and chromosome aberrations were examined on 100 metaphases for each dose, and frequency of aberrations, excluding gaps, was indicated by the number of breaks per cell.	Test results: negative for mammalian cell line, other: pseudodipoid Chinese hamster cell line (Don)(all strains/cell types tested) ; cytotoxicity: yes	–	3 (not reliable) Test material dipotassium disulphite	Abe, S. & Sasaki, M. (1977)
in vitro mammalian chromosome aberration test (chromosome aberration) mammalian cell line, other: human peripheral lymphocytes Test concentrations: 0.375 mM Human peripheral blood lymphocytes were tested for the induction of chromosome aberrations after sodium hydrogen sulfite exposure. The stimulated lymphocytes were cultured for 70-72 hours at 37°C, during the last 48 hours incubation the cells were exposed to 0.375 mM sodium hydrogen sulfite. Cell were arrested by addition of Colcemid, fixed and stained. 200 cells per culture from coded slides were analysed for the presence of chromosomal aberrations.	Test results: positive for mammalian cell line, other: human peripheral lymphocytes(all strains/cell types tested)	+	3 (not reliable) weight of evidence experimental result Test material (EC name): sodium hydrogensulfite	Nordenson, I. & Beckman, L. (1984)

STUDY 1 (Beckman & Nordenson, 1986): Sister chromatid exchanges: lymphocytes exposed to sodium hydrogensulfite (concentration: 0.0004 M) STUDY 2 (Chen & Shaw, 1994): base substitution mutations, bacteriophage M13mp2 C141, DNA base substitution in transfected cells followed by isolation and sequencing was investigated. Cells were incubated for a maximum of 54 days at hydrogen sulfite concentrations up to 50 mM. STUDY 3 (Peden & Nathans, 1982): BD1528 cells (strain of E. coli) Mechanistic study on in vitro DNA base-substitution in deletion loops. Sodium bisulfite was used as deamination reagent of the DNA bases at 2 M. STUDY 4 (MacRae & Stich, 1979): Sister chromatid exchanges (SCEs) are studied in Chinese hamster cells exposed to bisulfite during a 2-3 hour or a 24 hour exposure period of .0.000030, 0.000090, 0.00027, 0.00081, 0.0024, and 0.0073 M STUDY 5 (Meng & Zhang, 1992): The frequency of sister-chromatid exchanges (SCE) in human blood lymphocytes exposed to a sodium bisulfite solution at various concentrations (0.05, 0.1, 0.5, 1.0, and 2.0 x 10^-3 M) was studied. STUDY 6 (Doniger et al., 1982): Study investigates substance induced DNA lesions and DNA replication rate in mammalian cells. 10, 20, or 50 mM	Test results: STUDY 1: positive: human peripheral lymphocytes(all strains/cell types tested) ; cytotoxicity: not clearly stated in the publication; STUDY 2:positive bacteriophage M13mp2 C141(all strains/cell types tested) STUDY 3: positive: BD1528 cells (strain of E. coli)(all strains/cell types tested) STUDY 4: positive: Chinese hamster ovary (CHO) cells(all strains/cell types tested) ; cytotoxicity: yes STUDY 5: positive: human blood lymphocytes(all strains/cell types tested) ; cytotoxicity: yes STUDY 6: negative: Syrian hamster embryo cells (HEC)(all strains/cell types tested) ; cytotoxicity: yes	S1:+ S2:+ S3:+ S4:+ S5:+ S6:- S7:+ S8:+	3 (not reliable) disregarded study experimental result Test material (common name): STUDY 1 -5 & 7: sodium hydrogensulfite; STUDY 6: bisulfite; STUDY 8: dipotassium disulfite Form: STUDY 5: solution; STUDY 1, 2, 3, 4, 6, & 7: no data; STUDY 8: powder	Beckman, L. & Nordenson, I. (1986) Chen, H. & Shaw, B.R. (1994) Peden, K.W.C. & Nathans, D. (1982) MacRae, W. D. & Stich, H. F. (1979) Meng, Z. & Zhang, L. (1992) Doniger, J. et al. (1982) Popescu, N.C. & DiPaolo, J.A. (1988) Yavuz-Kocaman et al. (2008)

STUDY 7: (Popescu & DiPaolo, 1988): In this study the effect of bisulfite on sister chromatid exchange in normal Syrian hamster foetal cells was determined. STUDY 8 (Yavuz-Kocaman et al., 2008): The effect of potassium metabisulfite on sister chromatid exchanges in human lyphocytes was investigated.The human lymphocytes were treated with 25, 50, 100, and 200 µg/mL of the test substance for 24 and 48 hours. Positive control substance(s): u.v. irradiation Positive control substance(s): ethylmethanesulphonate (SCE) in cultured human peripheral	STUDY 7: positive: Syrian hamster foetal cells (HFC)(all strains/cell types tested) STUDY 8: positive for human blood lymphocytes(all strains/cell types tested) ; cytotoxicity: yes ; vehicle controls valid: not examined			

STUDY 1 (Kunz & Glickman, 1983): Detection of amber and ochre mutations at 65 individual sites within the E. coli lacI system (G:C to A:T transition). 1M sodium bisulfite STUDY 2 (Mukai et al., 1970): Reversion studies with Escherichia coli mutants E. coli K12 and E. coli 15 investigated the bactericidal effects of 1M sodium bisulfite. STUDY 3 (De Giovanni-Donnelly, R., 1985): The bactericidal effects of sodium bisulfite on Salmonella typhimurium LT2 strains carrying the hisG46 allele were investigated. Salmonella typhimurium strains TS24, GW 19, hisG46, TA 92, TA1950 & TA2410 (met. act.: without) 1 M (hisG46 strain was also tested with the concentrations 0.1, 0.5, 1.5, & 2.0 M) STUDY 4 (Valencia et al., 1973): In this study the genotoxic effects of sodium sulfite 0.04 and 0.08 M in Drosophila was tested. Drosophila melanogaster sex-linked recessive lethal test; STUDY 5 (Jagiello et al., 1975): Investigated the induction of chromosomal damage caused by sodium sulfite in oocytes freshly isolated from mice, ewe and cows. Cells were incubated for 5 and 15 hours for mice and 28 hours for ewe and cow. - mice (in vitro study): 0, 5, 10, 25, 50, 100, 150, 200, 250, 350, 500, 750, 1000, and 10,000 µg/cm³ - mice, (14- hour in vitro study): 12.6 and 1260 µg/cm³ - mice (in vivo) study, first series): 1.0, 2.5, or 5.0 mg Na2SO3 - mice (in vivo) study, second series): 5.0 mg Na2SO3 - mice (in vivo) study, third series): 5.0 mg Na2SO3 - ewe: (in vitro study): 0, 50, 100, 250, 350, 500, and 1250 µg/cm³ - cow (in vitro study): 0, 100, 250, 350, 500, 1000, 1250, and 1500 µg/cm³ STUDY 6 (Clark, 1953): Sodium sulfite was tested in a bacterial forward mutation test in Micrococcus pyogenes var, aureus strain FDA209 using a preincubation method at a single concentration.0.1% sodium bisulfite & 0.06% sodium sulfite Positive control substance(s): sodium azide	Test results: STUDY 1: not determined: E. coli lacI system(all strains/cell types tested) ; cytotoxicity: yes STUDY 2: not determined: E. coli K12 and E. coli 15 ; cytotoxicity: yes STUDY 3: not determined for Salmonella typhimurium strains cytotoxicity: yes STUDY 4: negative: Drosophila melanogaster(all strains/cell types tested) STUDY 5: positive for oocytes from mouse, ewe, and cow STUDY 6: positive for Micrococcus pyogenes var	S1: / S2:/ S3:/ S4:- S5:+ S6:+	3 (not reliable) disregarded study due to cytotoxicty Test material STUDY 1 - 3 & 6: sodium hydrogensulfite; STUDY 4 - 6: sodium sulfite Form: STUDY 1, 2, 3, 4, 5 & 6: no data	Kunz, B.A. & Glickman, B.W. (1983) Mukai, F. et al. (1970) De Giovanni-Donnelly, R. (1985) Valencia, R. et al. (1973) Jagiello, G.M. et al. (1975) Clark, J.B. (1953)

7.9.5.2. Overview of experimental in vivo genotoxicity studies

Table 25: Overview of experimental in vivo genotoxicity studies

Method	Description of results	+/- Outcome in vivo gentox	Remarks	Reference
micronucleus assay (chromosome aberration) mouse (NMRI) male subcutaneous 250 mg/kg body weight/day (nominal conc.) 500 mg/kg body weight/day (nominal conc.) 1000 mg/kg body weight/day (nominal conc.) Positive control substance(s): Cyclophosphamide (20 mg/kg bw) and vincristine sulfate (0.15 mg/kg bw) OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test) EU Method B.12 (Mutagenicity In Vivo Mammalian Erythrocyte Micronucleus Test) EPA OPPTS 870.5395 (In Vivo Mammalian Cytogenics Tests: Erythrocyte Micronucleus Assay)	Evaluation of results: negative Test results: Genotoxicity: negative (male); toxicity: no effects ; vehicle controls valid: yes; negative controls valid: not examined; positive controls valid: yes	_	1 (reliable without restriction) key study read-across from supporting substance (structural analogue or surrogate) Test material (EC name): sodium sulfite (See endpoint summary for justification of read-across)	Schulz, M. (2008)
micronucleus assay (chromosome aberration) mouse (NMRI) male subcutaneous 250 mg/kg body weight/day (nominal conc.) 500 mg/kg body weight/day (nominal conc.) 1000 mg/kg body weight/day (nominal conc.) Positive control substance(s): Cyclophosphamide (20 mg/kg bw) and vincristine sulfate (0.15 mg/kg bw) OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test) EU Method B.12 (Mutagenicity - In Vivo Mammalian Erythrocyte Micronucleus Test) EPA OPPTS 870.5395 (In Vivo Mammalian Cytogenics Tests: Erythrocyte Micronucleus Assay)	Evaluation of results: negative Test results: Genotoxicity: negative (male); toxicity: yes ; vehicle controls valid: yes; negative controls valid: not examined; positive controls valid: yes	_	1 (reliable without restriction) weight of evidence experimental result Test material (EC name): sodium sulfite	Schulz, M. (2008)

dominant lethal assay (chromosome aberration) rat (Sprague-Dawley) male oral: feed 4.5, 15, and 45 (maximum tolerated dose) mg/kg bw/day (nominal in diet) 4.43, 14.76, and 44.23 mg/kg/day (average actual) (analytical conc.) Positive control substance(s): Triethylenemelamine (TEM) - Route of administration: drinking water - Doses / concentrations: approximately 0.04 mg/kg/day The positive control group received the positive control substance, administered in the drinking water at a concentration of 0.60 mg/liter throughout the 10-week treatment period. Solutions were preapred fresh daily and the previous day's water was discarded. Based on the average rat weight of 369 g for the 10-week exposure period and an estimated intake of approximately 25 mL per rat per day, the average TEM dose was approximately 0.04 mg/kg/day. In a dominant lethal assay, the induction of dominant lethal mutations in rat after sodium bisulfite administration was investigated. Male Sprague-Dawley rats (53 to 62 days old, bodyweight 247-339 g) were given sodium bisulfite in diet, ad libitum at doses of 45, 15 and 4.5 mg/kg/day over a period of 10 weeks. Animals in the positive control group received triethylenemelamine (TEM). After the 10-week treatment period, 40 male rats from the vehicle control group and 20 male rats from each treatment group were selected and mated with two adult virgin females for seven days. These females were replaced with two new females for an additional 7-day mating period. Each female was sacrificed 15-19 days after the first day of cohabitation.	Evaluation of results: negative Test results: Genotoxicity: negative (male); toxicity: no effects ; negative controls valid: not examined; positive controls valid: yes	_	2 (reliable with restrictions) weight of evidence experimental result Test material (EC name): sodium hydrogensulfite Form: granular	Unpublished study report (1979)

micronucleus assay (chromosome aberration) rat male/female intraperitoneal 150, 300, and 600 mg/kg bw (injected) Positive control substance(s): Urethane (Sigma, U2500) - Doses / concentrations: 400 mg/kg bw In this study the induction of chromosomal aberrations in the bone marrow of albino rats was investigated. Four animals (2M/2F) per group were given a single intraperitoneal injection of 150, 300, 600 mg/kg bw potassium disulfite 12 and 24 hours before sacrifice. Colchicine was given 2 hours prior sacrifice via intraperitonal injection. Urethane was used as positive control substance. Bone marrow of femurs was fixed and stained for microscopic analysis. 100 metaphases per animal (400 per dose group) were scored. Mitotic index was determined by scoring 3000 cells from each animal.	Evaluation of results: positive Test results: Genotoxicity: positive (male/female); toxicity: yes	+	3 (not reliable) weight of evidence experimental result Test material (EC name): dipotassium disulphite Form: powder	Yavuz-Kocaman, A. et al. (2008b)

dominant lethal assay (chromosome aberration) mouse (males: (101 x C3H)F1; females: (C3H X 101)F1) male/female intraperitoneal 300 or 400 mg/kg/day (males only) (injected) 550 mg/kg (females only) (injected) Positive control substance(s): no data The clastogenic effects of sodium bisulfite was investigated via dominant lethal assay in male and female mice. Male (101 X C3H)F1 mice were intraperitoneal administered 20 times doses of 300 mg/kg bw/day during a 26-day period and 38 times during a 54-day period. Males were paired with two (SEC X C57BL)F1 females after last injection. Female (C3H X 101)F1 mice were intraperitoneal administered a single doses of 550 mg/kg bw/day and mated with untreated male (101 X C3H)F1 mice.	Evaluation of results: negative Test results: Genotoxicity: negative (male/female)	_	3 (not reliable) weight of evidence experimental result Test material (EC name): sodium hydrogensulfite	Generoso, W.M. et al. (1978)

chromosome aberration assay (chromosome aberration) rat male/female Experiment 1: oral: gavage / Experiment 2: intraperitoneal Experiment 1: 250, 500, 750 and 1000 mg/kg bw (actual ingested) Experiment 2:250, 500, 750 and 1000 mg/kg bw (actual injected) Positive control substance(s): Experiment 1 and Experiment 2: Ethyl carbamate (obtained from Sigma) The substance was injected to rats for 6, 12 and 24 hour treatment period by intraperitoneal injection. This study is designed to investigate the genotoxic effect of sodium metabisulfite on bone marrow cells of rats. Four different concentrations of sodium metabisulfite (250, 500, 750 and 1000 mg/kg bw) were given to rats for 6, 12 and 24 hour treatment periods by intraperitoneal or gavage administrations. An examination for different types of chromosomal aberrations was carried out. In addition, a mitotic index was determined.	Evaluation of results: positive Test results: Genotoxicity: positive (Experiment 1 and Experiment 2) (male/female); toxicity: yes (Experiment 1 and Experiment 2)	+	3 (not reliable) weight of evidence experimental result Test material (EC name): disodium disulphite	Kayraldiz, A. & Topaktas, M. (2007)
micronucleus assay (chromosome aberration) mouse (CF-1) male/female oral: gavage 0.5, 1, or 2 g/kg bw (actual ingested) Positive control substance(s): Cyclophosphamide - Route of administration: gavage - Doses / concentrations: 25 mg/kg In this study the genotoxic effect of sodum metabisulfite (concentrations: 0.5, 1, or 2 g/kg bw) on different tissues of the mouse was investigated by use of the micronucleus test (blood and bone marrow cells).	Evaluation of results: positive Test results: Genotoxicity: positive (male/female); toxicity: yes	+	3 (not reliable) weight of evidence experimental result Test material (EC name): disodium disulphite	Carvalho, I.M.C.M.M. et al. (2011)

Experiment 1: chromosome aberration assay; Experiment 2: micronucleus assay (chromosome aberration) mouse (Swiss) Experiment 1: intraperitoneal, subcutaneous, and oral; Experiment 2: intraperitoneal Experiment 1 & 2: 200, 300, and 400 mg/kg bw (nominal conc.) Positive control substance(s): Experiment 1 & 2: no data This study investigated the clastogenic effect of sodium disulfite (aka sodium metabisulfite) in a bone marrow chromosome aberration and micronucleus test in mouse. Albino Swiss mice were given doses of 200, 300 and 400 mg/kg bw via subcutaneous, intraperitoneal injection or oral administration. A total of 4-6 animals of unknown sex were used per dose group, control groups contained 6 or 10 animals. The animals were given the test item at different routes and different doses. A total of 75 metaphases were scored per animal. For the micronucleus experiments, the number of MN PCE and NCE was determined by scoring 1000 PCE and NCE per animal.	Evaluation of results: positive Test results: Genotoxicity: positive (Experiment 1: intraperitoneal & subcutaneous route) Genotoxicity: positive (Experiment 2)	+	3 (not reliable) weight of evidence experimental result Test material (EC name): disodium disulphite	Pal, B.B. & Bhunya, S.P. (1992)
in vivo cytogenicity and dominant lethal assay (chromosome aberration) rat & human not reported 30 mg/kg 700 mg/kg 1200 mg/kg Positive control substance(s): triethylenemelamine In a range of genetic toxicity test, sodium metabisulfite was tested in an in vivo cytogenicity and dominant lethal assay in rats.	Test results: Genotoxicity: results cannot be interpreted, since it is unclear under which experimental conditions these were obtained	?	4 (not assignable) disregarded study experimental result Test material (EC name): disodium disulphite	Unpublished study report (1972a)

single cell gel/comet assay in rodents for detection of DNA damage (DNA damage and/or repair) mouse (CF-1) male/female oral: gavage 0.5, 1, or 2 g/kg bw (actual ingested) Positive control substance(s): Cyclophosphamide - Route of administration: gavage - Doses / concentrations: 25 mg/kg In this study the genotoxic effect of sodum metabisulfite (concentrations: 0.5, 1, or 2 g/kg bw) on different tissues of the mouse was investigated by use of the comet assay (liver and blood cells).	Evaluation of results: positive Test results: Genotoxicity: positive (male/female); toxicity: yes	+	3 (not reliable) weight of evidence experimental result Test material (EC name): disodium disulphite	Carvalho, I.M.C.M.M. et al. (2011)
single cell gel/comet assay in rodents for detection of DNA damage (DNA damage and/or repair) mouse (Kunming albino) male intraperitoneal 125, 250 or 500 mg/kg bw (injected) Positive control substance(s): none This study describes a study investigating the DNA damage of a 3:1 sodium sulfite and sodium bisulfite mixture in male mice via comet assay. Three groups of six male mice each received an intraperitoneal. dose of a mixture of sodium sulfite and sodium bisulfite (3:1 M/M) (125, 250 or 500 mg/kg bw) in 200 mL of 0.9% sodium chloride daily for 7 days, maximum dose equals the half LD50.	Evaluation of results: positive Test results: Genotoxicity: positive (male); toxicity: no effects	+	disregarded study by registrant Test material: sodium sulfite & sodium hydrogensulfite	Meng, Z. et al. (2004)

Male and female mice were housed in exposure chambers and treated with 14.00 6 1.25, 28.00 6 1.98, 56.00 6 3.11, and 112.00 6 3.69 mg/m3 SO2 for 6 hr/day for 7 days, while control groups were exposed to filtered air. Comet assays were performed on blood lymphocytes and cells from the brain, lung, liver, spleen, kidney, intestine, and testicles of the animals.	SO2 caused significant, dose-dependent increases in DNA damage, as measured by Olive tail moment, in all the cell types analyzed from both sexes of mice. The results indicate that inhalation exposure to SO_2 damages the DNA of multiple organs in addition to the lung, and suggests that this damage could result in mutation, cancer, and other diseases related to DNA damage.	+	study not listed by registrant 2 Test material: SO_2	Meng et al. 2005
human workers exposed at a sulphite pulp factory chromosome aberrations in human lymphocytes	A significantly increased frequency of chromosomal aberrations was found among workers at a sulphite pulp factory in northern Sweden. This increase was found to be associated mainly with exposure to sulphur dioxide (boiling of sulphite pulp and handling of sulphuric acid), and not with exposure to chlorine and dust in other workplaces within the factory.	+	study not reported from registrant	Nordenson et al. 1980

Summary on mutagenicity

The registrant discusses data on mutagenicity in vitro and in vivo very extensively. One gene mutation assay has been performed with Sodium dithionite, dated with 1989, which was negative. An in vivo micronucleus assay in mice at dose levels of 250 mg/kg, 500 mg/kg and 1 000 mg/kg bw. Sodium dithionite, dated 2008, was negative as well.

Inconsistent results were reported for the mutagenicity of sulphites in bacterial reverse mutation assays. However, there were limitations in reporting of e.g. cell viability, the lack of positive controls used or limited reliability due to other reasons.

Whereas the majority of studies report negative results, there were at least 8 in vitro chromosome aberration tests with human peripheral lymphocytes available, all tests were positive but judged as not reliable by the registrant. Due to the higher sensitivity of humans due to lower sulphite oxidase capacity in humans compared to laboratory rodents, these results give some reason for concern. Positive findings were reported from in vitro assays (chromosomal aberration, micronucleus, Sister Chromatid exchange and DNA-protein crosslinks) from humans occupationally exposed to sulfur dioxide (Meng and Zhang, 1990 a, b; Yadav and Kaushik (1996), Nordenson et al. 1980). Although the studies have certain deficiencies such as inaccurately reported exposure (e.g. exposure only based on the range of SO2 in the air) they indicate cause of concern.

Further, DNA damage has been observed after intraperitoneal exposure in a dose dependent manner in mice administered 125, 150 and 500 mg/kg bw. of a mixture of sodium sulfite and sodium bisulfite, 3:1M/M in multiple organs (brain, lung, heart, liver, stomach, spleen, thymus, bone marrow and kidney) (Meng et al. 2004). Due to the lack of a positive control and the test was not following the criteria of Tice et al. 2000 the study

has limitations was judged as "not rateable" by the registrant. In another study DNA damage was investigated in mice treated with sulfur dioxid (14.00 +/- 1.25, 28.00+/- 1.98, 56.00 +/- 3.11, and 112.00 +/- 3.69 mg/m3 SO2 for 6 hr/day for 7 days (Meng et al. 2005). SO2 exposure caused significant, dose-dependent increases in DNA damage, as measured by Olive tail moment, in all the cell types analyzed from both sexes of mice. The authors concluded that inhalation exposure to SO2 damages the DNA of multiple organs in addition to the lung, and suggest that this damage could result in mutation, cancer, and other diseases related to DNA damage (Meng et al. 2005). Also rated as not reliable by the registrant, the result needs further consideration.

Although the majority of the genotoxicity assays gave negative results, some positive results, like for instance the positive findings in humans after occupational exposure and positive findings in human lymphocytes, but also positive results in in vivo micronucleus and chromosomal aberration tests after oral, i.p., s.c. and inhalation exposure as well as positive in vivo COMET assays via oral, inhalation and i.p. route give rise to concern for mutagenicity. Though some of these studies have drawbacks (e.g. lacking positive controls, missing information on cytotoxicity) especially the positive results obtained in human cells are of concern also with regard to the lower sulphite oxidase capacity in humans compared to rodents. Further, there is indication from epidemiological studies that exposure to sulfites and SO2 may enhance the risk for certain cancers. Tumour promoting activity has been demonstrated in animal experiments.

Conclusions on mutagenicity:

Based on the reported findings, , and the recent classification proposal for sulphur dioxide as Muta 2, the eMSCA is of the opinion that certain standard requirements for mutagenicity according to REACH are not fulfilled and are thus proposed to be requested in a potential CCH:

- Annex VII, 8.4.1: Bacterial Reverse Mutation Test (Ames-Test), OECD 471, with 4 strains of Salmonella typhimurium (TA 98, TA 100, TA 1535, TA 1537) and 1 strain Escherichia coli (WP2 uvrA)'
- Annex VIII, 8.4.2.: in vitro cytogenicity study (OECD 487)
- Annex VIII, 8.4.3.: in vitro gene mutation in mammalian cells (OECD 476)

7.9.6. Carcinogenicity

7.9.6.1. Carcinogenicity in animal experiments

7.9.6.1.1. **Carcinogenicity in animal experiments after oral exposure**

Table 26: Overview of experimental studies on carcinogenicity after oral administration

Method	Results	Remarks	Reference
mouse (ICR/JCL) male/female oral: drinking water Exposure: 24 months Groups of 100 ICR/JCL mice received 0, 1 or 2% K2S2O5 in the drinking water for 24 months. According to a sub-acute test the 2% dose was the maximum tolerated dose. Mice were necropsied at death or at termination of the study.	no NOAEL identified (carcinogenicity): > 2500 mg/kg bw/day (nominal) (male/female) based on: test mat. (Assuming an average mice body weight of 25 g and a daily water intake of 5 ml, the K2S2O5 dose has been estimated to correspond to 2500 mg/kg bw/d (or about 1450 mg/kg bw/d as SO2 equivalents).) Neoplastic effects: no effects	2 (reliable with restrictions) key study Test material dipotassium disulfite	Tanaka, T.; et al. (1994)
rat (Wistar) male/female oral: feed n a three-generation feeding study, groups of 20 male and 20 female Wistar rats received 0, 0.125, 0.25, 0.5, 1.0 and 2.0% sodium metabisulfite, i.e. 49, 108, 220, 460, and 955 mg/kg bw/d as actual dose in a thiamine-containing diet over periods of 2 years. Exposure: Exposure period: 104 weeks (F0 and F1 generation) and 30 weeks (F2 generation) Premating exposure period (males): 21 weeks Premating exposure period (females): 21 weeks Duration of test: until the weaning of the F3 animals	no NOAEL identified (carcinogenicity): > 955 mg/kg bw/day (nominal) (male/female) based on: test mat. There was no indication that metabisulfite had any carcinogenic effect at the highest dietary dose of 2% Na2S2O5 (955 mg/kg bw/d or 640 mg/kg bw/d as SO2 equivalents).) NOAEL (local effects) (toxicity): 108 mg/kg bw/day (nominal) (male/female) Based on the occurrence of occult blood in the faeces and changes in gastric morphology at a dose level of 0.5% or more	2 (reliable with restrictions) supporting study Test material disodium disulfite	Til, H.P., et al. (1972b)
rat (Wistar) male/female chronic (oral: in the diet short term (10- 56 days): 1.0, 2.0 % Long term: for and 8, 12 and 24 months 0.125, 0.25, 0.5, 1.0, 2.0 % for 8, 12 and 24 months Pathological and microscopic examinations of the stomach of the rats were performed after treatment periods of 10, 28 and 56 days (short-term) and after 8, 12 and 24 months.	no NOAEL identified (local stomach) (carcinogenicity): > 955 mg/kg bw/day (nominal) (male/female) based on: test mat. NOAEL (local) (toxicity): 108 mg/kg bw/day (nominal) (male/female) based on: test mat. According to the induction of hyperplastic and inflammatory changes in the forestomach at dietary levels of 0.5% and higher, the sodium metabisulfite level of 0.25% (corrected to 0.215% based on analytical verifications) can be considered as a NOAEL, which can be recalculated to 108 mg/kg bw/d Na2S2O5 (or about 72 mg/kg bw/d as SO2 equivalents).) Neoplastic effects: no effects	2 (reliable with restrictions) supporting study Test material disodium disulfite	Feron, V.J.; Wensvoort, P. (1972)

Three generation study rat (uniform strain bred for cancer research) male/female combined repeated dose and reproduction 375 and 750 ppm SO2 as sodium metabisulfite (nominal in water) Exposure: Up to 2.5 year (over 3 generations) before and throughout matin and during pregnancy an lactation Generation I consisted c three groups: group 1 (ta water controls), group 2 (750 ppm as SO2) and group (375 ppm as SO2). The metabisulfite drinkin water groups of generation were produced from matinc of the sulfite drinking wate groups of generation Generation III was derive similarly from generation I Observations on growth, fee consumption, fluid intake faecal output, reproductior lactation and the incidence c tumours were recorded Detailed post-mortem an histological examinatior were made.	no NOAEL identified (carcinogenicity): > 53 mg/kg bw/day (nominal) (male/female) based on: element The incidences of tumours were unaffected by the addition of 750 ppmSO2 as sodium metabisulfite to drinking water (corresponding to 53 mg/kg bw/d)) no NOAEL identified (toxicity): > 53 mg/kg bw/day (nominal) (male/female) based on: element (No evidence of systemic toxicity.) Neoplastic effects: no effects (no treatment related effects)	2 (reliable with restrictions) supporting study Test material disodium disulfite	Lockett, M.F.; Natoff, I.L. (1960)
rat (Fischer 344/DuCrj) male oral: gavage In vivo study on the tumour-promoting activity of sulfites in the stomach of rats. 0.45, 0.89, 1.34 g/kg bw (actual ingested (2.45 - 8.45 mmol K2SO3/kg bw)) 0.5, 0.8, 1.1, 1.4 g/kg bw (actual ingested (2.25 -6.75 mmol K2S2O5/kg bw)) Exposure: single dose (once) Male Fischer rats (7 to 8 weeks old) were given single doses of 2.45 – 8.45 mmol K2SO3/kg bw or doses of 2.25 - 6.75 mmol K2S2O5/kg bw by gastric intubation. Ornithine decarboxylase (ODC) activity and induction of DNA synthesis via deoxythymidine incorporation were determined in tissue extracts of the pyloric mucosa of rat stomach during the time range from 4 to 48 hours after administration.	tumour promotion: (Based on these results it was suggested that both K2SO3 and K2S2O5 may have tumour-promoting activities in glandular stomach carcinogenesis.) Neoplastic effects: not examined	2 (reliable with restrictions) supporting study Test material dipotassium disulfite Test material potassium sulfite	Furihata, C.; et al. (1989)

rat (Wistar) male oral: drinking water Initiation-promotion study Exposure: - 8 weeks of exposure to MNNG (N-methyl-N'-nitro-N-nitrosoguanidine) a diet supplemented with 1% NaCl for 8 weeks. - 32 weeks of exposure to K2S2O5 (group 3 and 8) - 40 weeks total exposure time (MNNG/K2S2O5) Thirty animals were treated with the initiation procedure alone. Ten rats serving as negative controls without MNNG treatment were given drinking water with 1% K2S2O5 during the promotion stage. At the end of the 40th experimental week, all surviving animals were sacrificed for necropsy. The stomach and other organs in the peritoneal cavity were examined carefully.	tumour initiation and promotion : Potassium metabisulfite was considered to exert tumour-promoting activity in the rat glandular stomach. Neoplastic effects: yes (significant effects by K2S2O5)	2 (reliable with restrictions) supporting study Test material: dipotassium disulfite	Takahashi, M.; et al. (1986)

7.9.6.1.2. **Carcinogenicity in animal studies: inhalation**

Table 27: Overview of experimental studies on carcinogenicity after inhalation exposure

Method	Results	Remarks	Reference
rat (Sprague-Dawley) male inhalation: gas (whole body) To study the effect of inhaled sulphur dioxide and systemic sulfite on the induction of lung carcinoma in rats by benzo[a]pyrene (BaP), groups of 20 male Sprague-Dawley rats were exposed in chambers at nominal concentrations of 0, 10 or 30 ppm SO2 for 6 hr per day, 5 days per week for 21 weeks. In the combination experiments, 76 rats per group were exposed simultaneously to BaP by intratracheal instillation (1.0 mg BaP in 0.2 ml vehicle weekly). The BaP treatment was started on the 4th week of SO2 treatment and continued for 15 consecutive weeks. Thereafter, the rats were observed for the development of tumours in the respiratory tract for 737 days. Systemic exposure to sulfite/bisulfite was accomplished by inducing sulfite oxidase deficiency by means of high tungsten to molybdenum ratio in the diet. Complete necropsy was performed on all animals with particular attention given to the respiratory tract.	no NOAEC identified : no eoplastic effects of sulfur dioxide were observed Sulfite oxidase deficiency resulted in an accumulation of endogenously generated sulfite	2 (reliable with restrictions) supporting study Test material sulfur dioxide	Gunnison, A.F.; et al. (1988)

Summary of animal carcinogenicity data

Repeated dose experiments in combination with carcinogenicity/cocarcinogenicity assessments have revealed that sulphites may exert some tumour promoting, cocarcinogenic activity. 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. In a repeated dose study, 4 out of 149 female rats with low SOX activity displayed mammary adenocarcinomas after 9 weeks of treatment with sodium metabisulfite in their drinking water (Gunnison et al., 1981). No tumours were seen when the animals were not exposed to exogenous sulfite and in control animals with normal levels of sulfite oxidase.

7.9.6.2. Carcinogenicity – Human data

Table 28: Overview of exposure-related observations on carcinogenicity in humans

Method	Results	Remarks	Reference
Study type: cohort study (retrospective) Occupational exposure Details on study design: Data collection: - Type: Record review - Details: Copies of the death Study period: deaths between 1935 and 1963 were evaluated Setting: Pulp and Paper Mills Study population: - Total population (Total no. of persons in cohort from which the subjects were drawn): a total of 2,689 death notices were abstracted from the journals published between 1935 and 1964 - Total number of subjects participating in study: The analysis was based on 2113 death records (79% of the original death notices). - Sex/age/race: Males, North Americans Death records were requested from various states and Canadian provinces. The final record for each worker contained case number, name, union local number, state, year of death, age at death, cause of death and mill type. Cause of death was coded by the Washington State nosologist to the eight "International Classification of Diseases (ICDA). Monson's PMR program was employed for analysis of the data. Expected numbers of lymphosarcoma deaths before 1950 and small-bowl cancer were estimated using New York State cancer mortality data and an age- and year-of-death-specific proportionate mortality model. Canadian deaths were treated as if they were U.S. deaths. The analysis was based on 2113 death records (79% of the original death notices). Endpoint addressed: carcinogenicity	Findings- Higher proportionate mortality ratios (PMRs) for lymphosarcoma (statistically significant) and kidney, pancreatic and rectal cancers were associated with jobs in the sulfite process. - A statistically significant excess of mortality due to gastric cancer was seen among men with both sulfite and sulfate pulping. - Hodgkin´s disease deaths occurred primarily in sulfate (Kraft) process workers.	reliable report, supporting study Test material (Common name): sulfite (not specified)	Milham, S.;Demers, R.Y. (1984)

Study type: cohort study (retrospective) Type of population: occupational SETTING: five pulp and paper mills, located in the north-western part of the USA, of which two produced sulfite pulp STUDY POPULATION - Total population (Total no. of persons in cohort from which the subjects were drawn): - Selection criteria: pulp and paper mill workers, white, males, employed for at least one year between 1945 and 1955, in the two major processes: sulfate and sulfite - Total number of subjects participating in study: 3572, sulfite process subcohort consisted of 1779 men - Sex/age/race: white males Details on study design: HYPOTHESIS TESTED (if cohort or case control study): The study was designed to evaluate the chronic health effects of employment in pulp and paper mill industries and to test the hypotheses of increased mortality due to malignancies of the buccal cavity, pharynx, stomach, intestine/rectum, larynx, lung, prostate, bladder, kidney, and the hematopoitic and lymphatic systems, as well as that due to coronary heart disease and diseases of the blood-forming organs. METHOD OF DATA COLLECTION - Type: Interview / Questionnaire / Record review / Work history / Clinical tests / other: employment records of the workers - Details: A cohort of 3572 pulp and paper mill workers (white males) employed for at least one year between 1945 and 1955 was followed from their last day of employment through 31 March 1977. Vital status was determined for 99% of the cohort. Death certificates were obtained for 99 % of those deceased. Underlying causes of death were coded according to ICD. COMPARISON POPULATION - Type: National registry - Details: The observed numbers of deaths in the study cohort and subcohorts were compared to the numbers expected as	EXPOSURE - an analysis of exposure-response relationships was not possible because no exposure levels were available FINDINGS - The 915 deaths observed were 79% of the number expected on the basis of comparable United States mortality rates. Statistically non-significant excesses of deaths due to lymphosarcoma and reticulosarcoma and stomach cancer were observed. - No deaths due to nasal cancer were observed, but only 0.6 were expected. - When process-specific analyses were conducted, **the excess risk of lymphosarcoma and reticulosarcoma was increased only for men who worked in sulfate mills.** - The **excess risk of stomach cancer was limited to men who worked in sulfite mills; it was the most elevated after a 20-year latency period** (9 observed, 5.1 expected, SMR 176), but during latency period did not increase with duration of employment. - Process-specific SMRs for these causes were highest after 20 years since first employment in the mills.	reliable report, however not rated according to Klimisch supporting study Test material name): Sulfite (not specified)	Robinson, C.F.; et al. (1986)

derived from sex., age., race., calendar-time-, and cause-specific US mortality rates. SMR values were calculated by dividing the number of observed deaths by the number of expected deaths and multiplying by 100.			
Study type: cohort study (retrospective) Type of population: occupational Details on study design: HYPOTHESIS TESTED (if cohort or case control study): Studies have indicated an excess risk of stomach cancer, pancreas cancer, and non-Hodgkin's lymphoma for sulfite pulp mill workers, and therefore cancer incidence was evaluated for 2 Danish sulfite mills. METHOD OF DATA COLLECTION - Type: Record review - Details: Cancer incidence was evaluated for 2 Danish sulfite mills. 2238 persons (only 102 women) employed in 1955-1990 were included in a historic cohort and followed until December 1993 STUDY PERIOD: 1955 - 1990, follow up until 1993 SETTING: 2 Danish sulfite mills STUDY POPULATION - Total population (Total no. of persons in cohort from which the subjects were drawn): 2238 persons (102 women) were included and followed up to 1993 - Selection criteria: availability of personal identification number for vital status, date of death in the National Mortality Register - Total number of subjects participating in study: 2198 persons - Other: For each person, the person-years at risk were calculated from start of employment until death or end of follow-up. COMPARISON POPULATION - Type: National registry - Details: National cancer rates were uses to calculated expected cancer cases. Endpoint addressed: carcinogenicity	FINDINGS - There was an **increased mortality from asthma (OR=2.8) and brain tumours** (OR=3.3) among the sulfite workers. - **The risk of stomach cancer (10 observed) and pancreatic cancer (7 observed) was doubled** (SIR 1.99 and 1.88, respectively). - For the men with **known pulp exposure, lung cancer was slightly increased** (SIR 1.53). - **Other cancers with elevated risks were leukaemia (7 cases; SIR 1.84) and soft-tissue sarcomas** (4 observed; SIR 2.37). - The excess risk of stomach cancer and pancreatic cancer found in this study was in accordance with that of other studies from sulfite pulp mills.	reliable report, not rated according to Klimisch supporting study Test material:sulfite / sulphur dioxide	Andersson E,; et al. (1998)

Cohort study to investigate associations between various malignancies and work in the pulp and paper industry Cohort: 18,113 males and 2,292 females enrolled from 1939 to 1999 with >1 year of employment was followed up for cancer incidence from 1958 to 2001. Data were obtained from the mills personel files and standardized incidence ratios(SIRs) were calculated using the Swedish population as reference.	Overall cancer incidence was not increased, but risks of pleural mesothelioma were increased among males employed in sulphate pulping , (SIR, 8.38; 95% CI, 3.37-17) and maintainance (SIR, 6.35, 95% CI 3.47-11) with no corresponding increase of lung cancer. Testicular cancer risks were increased among males employed in sulphate pulping (SIR, 2.92, 95% CI 1.18-6.02)	Reliable report, Not rated to Klimisch due to potential co exposure	Andersson et al, (2013)

Positive findings were reported from in vitro assays (chromosomal aberration, micronucleus, Sister Chromatid exchange and DNA-protein crosslinks) from humans occupationally exposed to sulfur dioxide (Meng and Zhang, 1990 a, b; Xie et al., 2007, Yadav and Kaushik (1996) Nordenson et al. 1980). Although the studies have certain deficiencies such as inaccurately reported exposure (e.g. exposure only based on the range of SO2 in the air) they indicate cause of concern.

The mortality of workers exposed to sulfur dioxide in the pulp and paper industry was investigated in a cohort study including 57,613 workers employed for at least 1 year in the pulp and paper industry in 12 countries. After adjustment for occupational coexposures, the lung cancer risk was increased compared with unexposed workers (odds ratio =1.49; 95% CI, 1.14–1.96). Mortality from non-Hodgkin lymphoma and from leukemia was increased among workers with high SO2 exposure; a dose–response relationship with cumulative SO2 exposure was suggested for non-Hodgkin lymphoma (Lee et al., 2002). Andersson et al, 2013 investigated a cohort (18,113 males and 2,292 females pulp and paper industry worker from Sweden) and reported elevated risks of pleural mesothelioma among males employed in sulphate pulping (SIR, 8.38; 95 % CI, 3.37-17) and maintenance (SIR, 6.35; 95 % CI, 3.47-11), with no corresponding increase of lung cancer. Testicular cancer risks were increased among males employed in sulphate pulping (SIR, 4.14; 95 % CI, 1.99-7.61) and sulphite pulping (SIR, 2.59; 95 % CI, 0.95-5.64). Female paper production workers showed increased risk of skin tumours other than malignant melanoma (SIR, 2.92; 95 % CI, 1.18-6.02 (Andersson et al., 2013). The study of Robinson et al., 1986 investigating 3572 workers in the pulp and paper industry in the US reported excess risk of lymphosarcoma and reticulosarcoma for men who worked in sulfate mills. Excess risk of stomach cancer was limited to men who worked in sulfite mills (Robinson et al. 1986). A Danish study including 2238 workers found the risk of stomach cancer was doubled (110 observed, SIR 1.99, (95% CI) 0.95-3.661), as was the risk of pancreatic cancer (7 observed, SIR 1.88, 95% CI 0.75-3.88). For the men with known pulp exposure, lung cancer was slightly increased (SIR 1.53, 95% CI 0.94-2.37). Other cancers with elevated risks were leukemia (7 observed, SIR 1.84) and soft-tissue sarcomas (4 observed, SIR 2.37) (Rix et al. 1997). Another occupational study investigating 780 workers from Swedish pulp mills found an increased mortality from asthma (odds ratio (0R) 2.8, 90% confidence interval (90% CI) 1.1-6.81) and brain tumors (OR 3.3, 90% CI 1.2-8.9) among the sulfite workers. The mortality due to lung cancer was not significantly increased (OR 1.4, 90% CI 0.7-2.6), and there was a reduced mortality from stomach cancer (OR 0.4, 90% CI 0.2-0.9) (Andersson et al., 1998).

7.9.6.2.1. **In vitro studies related to Carcinogenicity:**

Table 29

Method	Results	Remarks	Reference
Endpoint addressed: carcinogenicity Type of effects studied: morphologic transformation of HEC (in vitro) 1 mM K2S2O5 (nominal conc.) 5 mM K2S2O5 (nominal conc.) 10 mM K2S2O5 (nominal conc.) 20 mM K2S2O5 (nominal conc.) Transformation of Syrian hamster embryo cells (HEC) by bisulfite and after treatment with UV irradiation. Two-day-old secondary hamster cultures were treated 24 h after plating for 15 min with varying concentrations of sodium bisulfite in PBS (0, 1, 5, 10, or 20 mM). With this procedure, all bisulfite treatment was at neutral pH. After treatment incubation was for 6 more days before determination of cell survival of HEC and transformation.	- Concentrations of sodium bisulfite between 1 to 20 mM that caused minimal toxicity caused a dose-dependent increase in the transformation of Syrian hamster embryo cells. - Exposure of the cells to bisulfite (up to 10 mM) either before or after UV irradiation with 3 J/m2 did not result in an increased transformation frequency. - The results suggest that bisulfite transformation of HEC may not occur by a mechanism involving mutation.	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) Test material (EC name): sodium hydrogensulfite (See endpoint summary for justification of read-across)	DiPaolo, J.A.; et al. (1981)

Endpoint addressed: carcinogenicity Type of effects studied: (in vitro) 1 mM NaHSO3 (nominal conc.) 5 mM NaHSO3 (nominal conc.) 10 mM NaHSO3 (nominal conc.) 20 mM NaHSO3 (nominal conc.) Proteomic analysis of changed polypeptide expression in Syrian hamster foetal cells (HFC). Cells from a secondary culture were exposed 24 h after plating for 15 min to 20 mM sodium bisulfite in PBS (0, 1, 5, 10, or 20 mM). After being incubated for a total of 7 days, morphologically transformed colonies were ring isolated and expanded. Within 6 weeks cell populations were injected subcutaneously into nu/nu mice to determine tumorigenicity. Cell lines of proven malignancy but which had not been derived from tumours were used exclusively. Amino acid-labelled [14C]-polypeptides from neoplastic and non-transformed parental foetal cells were separated by two-dimensional gel electrophoresis and analyzed by computerized microdensitometry of autoradiographic patterns.	- Approximately 1000 polypeptides from parental fibroblasts at population doublings ranging from 4 to 20 and those from colony-derived malignant cell lines were compared. - Sodium bisulfite caused neoplastic transformation of HFC which was associated with qualitative and quantitative changes of the polypeptide pattern. Seven malignant lines exhibited 4 qualitative polypeptide changes: two polypeptides shifted slightly to the acidic side, one new polypeptide was observed, and one polypeptide was absent. - The transformed bisulfite lines differed quantitatively from control cells in that 10-25% and 2-4% of the polypeptides had differences in expression greater than 2- and 4-fold, respectively. 21 specific polypeptides in all transformed lines had coordinate quantitative changes. - Because bisulfite at neutral pH fails to induce any significant DNA changes at concentration that cause transformation, polypeptides expressed immediately or 48 h after bisulfite treatment were compared to those of non-treated controls, and no differences were found. - No differences were found in the polypeptides expressed immediately or 48 hours after bisulfite treatment. Even though bisulfite does not induce detectable DNA changes or early post-treatment polypeptide changes in polypeptide expression, a consistent set of qualitative and quantitative changes were observed after transformation. - The qualitative polypeptide changes found in the bisulfite-induced malignant lines were similar to those seen in a benzo(a)pyrene induced malignant line (OBP). - The results suggest that there is a convergence of pathways responsible for carcinogenesis independent of the nature of initiation	2 (reliable with restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) Test material (EC name): sodium hydrogensulfite (See endpoint summary for justification of read-across)	Wirth, P.J. et al. (1986)

Evaluation of the International Agency for Research on Cancer

The International Agency for Research on Cancer (IARC, 1992) has evaluated the evidence for carcinogenicity and concluded: There is limited evidence for sulphur dioxide carcinogenicity in experimental animals. There is however inadequate evidence for sulfites, bisulfites and metabisulfites for carcinogenicity in experimental animals.

As depicted above occupational exposure to sulphites is mostly reported from the pulp and paper industry, where elevated cancer rates are recorded. There is however co-exposure with various substances documented as well.

IARC has evalutated the evidence for carcinogenicity to humans exposed in pulp and paper manufacture and concluded in 1981 exposures that are not classifiable as to their carcinogenicity to humans (Group 3).

Conclusion on Carcinogenicity:

Based on the available evience sulphites may exert tumour promoting properties, but can not be classified as carcinogens. This applies also to sodium dithionite. At this stage no further data for the endpoint carcinogenity are requested.

The recent CLH report on SO2, proposes no classification for carcinogenicity. "In summary, taking into account the limitations of the available data on carcinogenicity, the evaluating body does not see sufficient evidence to propose classification for carcinogenic hazards, even though sulfur dioxide is proposed to be a genotoxic compound" (BAuA, 2020).

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

7.9.7.1. Effects on fertility

Table 30: Overview of experimental studies on fertility

Method	Results	Remarks	Reference
rat (Wistar) male/female multi-generation study oral: feed, thiamine supplemented diet 0.125% (ca. 50 mg/kg bw) 0.25% (ca. 110 mg/kg bw) 0.5 % (ca. 220 mg/kg bw) 1.0% (ca. 460 mg/kg bw) 2.0% (ca. 960 mg/kg bw) Exposure period: 104 weeks (F0 and F1 generation) and 30 weeks (F2 generation) Premating exposure period (males): 21 weeks Premating exposure period (females): 21 weeks Duration of test: until the weaning of the F3 animals Frequency of treatment: Continuously In a three-generation feeding study, groups of 20 male and 20 female Wistar rats received 0, 0.125, 0.25, 0.5, 1.0 and 2.0% sodium metabisulfite, i.e. 49, 108, 220, 460, and 955 mg/kg bw/d as actual dose in a thiamine-containing diet over periods of 2 years.	NOAEL (local toxicity) (F1, F2a and F2b): 108 mg/kg bw/day (nominal) (male/female) based on: test mat. (Based on the occurrence of occult blood in the faeces and changes in gastric morphology at dose levels of 0.5% or more, the NOAEL for local effects is represented by the dose of 0.25% metabisulfite (or 0.215% accounting for the loss of metabisulfite). The corrected dose level corresponds to a dose of 108 mg/kg bw/d Na2S2O5 or an equivalent dose of 72 mg SO2/kg bw/day.) NOAEL (fertility) (F1, F2a, F2b and F3): > 955 mg/kg bw/day (nominal) (male/female) based on: test mat. (No effects on fertility and reproduction were observed up to a dose level of 2%, and the NOAEL for fertility can be expected above the highest dose of 2% metabisulfite corresponding to 955 mg/kg bw/d of Na2S2O5 (or 640 mg/kg bw/d as SO2 equivalents).)	2 (reliable with restrictions) key study Test material sodium metabisulfite	Til, H.P., Feron, V.J., de Groot, A.P. (1972)

Three generation study rat (uniform strain bred for cancer research) male/female combined repeated dose and reproduction 375 and 750 ppm SO2 as sodium metabisulfite (nominal in water) Exposure: Up to 2.5 yea (over 3 generations) before and throughout matir and during pregnancy ar lactation Generation I consisted three groups: group 1 (ta water controls), group 2 (75 ppm as SO2) and group (375 ppm as SO2). The metabisulfite drinkir water groups of generation were produced from matin of the sulfite drinking wat groups of generation Generation III was derive similarly from generation I Observations on growth, fe consumption, fluid intak faecal output, reproductio lactation and the incidence tumours were recorde Detailed post-mortem ar histological examinatior were made.	no NOAEL identified (toxicity) (F1, F2a and F2b): > 53 mg/kg bw/day (nominal) (male/female) based on: element (SO2) No evidence of systemic toxicity no NOAEL identified (reproduction toxicity) (F1, F2a, F2b and F3): > 53 mg/kg bw/day (nominal) (male/female) based on: element (SO2) No significant difference in the number of offspring of either generation 1 and 2; the proportion surviving to the end of lactation did not differ.	2 (reliable with restrictions) supporting study Test material disodium disulfite	Lockett, M.F.; Natoff, I.L. (1960)

7.9.7.2. Developmental toxicity

Table 31: Overview of experimental studies on developmental toxicity

Method	Results	Remarks	Reference
rat (Wistar) oral: feed 0.1% (nominal in diet (K2S2O2 uptake: 130 mg/kg body weight/day)) 1.0% (nominal in diet (K2S2O2 uptake: 1320 mg/kg body weight/day)) 10% (nominal in diet (K2S2O2 uptake: 2860 mg/kg body weight/day)) Exposure: 8 consecutive days from	NOAEL (maternal toxicity): 1320 mg/kg bw/day based on: test mat. (K2S2O5) (Maternal body weight gain during pregnancy and food intake during the administration period were markedly suppressed at a dietary level of 10%. Thus, the dietary dose level of 1% represents the NOAEL (2860 mg K2S2O5/kg bw/d or 1659 mg/kg bw/d SO2 equivalents).) NOAEL (foetotoxicity): 1320 mg/kg bw/day	2 (reliable with restrictions) key study Test material dipotassium disulfite	Ema M.; et al. (1985)
rat (Wistar) oral: feed 0.32 %, 0.63 % 1.25 %, 2.5 %, 5 % (nominal in diet) Na2SO3 x 7H2O uptake: approx. 200, 400, 900,1750 2900 mg/kg body weight/day) Exposure: from day 8 to day 20 of gestation (daily) equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)	NOAEL (maternal toxicity): 850 mg/kg bw/day (nominal) based on: test mat. (Na2SO3 x 7H2O) Effects on body weight gain and food consumption at a dietary level of 5%. The NOAEL for maternal toxicity can be established at 2.5% in the diet (approx. 850 mg/kg bw/d Na2SO3 x 7 H2O or approx. 440 mg/kg bw/d as SO2 equivalents).) no NOAEL identified (teratogenicity): > 1450 mg/kg bw/day (nominal) based on: test mat. (Na2SO3 x 7H2O) (No evidence of a teratogenic potential.) (slight growth retardation): 100 mg/kg bw/day (nominal) based on: test mat. (Na2SO3 x 7H2O) (Some evidence of growth retardation was observed in all treatment groups at and above a dietary dose of 0.32% (approx. 100 mg/kg bw/d Na2SO3 x 7 H2O or approx. 50 mg/kg bw/d as SO2 equivalents); no dose-relationship was seen and these effects were not observed in the live-birth part of the study	2 (reliable with restrictions) key study Test material: Sodium sulfite heptahydrate	Itami, T.; et al. (1989) Unpublished study report (1992) JECFA (Joint FAO/WHO Expert Committee on Food Additives) (1999): Sulfur (1999)

equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study) rat (Wistar) oral: gavage 4.0, 19.0 , 86.0, 400 mg/kg body weight (nominal conc.) Exposure: from day 6 to 15 of gestation (daily)	no NOAEL identified (maternal toxicity): > 400 mg/kg bw/day based on: test mat. (No clearly discernible effect on nidation or on maternal survival were observed at dose level up to and including the highest dose of 400 mg/kg bw/d sodium thiosulfate.) no NOAEL identified (developmental toxicity): > 400 mg/kg bw/day based on: test mat. (Treatment of dams with dose level up to 400 mg/kg bw/d sodium thiosulfate did not cause effects on the number of live and dead foetuses, sex ratio and average foetal weights.) no NOAEL identified (teratogenicity): > 400 mg/kg bw/day based on: test mat. (No evidence of a teratogenic potential.)	2 (reliable with restrictions) key study Test material sodium thiosulfate	Unpublished study report (1972a)
equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study) rat (Wistar) oral: gavage 1, 5, 24, 110 mg/kg body weight (nominal conc.) Exposure: from gestation day 6 to 15 (daily)	no NOAEL identified (maternal toxicity): > 110 mg/kg bw/day based on: test mat. (No clearly discernible effect on nidation or on maternal survival were observed at dose level up to and including the highest dose of 110 mg/kg bw/d sodium bisulfite.) no NOAEL identified (developmental toxicity): > 110 mg/kg bw/day based on: test mat. (Treatment of dams with dose level up to 110 mg/kg bw/d sodium bisulfite did not cause effects on the number of live and dead foetuses, sex ratio and average foetal weights.) no NOAEL identified (teratogenicity): > 110 mg/kg bw/day based on: test mat. (No evidence of a teratogenic potential.)	2 (reliable with restrictions) key study read-across Test material sodium hydrogensulfite	Unpublished study report (1972b)
equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study) rat (Wistar) oral: gavage 1.55 , 7.19, 33.4, 155 mg/kg body weight (nominal conc.) Exposure: from day 6 to 15 of gestation (daily)	no NOAEL identified (maternal toxicity): > 155 mg/kg bw/day based on: test mat. (No clearly discernible effect on nidation or on maternal survival were observed at dose level up to and including the highest dose of 155 mg/kg bw/d potassium metabisulfite.) no NOAEL identified (developmental toxicity): > 155 mg/kg bw/day based on: test mat. (Treatment of dams with dose level up to 155 mg/kg bw/d potassium metabisulfite did not cause effects on the number of live and dead foetuses, sex ratio and average foetal weights.) no NOAEL identified (teratogenicity): > 155 mg/kg bw/day based on: test mat. (No evidence of a teratogenic potential.)	2 (reliable with restrictions) key study Test material dipotassium disulfite	Unpublished study report (1975)

equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study) rabbit (Dutch) oral: gavage 1.23, 5.71, 26.5, 123 mg/kg body weight (nominal conc.) Exposure: from gestation day 6 to 18 (daily)	no NOAEL identified (maternal toxicity): > 123 mg/kg bw/day based on: test mat. (No clearly discernible effect on nidation or on maternal survival were observed at dose level up to and including the highest dose of 123 mg/kg bw/d sodium metabisulfite.) no NOAEL identified (developmental toxicity): > 123 mg/kg bw/day based on: test mat. (Treatment of dams with dose level up to 123 mg/kg bw/d sodium metabisulfite did not cause effects on the number of live and dead foetuses, sex ratio and average foetal weights.) no NOAEL identified (teratogenicity): > 123 mg/kg bw/day based on: test mat. (No evidence of a teratogenic effect.)	2 (reliable with restrictions) key study Test material disodium disulfite	Unpublished study report (1974a)
equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study) rabbit (Dutch-belted) oral: gavage 2.5, 5.8, 27.0 , 125.4, 580 mg/kg body weight (nominal conc.) Exposure: from day 6 through day 18 of gestation (daily)	no NOAEL identified (maternal toxicity): > 580 mg/kg bw/day based on: test mat. (No clearly discernible effect on nidation or on maternal survival were observed at dose level up to and including the highest dose of 580 mg/kg bw/d sodium thiosulfate.) no NOAEL identified (developmental toxicity): > 580 mg/kg bw/day based on: test mat. (Treatment of dams with dose level up to 580 mg/kg bw/d sodium thiosulfate did not cause effects on the number of live and dead foetuses, sex ratio and average foetal weights.) no NOAEL identified (teratogenicity): > 580 mg/kg bw/day based on: test mat. (No evidence of a teratogenic effect.)	2 (reliable with restrictions) key study Test material sodium thiosulfate	Unpublished study report (1974b)
equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study) rabbit (Dutch-belted) oral: gavage 1, 4.64, 21.6, 100 mg/kg body weight (nominal conc.) Exposure: from gestation day 6 to 18 (daily)	no NOAEL identified (maternal toxicity): > 100 mg/kg bw/day based on: test mat. (No clearly discernible effect on nidation or on maternal survival were observed at dose level up to and including the highest dose of 100 mg/kg bw/d sodium bisulfite.) no NOAEL identified (developmental toxicity): > 100 mg/kg bw/day based on: test mat. (Treatment of dams with dose level up to 100 mg/kg bw/d sodium bisulfite did not cause effects on the number of live and dead foetuses, sex ratio and average foetal weights.) no NOAEL identified (teratogenicity): > 100 mg/kg bw/day based on: test mat. (No evidence of a teratogenic potential.)	2 (reliable with restrictions) key study Test material sodium hydrogensulfite	Unpublished study report (1974c)

equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study) mouse (CD-1) oral: gavage 5.5, 25.5, 118.0, 555.0 mg/kg body weight (nominal conc.) Exposure: from day 6 to 15 of gestation (daily)	no NOAEL identified (maternal toxicity): > 550 mg/kg bw/day based on: test mat. (No clearly discernible effect on nidation or on maternal survival were observed at dose level up to and including the highest dose of 550 mg/kg bw/d sodium thiosulfate.) no NOAEL identified (developmental toxicity): > 550 mg/kg bw/day based on: test mat. (Treatment of dams with dose level up to 550 mg/kg bw/d sodium thiosulfate did not cause effects on the number of live and dead foetuses, sex ratio and average foetal weights.) no NOAEL identified (teratogenicity): > 550 mg/kg bw/day based on: test mat. (No evidence of a teratogenic effect.)	2 (reliable with restrictions) key study read-across from supporting substance (structural analogue or surrogate) Test material (EC name): sodium thiosulfate (See endpoint summary for justification of read-across)	Unpublished study report (1972a)
equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study) mouse (CD-1) oral: gavage 2 , 7, 32, 150 mg/kg body weight (nominal conc.) Exposure: from gestation day 6 to 15 (daily)	no NOAEL identified (maternal toxicity): > 150 mg/kg bw/day based on: test mat. (No clearly discernible effect on nidation or on maternal survival were observed at dose level up to and including the highest dose of 150 mg/kg bw/d sodium bisulfite.) no NOAEL identified (developmental toxicity): > 150 mg/kg bw/day based on: test mat. (Treatment of dams with dose level up to 150 mg/kg bw/d sodium bisulfite did not cause effects on the number of live and dead foetuses, sex ratio and average foetal weights.) no NOAEL identified (teratogenicity): > 150 mg/kg bw/day based on: test mat. (No evidence of a teratogenic potential.)	2 (reliable with restrictions) key study Test material sodium hydrogensulfite	Unpublished study report (1972b)
Guideline 414 (Prenatal Developmental Toxicity Study) mouse (CD-1) oral: gavage 1.25, 5.47, 26.9 125 mg/kg body weight (nominal conc.) Exposure: from day 6 to 15 of gestation (daily) equivalent or similar to OECD	no NOAEL identified (maternal toxicity): > 125 mg/kg bw/day based on: test mat. (No clearly discernible effect on nidation or on maternal survival were observed at dose level up to and including the highest dose of 125 mg/kg bw/d potassium metabisulfite.) no NOAEL identified (developmental toxicity): > 125 mg/kg bw/day based on: test mat. (Treatment of dams with dose level up to 125 mg/kg bw/d potassium metabisulfite did not cause effects on the number of live and dead foetuses, sex ratio and average foetal weights.) no NOAEL identified (teratogenicity): > 125 mg/kg bw/day based on: test mat. (No evidence of a teratogenic potential.)	2 (reliable with restrictions) key study Test material dipotassium disulfite	Unpublished study report (1975)

equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study) hamster (Golden) oral: gavage 4.0, 19.0, 86.0, 400.0 mg/kg body weight (nominal conc.) Exposure: from day 6 to 10 of gestation (daily)	no NOAEL identified (maternal toxicity): > 400 mg/kg bw/day based on: test mat. (No clearly discernible effect on nidation or on maternal survival were observed at dose level up to and including the highest dose of 400 mg/kg bw/d sodium thiosulfate.) no NOAEL identified (developmental toxicity): > 400 mg/kg bw/day based on: test mat. (Treatment of dams with dose level up to 400 mg/kg bw/d sodium thiosulfate did not cause effects on the number of live and dead foetuses, sex ratio and average foetal weights.) no NOAEL identified (teratogenicity): > 400 mg/kg bw/day based on: test mat. (No evidence of a teratogenic potential.)	2 (reliable with restrictions) key study Test material sodium thiosulfate	Unpublished study report (1972a)
equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study) hamster (Golden) oral: gavage 1, 6, 26, 120 mg/kg body weight (nominal conc.) Exposure: from day 6 to 10 of gestation (daily)	no NOAEL identified (maternal toxicity): > 120 mg/kg bw/day based on: test mat. (No clearly discernible effect on nidation or on maternal survival were observed at dose level up to and including the highest dose of 120 mg/kg bw/d sodium bisulfite.) no NOAEL identified (developmental toxicity): > 120 mg/kg bw/day based on: test mat. (Treatment of dams with dose level up to 120 mg/kg bw/d sodium bisulfite did not cause effects on the number of live and dead foetuses, sex ratio and average foetal weights.) no NOAEL identified (teratogenicity): > 120 mg/kg bw/day based on: test mat. (No evidence of a teratogenic potential.)	2 (reliable with restrictions) key study Test material (EC name): sodium hydrogensulfite	Unpublished study report (1972b)

No studies on reproduction and development are available for Sodium dithionite. The available studies investigating sulphites are old and characterized by a lack of reporting. From the key study and supporting study provided for evaluating the effects of sodium metabisulfite on fertility and reproduction a NOAEL of 955 mg/kd bw/day for Na2S2O5 has been identified, based on the slight growth retardation during lactation in offspring. Several parameters were not examined (e.g. sperm parameters, estrous cyclicity, offspring pathology, etc.). Developmental toxicity studies did not show the occurrence of abnormalities or foetotoxicity below maternal toxic doses, which varied between species and experiments.

Conclusion of toxicity to reproduction:

Despite the shortcomings of the available studies the weight of evidence approach of assessing the available data showed no concern for the endpoint of fertility.

Conclusion of developmental toxicity

Despite the shortcomings of the available studies the weight of evidence approach of assessing the available data showed no concern for the endpoint developmental toxicity.

7.9.8. Other effects

7.9.8.1. Neurotoxicity

Table 32: Overview of experimental studies on neurotoxicity

Method	Results	Remarks	Reference
in-vitro test with human neuroblastoma cells 0.08 - 0.8 mM sodium bisulfite (nominal conc.) Exposure: 3 or 20 hours Plates of cells were incubated with sodium bisulfite concentrations in the range from 0.08 to 0.8 mM at pH 7.4 for 3 or 20 hours. After the exposure, the medium was discarded and drug-free medium was added to the experimental and control cultures. The cultures were left undisturbed at 37°C for 3, 6, and 9 days. Thereafter, the cultures were washed with saline and stained with Wright-Giemsa for cell counting. Cell multiplication was measured by the number of colonies that developed in each experimental and untreated culture.	Sodium bisulfite reduced cell multiplication in human neuroblastoma cells. Colony-forming ability (CFA) was reduced 72% to 92% by a 3-h exposure to one commercial sample of Sodium Bisulfite and 57% to 72% by another commercial sample (both tested at 0.8 × 10−3 M). When exposure time was lengthened to 20 h, both solutions inhibited CFA to the same extent (98%). No difference in the inhibition of CFA was observed between the two samples at <0.8 × 10−3 M.	2 (reliable with restrictions) supporting study Test material sodium hydrogensulfite	Seravalli, E.; Lear, E. (1987)

in-vitro system: increase of reactive oxygen species (ROS) 5-500 µM sodium sulfite (in-vitro applied concentration) Neuro-2a, PC12, HepG2 cell lines and human foetal liver cells were exposed to 5-500 µM freshly prepared sulfite for 30 min rat brain mitochondria used were prepared from Wistar rats	Exposure of Neuro-2a and PC12 cells to micromolar concentrations of sulfite caused an increase in reactive oxygen species and a decrease in ATP. Likewise, the biosynthesis of ATP in intact rat brain mitochondria from the oxidation of glutamate was inhibited by micromolar sulfite. Glutamate-driven respiration increased the mitochondrial membrane potential (MMP), and this was abolished by sulfite but the MMP generated by oxidation of malate and succinate was not affected. The increased rate of production of NADH from exogenous NAD+ and glutamate added to rat brain mitochondrial extracts was inhibited by sulfite, and mitochondria preincubated with sulfite failed to reduce NAD+. Glutamate dehydrogenase (GDH) in rat brain mitochondrial extract was inhibited dose-dependently by sulfite as was the activity of a purified enzyme. An increase in the Km (glutamate) and a decrease in Vmax resulting in an attenuation in Vmax/Km (glutamate) at 100 µm sulfite suggest a mixed type of inhibition. However, uncompetitive inhibition was noted with decreases in both Km (NAD+) and Vmax, whereas Vmax/Km (NAD+) remained relatively constant. We propose that GDH is one target of action of sulfite, leading to a decrease in α-ketoglutarate and a diminished flux through the tricarboxylic acid cycle accompanied by a decrease in NADH through the mitochondrial electron transport chain, a decreased MMP, and a decrease in ATP synthesis. Because glutamate is a major metabolite in the brain, inhibition of GDH by sulfite could contribute to the severe phenotype of sulfite oxidase deficiency in human infants.	2 (reliable with restrictions) supporting study Test material sodium sulfite	Zhang, X.; et al. (2005)

rat male subchronic (oral: drinking water): Metabisulfite effects on cognitive function 25 mg/kg*d Na2S2O5 (nominal in water) Exposure: 6 weeks Groups of adult male albino rats were treated as follows: drinking water with Na2S2O5 (25 mg/kg), vitamin E, and Na2S2O5 plus vitamin E. A 4th group served as control. The same set of experiments was performed on sulfite oxidase-deficient (SOXD) rats. Active avoidance behaviour was studied. Prior to this, motor function of the rats was tested using the Hanging Wire Test. At the end, blood was collected for determination of plasma S-sulphonate levels. After exsanguination brain and liver tissues were removed immediately for measuring hippocampus TBARS levels and assay of SOX activity, respectively.	Sulfite (25 mg/kg bw) induced impairment of active avoidance learning in SOX deficient but not in normal rats. The index of lipid peroxidation level thiobarbituric acid reactive substances (TBARS) was significantly increased in SOX deficient rats.	2 (reliable with restrictions) supporting study Test material: disodium disulfite	Kücükatay, V.; et al. (2005)

Conclusion:
No studies on neurotoxicity of Sodium dithionite are available. Several studies, some of those also reported within the registration dossier, demonstrate potential neurotoxicity. Although EFSA considers the health consequence (for the use of sulphites a food additive) not relevant due to the required high doses to reach neurotoxicty, it is stated that more data are needed before a clear conclusion on the possible neurotoxic effects of sulphites (used as food additives) can be reached (EFSA, 2016).

7.9.8.2. Specific investigations:

Other effects from sulphites which were observed in different studies have been discussed in the EFSA assessment on food additives (EFSA, 2016).

7.9.8.2.1. **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.

7.9.8.2.2. **Hepatotoxicity**

The only available 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.

7.9.8.2.3. **Potential roles of hydrogen sulfide**

The Panel noted that hydrogen sulfide (H2S) 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 H2S and the use of sulfites as food additives are needed before a conclusion can be drawn on their beneficial or detrimental roles in modulating H2S activities, if any. 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.

7.9.8.2.4. **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).

7.9.8.2.5. **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 SO2 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.

7.9.8.2.6. **Biological and toxicological data on reaction products of sulfites.**

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). 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.

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).

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 SO2 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.

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).

Conclusion from EFSA 2016: The Panel noted several uncertainties and limitations in the database and concluded that the current group acceptable daily intake (ADI) of 0.7 mg SO2 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 SO2 equivalent/kg bw per day for all population groups.

7.9.9. Hazard assessment of physico-chemical properties

Not assessed.

7.9.10. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

Not derived.

7.9.11. Conclusions of the human health hazard assessment and related classification and labelling

Several concerns have been identified by the eMSCA. The final conclusion on human health hazard assessment and related classification will be postponed until the outcome of the CCH and the decision on harmonised classification of sulphur dioxide.

7.10. Assessment of endocrine disrupting (ED) properties

7.10.1. Endocrine disruption – Environment

For potential endocrine effects in the environment no data are available, but no indicitations for endocrine disruption were noticed so far.

7.10.2. Endocrine disruption - Human health

For potential endocrine effects in humans no data are available, but no indicitations for endocrine disruption were noticed so far.

7.10.3. Conclusion on endocrine disrupting properties (combined/separate)

For potential endocrine effects in humans and the environment no data are available, but no indicitations for endocrine disruption were noticed so far.

7.11. PBT and VPVB assessment

The assessment of the PBT/vPvB properties resulted in the conclusion that the P/vP and B/vB-criteria are not fulfilled as the substance is inorganic with rapid hydrolysis resulting in polar hydrolysis products. No bioaccumulation is expected. The T-criterion is currently not fulfilled but might be fulfilled when the required data are available.

Overall, it can be concluded the sodium dithionite is not a PBT or vPvB substance.

7.12. Exposure assessment

Not presented.

7.13. Risk characterisation

Not prepared at this stage.

7.14. References

Agard C, Nicolet-Akhavan F, Bouillard J, Sandron D (1998). Occupational asthma to metabisulfites . Three cases [Article in French]. Rev Mal Respir. 1998 Sep;15(4):537-40.

Allen D, Delohery J. (1985). Metabisulfite-induced asthma. J. Allergy Clin. Immunol., 75., 145

Andersson, E., Westberg, H., Bryngelsson, L., Magnuson, A., & Persson, B. (2013). Cancer incidence among Swedish pulp and paper mill workers: a cohort study of sulphate and sulphite mills. International archives of occupational and environmental health, 86(5), 529-540.

Andersson E, Nilsson T, Persson B, Wingren G, Torén K (1998). Mortality from asthma and cancer among sulfite mill workers. Scand J Work Environ Health. 1998 Feb;24(1):12-7.

Andersson E, Knutsson A, Hagberg S, Nilsson T, Karlsson B, Alfredsson L, Torén K (2006). Incidence of asthma among workers exposed to sulphur dioxide and other irritant gases. Eur Respir J. 2006 Apr;27(4):720-5.

Angelini G, Vena GA, Foti C, Grandolfo M (1997). Contact allergy to preservatives and perfumed compounds used in skin care products. J.Appl. Cosmetol. 15, 49-57.

OECD (2001). SIDS Dossier on Sodium Disulfite. Final Draft for Publication, available on http://www.oecd.org/document/63/0,2340, en_2649_34379_1897983_1_1_1_1,00.html

ATSDR (1998). Toxicological profile for sulfur dioxide. Agency for Toxic Substances and Disease Registry. https://www.atsdr.cdc.gov/toxprofiles/tp116.pdf

Basketter D, Cockshott A, Corsini E, Gerberick GF, Idehara K, Kimber I, Van Loveren H, Matheson J, Mehling A, Omori T, Rovida C, Sozu T, Takeyoshi M, Casati S (2008). An evaluation of performance standards and non-radioactive endpoints for the local lymph node assay. The report and recommendations of ECVAM Workshop 65. Altern Lab Anim 2008 May;36(2):243-57. https://eurl-ecvam.jrc.ec.europa.eu/validation-regulatory-acceptance/docs-skin-sen/WorkshopReport65.pdf

Basketter D, Kolle SN, Schrage A, Honarvar N, Gamer AO, van Ravenzwaayb B, Landsiedel R (2012). Experience with local lymph node assay performance standards using standard radioactivity and nonradioactive cell count measurements. J. Appl. Toxicol. 2012; 32: 590–596.

Boner AL, Guarise A, Vallone G, Fornari A, Piacentini F, Sette L (1990). Metabisulfite oral challenge: incidence of adverse responses in chronic childhood asthma and its relationship with bronchial hyperreactivity. J Allergy Clin Immunol. 1990 Feb;85(2):479-83.

Boxer MB, Bush RK, Harris KE, Patterson R, Pruzansky JJ, Yang WH (1988). The laboratory evaluation of IgE antibody to metabisulfites in patients skin test positive to metabisulfites. J Allergy Clin Immunol. 1988 Oct;82(4):622-6.

Buckley CE, Saltzman HA, Sieker HO (1985) The prevalence and degree of sensitivity to ingested sulfites. J. Allergy and Clinical Immun. January,Volume 75, Issue 1, Part 2, Page 144

Bush RK, Taylor SL, Busse W (1986). A critical evaluation of clinical trials in reactions to sulfites. J Allergy Clin Immunol. 1986 Jul;78(1 Pt 2):191-202.

Campbell JR1, Maestrello CL, Campbell RL (2001). Allergic response to metabisulfite in lidocaine anesthetic solution. Anesth Prog. 2001 Winter;48(1):21-6.

Chen, L. C., Lam, H. F., Ainsworth, D., Guty, J., & Amdur, M. O. (1987). Functional changes in the lungs of guinea pigs exposed to sodium sulfite aerosols. Toxicology and applied pharmacology, 89(1), 1-8.

Cohen HJ, Johnson HJ and Rajagopalan KV, 1974. Molecular basis of the biological function of molybdenum. Developmental patterns of sulphite oxidase and xanthine oxidase in the rat. Archives of Biochemistry and Biophysics 164, 440–446.

Constantin, D. et al. (1994). Alternative pathways of sulfite oxidation in human polymorphonuclear leukocytes. Pharmacology & Toxicology. 74: 136 - 140.

Cosmetic Ingredient Expert Review Panel (2003). Final Report on the Safety Assessment of Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite, Sodium Bisulfite, Ammonium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite. International Journal of Toxicology, 22 (S2): 63-88.

DeLuque Pinana, V. et al (2013). Sulfite-dependent excercise urticaria. Allergy 68, suppl.97: 260-261.

DiPaolo, J. A.; et al. (1981). Transformation of Syrian hamster embryo cells by sodium bisulfite. Cancer Letters, 12, 203- 208.

Dooms-Goossens A, de Alam AG, Degreef H, Kochuyt A. (1989). Local anesthetic intolerance due to metabisulfite. Contact Dermatitis. 1989 Feb;20(2):124-6.

EBRC Consulting GmbH (2017) HERAG fact sheet. Assessment of dermal absorption for metals and inorganic metal compounds. Updated working draft, status January 2017, original document, 2007.

ECHA (2016): Decision on a Compliance Check of a registration pursuant to article 41(3) of regulation (EC) 1907/2006 for sodium dithionite EC No 231-890-0 (CAS No 7775-14-6) https://echa.europa.eu/documents/10162/60183cc0-8d3f-9bfa-ac79-3d0027111c00

EFSA (2016): 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: EFSA Panel on Food additives and Nutrient Sources added to Food (ANS)2, 3 European Food Safety Authority (EFSA), Parma, Italy

Ehling G, Hecht M, Heusener A, Huesler J, Gamer AO, van Loveren H, Maurer T, Riecke K, Ullmann L, Ulrich P, Vandebriel R, Vohr HW. (2005a). An European inter-laboratory validation of alternative endpoints of the murine local lymph node assay: first round. Toxicology. 2005 Aug 15;212(1):60-8.

Ehling G, Hecht M, Heusener A, Huesler J, Gamer AO, van Loveren H, Maurer T, Riecke K, Ullmann L, Ulrich P, Vandebriel R, Vohr HW (2005b). An European inter-laboratory validation of alternative endpoints of the murine local lymph node assay: 2nd round. Toxicology. 2005 Aug 15;212(1):69-79. Epub 2005 Feb 12.

Ema M.; et al. (1985). Effect of potassium metabisulfite on pregnant rats and their offspring. J. Fd. Hyg. Soc. Japan, 26, 454-459.

eMSCA (2014) Substance evaluation report 2014 Disodium disulphite. Submitting Member State Competent Authority: National Institute of Chemical Safety, Hungary.

Febriana SA, Jungbauer F, Soebono H, Coenraads PJ (2012). Occupational allergic contact dermatitis and patch test results of leather workers at two Indonesian tanneries. Contact Dermatitis. 2012 Nov;67(5):277-83.

Feron, V. J.; Wensvoort, P. (1972). Gastric lesions in rats after the feeding of sulfite. Path. Europ. 7, 103-111.

Ferron, G. A.; et al. (1990). Long-term exposure of dogs to a sulfite aerosol. J. Aerosol Sci. 21, S479-S482.

Fisher AA (1989). Reactions to injectable local anesthetics. Part IV: Reactions to sulfites in local anesthetics. Cutis. 1989 Oct;44(4):283-4.

Fitzhugh, G. O.; et al. (1946). The chronic toxicity of sulfites. J. Pharmacol. Exp. Ther. 86, 37-48.

Frick W.E and Robert F. Lemanske RF (1991). Clinical Acumen: Oral Sulfite Sensitivity and Provocative Challenge in a 2 Year Old. Journal of Asthma Vol. 28 , Iss. 3,1991

Friedman ME, Easton JG (2009). Prevalence of Positive Metabisulfite Challenges in Children with Asthma. Pediatric Asthma, Allergy & Immunology. March 2009, 1(1): 53-59.

Furihata, C.; et al. (1989). Various sodium salts, potassium salts, a Calcium salt and an Ammonium salt induced ornithine decarboxylase and stimulated DNA synthesis in rat stomach mucosa. Jpn. J. Cancer Res. 80, 424-429.

García-Gavín J, Parente J, Goossens A. (2012). Allergic contact dermatitis caused by sodium metabisulfite: a challenging allergen: a case series and literature review. Contact Dermatitis. Nov;67(5):260-9.

García Ortiz JC, Vega Gutiérrez JM, Pérez Velesar MJ, Medina AA (2014). Occupational allergic contact dermatitis from potassium metabisulfite. Dermatitis. 2014 May-Jun;25(3):150-1.

German MAK Commission (2014). Sulfites. The MAK-Collection Part I, MAK Value Documentations 2014. DFG, Deutsche Forschungsgemeinschaft. Wiley-VCH Verlag GmbH & Co. KGaA

Gibson W. B.; Strong, F. M. (1973). Metabolism and Elimination of Sulfite by rats, Mice and Monkeys. Fd Cosmet. Toxicol., 11, 185-198.

Gunnison, A. F. & Benton, A. W. (1971). Sulfur dioxide: Sulfite. Arch. Environ. Health, Vol. 22: 381 - 388.

Gunnison, A. F. and Palms, E. D. (1976). A model for the metabolism of sulfite in mammals. Toxicology and Applied Pharmacology, 38: 111-126.

Gunnison, A. F. et al. (1977). Comparative sulfite metabolism in the rat, rabbit, and rhesus monkey. Toxicology and Applied Pharmacology, 42: 99-109.

Gunnison, A. F.; et al. (1981). A sulfite-oxidase-deficient rat model: subchronic toxicology. Fd. Cosmet. Toxicol. 19, 221-232.

Gunnison, A. F. et al. (1987). Distribution, metabolism and toxicity of inhaled sulfur dioxide and endogenously generated sulfite in the respiratory tract of normal and sulfite oxidase. deficient rats. Journal of Toxicology and Environmental Health, 21: 141-162.

Gunnison, A. F.; et al. (1988). The effect of inhaled sulfur dioxide and systemic sulfite on the induction of lung carcinoma in rats by benzo[a]pyrene. Environ. Res. 46, 59-73.

Harrison DA, Smith AG Concomitant sensitivity to sodium metabisulfite and clobetasone butyrate in Trimovate® cream. Contact Dermatitis. 2002 May;46(5):310.

Hoffmann, Rüdorff (1969), Anorganische Chemie, 20. Auflage

Hollemann, Wiberg (1995), Lehrbuch der Anorganischen Chemie, 101. Auflage

ECHA (2017) Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7c: Endpoint specific guidance. Version 3.0. June 2017 https://echa.europa.eu/documents/10162/13632/information_requirements_r7c_en.pdf/e2e23a98-adb2-4573-b450-cc0dfa7988e5

Hui, J. Y.; et al. (1989). Comparative subchronic oral toxicity of sulfite and acetaldehyde hydroxysulphonate in rats. Food Chem. Toxicol., 27, 349-359.

Huston RK, Baxter LM, Larrabee PB (2009). Neonatal parenteral nutrition hypersensitivity: a case report implicating bisulfite sensitivity in a newborn infant. JPEN J Parenter Enteral Nutr. 2009 Nov-Dec;33(6).

IARC (1981) International Agency for Research on Cancer: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol.25. Wood, Leather and Some Associated Industries.

IARC (1998) International Agency for Research on Cancer: Summaries & Evaluations. Pulp and paper manufacture (Group 3).

IARC (1992) International Agency for Research on Cancer: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 54. Sulfur dioxide and some sulfites, bisulfites and metabisulfites.

ICCVAM (2009). Draft ICCVAM test method recommendations updated assessment of the validity of the LLNA for testing pesticide formulations and other products, metals, and substances in aqueous solutions. http://iccvam.niehs.nih.gov/methods/immunotox/LLNA-app/recs.pdf

Ikehata, K. et al (1996). Two cases opf allergic contact dermatitis caused by sodium sulfite in the vehicle of antifungal cream. Skin Research 38: 198 - 202

Itami, T.; et al. (1989). Evaluation of teratogenic potential of sodium sulfite in rats.: Drug Chem. Toxicol., 12, 123-135 (cited in OECD SIDS sodium dithionite, Oct. 2004).

Jacobs MC, Rycroft RJ (1995). Contact dermatitis and asthma from sodium metabisulfite in a photographic technician. Contact Dermatitis. 1995 Jul;33(1):65-6.

JECFA (Joint FAO/WHO Expert Committee on Food Additives) (1999): Sulfur Dioxide and Sulfites. Safety evaluation of certain food additives, WHO Food Additives Series 42, 95-116 (1999). Sulfur Dioxide and Sulfites. Safety evaluation of certain food additives, WHO Food Additives Series 42, 95-116.

Kaaman AC, Boman A, Wrangsjö K, Matura M. (2010) Contact allergy to sodium metabisulfite: an occupational problem. Contact Dermatitis. Aug;63(2):110-2.

Kajiyama, H., Nojima, Y., Mitsuhashi, H., Ueki, K., Tamura, S., Sekihara, T., ... & Naruse, T. (2000). Elevated levels of serum sulfite in patients with chronic renal failure. Journal of the American Society of Nephrology, 11(5), 923-927.

Kim, H.-J. & Lee, M.-H. (2002). A case of allergic contact dermatitis due to sodium sulfite contained in Nizoral® cream. Korean J Dermatol 40(2): 206 - 208.

Köhler, A.V. & Gall, H. (2000). Kontaktallergisches Ekzem auf Natriumdisulfit in einer antimykotischen Ketoconazol-Creme. Dermatol. Beruf Umwelt 48, 11 - 12.

Kolle SN, Basketter D, Schrage A, Gamer AO, van Ravenzwaay B, Landsiedel R (2012). Further experience with the local lymph node assay using standard radioactive and nonradioactive cell count measurements. J Appl Toxicol. 2012 Aug;32(8):597-607.

Kolle SN, Basketter DA, Casati S, Stokes WS, Strickland J, van Ravenzwaay B, Vohr HW, Landsiedel R (2013). Performance standards and alternative assays: practical insights from skin sensitization. Regul Toxicol Pharmacol. 2013 Mar;65(2):278-85.

Kücükatay, V.; et al. (2005). Effect of sulfite on cognitive function in normal and sulfite oxidase deficient rats. Neurotox. Teratol. 27, 47-54.

Last JA, Dasgupta PK, Etchison JR (1980). Inhalation toxicology of sodium sulfite aerosols in rats. Toxicol Appl Pharmacol, 55(2):299-34.

Laudańska H, Lemancewicz A, Kretowska M, Reduta T, Laudański T (2002). Permeability of human skin to selected anions and cations--in vitro studies. Res Commun Mol Pathol Pharmacol. 2002;112(1-4):16-26.

Lee, W. J., Teschke, K., Kauppinen, T., Andersen, A., Jäppinen, P., Szadkowska-Stanczyk, I., ... & Kishi, R. (2002). Mortality from lung cancer in workers exposed to sulfur dioxide in the pulp and paper industry. Environmental health perspectives, 110(10), 991.

Levanti C, Ricciardi L, Isola S, Cilia M, Guarneri F, Purello D'Ambrosio F (1996). Burning mouth syndrome: hypersensitivity to sodium metabisulfite. Acta Derm Venereol. 1996 Mar;76(2):158-9.

Lockett, M. F.; Natoff, I. L. (1960). Study Of The Toxicity Of Sulfite. I. J. Pharm. Pharmacol. 12, 488-496.

Lodi A, Chiarelli G, Mancini LL, Crosti C. (1993). Contact allergy to sodium sulfite contained in an antifungal preparation. Contact Dermatitis. 1993 Aug;29(2):97.

Madan V, Walker SL, Beck MH (2007). Sodium metabisulfite allergy is common but is it relevant? Contact Dermatitis. Sep;57(3):173-6.

Madsen J, Sherson D, Kjøller H, Hansen I, Rasmussen K (2004). Occupational asthma caused by sodium disulphite in Norwegian lobster fishing Occup Environ Med 2004;61:873–874.

Malik MM, Hegarty MA, Bourke JF (2007). Sodium metabisulfite--a marker for cosmetic allergy? Contact Dermatitis. 2007 Apr;56(4):241-2.

Malo JL, Cartier A, Desjardins A (1995). Occupational asthma caused by dry metabisulphite. Thorax. 1995 May;50(5):585-6; discussion 589.

Meisel SB, Welford PK. (1992). Seizures associated with high-dose intravenous morphine containing sodium bisulfite preservative. Ann Pharmacother. 1992 Dec;26(12):1515-7.

Meng,Z. and Zhang,L. (1990) Chromosomal aberrations and sister-chromited exchanges in lymphocytes of workers exposed to sulphur dioxide. Mutat. Res. , 241, 15–20.

Meng,Z. and Zhang,L. (1990) Observation of frequencies of lymphocytes with micronuclei in human peripheral blood cultures from workers in a sulphuric acid factory. Environ. Mol. Mutagen. , 15, 218–220.

Meng,Z. and Zhang,L. (1992) Cytogenetic damage induced in human lymphocytes by sodium bisulfite. Mutat. Res. , 298, 63–69.

Meng,Z. and Zhang,B. (1999) Polymerase chain reaction-based deletion screening of bisufite (sulfur dioxide)—enhanced gpt-mutants in CHO-AS52 cells. Mutat. Res. , 425, 81–85.

Meng,Z. and Zhang,B. (2002) Induction effects of sulfur dioxide inhalation on chromosomal aberrations in mouse bone marrow cells. Mutagenesis, 17, 215–217.

Meng,Z. (2003) Oxidative damage of sulfur dioxide on various organs of mice: sulfur dioxide is a systemic oxidative damage agent. Inhal. Toxicol. , 15, 181–195.

Meng,Z. Zhang,B. Ruan,A. Sang,N. and Zhang,J. (2002) Micromuclei induced by sulfur dioxide inhalation in mouse bone-marrow cells in vivo. Inhal. Toxicol. , 14, 303–309.

Meng,Z., Sang,N. and Zhang,J. (2002) Effects of derivatives of sulfur dioxide on micronuclei formation in mouse bone marrow cells in vivo. Bull. Environ. Contam. Toxicol. , 69, 257–264.

Meng et al. (2005). Levels of sulfite in three organs from mice exposed to sulfur dioxide. Inhalation Toxicology,17: 309-313.

Meng, Z., & Zhang, L. (1992). Cytogenetic damage induced in human lymphocytes by sodium bisulfite. Mutation Research/Genetic Toxicology, 298(2), 63-69.

Merget R and Korn M (2005). Metabisulphite-induced occupational asthma in a radiographer. Eur Respir J 2005; 25: 386–388

Milham, S.; Demers, R. Y. (1984). Mortality among pulp and paper workers. J. Occup. Med. 26, 844-846.

Münchow V (1992). Chromatographische Bestimmung und Zersetzung von Dithionit in wässriger Lösung. Diplomarbeit, TU Berlin.

Nair B, Elmore AR (2003). The Cosmetic Ingredient Review Expert Panel. Final report on the safety assessment of sodium sulphite, potassium sulphite, ammonium sulphite, sodium bisulfite, ammonium bisulfite, sodium metabisulfite and potassium metabisulfite. Int. J. Toxicol. 22, 63-88.

Nordenson, I.; Beckmann, G.; Beckmann, L.; Rosenhall, L.; Stjernberg. N. (1980). Is exposure to sulphur dioxide clastogenic? Chromosomal aberrations among workers at a sulphite pulp factory. - Hereditas 93: 161-164. ISSN 0018-0661. 1980

OECD (2004). SIDS Initial Assessment Report Sodium dithionite. https://hpvchemicals.oecd.org/ui/handler.axd?id=728026e7-08dd-4926-b748-b534c9bb43e1

OECD (2007). SIDS Sodium dithionite, CAS: 7775-14-6, final 04/2007. UNEP Publications.

OECD (2010). TG 429 http://www.oecd-ilibrary.org/docserver/download/9742901e.pdf?expires=1472543885&id=id&accname=guest&checksum=238D6E37C34CD865D10650BBC36B8699

Oliphant T, Mitra A, Wilkinson M. (2012). Contact allergy to sodium sulfite and its relationship to sodium metabisulfite. Contact Dermatitis. 2012 Mar;66(3):128-30.

Pambor M (1996). Contact dermatitis due to ammonium bisulfite in a bleaching cream. Contact Dermatitis. 1996 Jul;35(1):48-9.

Petersen CS, Menné T (1992). Consecutive patch testing with sodium sulfite in eczema patients. Contact Dermatitis. 1992 Nov;27(5):344-5.

Polanska A, Jenerowicz D, Mudzinska A, Danczak-Pazdrowska A, Czarnecka-Operacz M, Silny W (2011). Adverse reactions caused by metabisulfites, with particular emphasis on contact allergy – a study of 396 patients and review of literature. Centr Eur J Immunol 2011; 36 (4): 293-297).

Pougnet R, Loddé B, Lucas D, Jégaden D, Bell S, Dewitte JD (2010). A case of occupational asthma from metabisulphite in a fisherman. Int Marit Health. 2010;62(3):180-4.

Rix, B. A.; et al. (1997). Cancer incidence of sulfite pulp workers in Denmark. Scand. J. Work Environ. Health 23, 458-461.

Roberts DW, Basketter D, Kimber I, White J, McFadden J, White IR (2012). Sodium metabisulfite as a contact allergen--an example of a rare chemical mechanism for protein modification. Contact Dermatitis. 2012 Mar; 66(3):123-7.

Robinson, C. F.; et al. (1986). Mortality among production workers in pulp and paper mills. Scand. J. Work Environ. Health 12, 552-560.

Rothenberg, S. J., Dahl, A. R., Barr, E. B., & Wolff, R. K. (1986). Generation, behavior, and toxicity of ammonium sulfite aerosols. Journal of the Air Pollution Control Association, 36(1), 55-59.

Rovida C (2011). Local Lymph Node Assay: How Testing Laboratories Apply OECD TG 429 for REACH Purposes. Altex 28, 2/11. http://www.altex.ch/resources/altex_2011_2_117_129_Rovida1.PDF

Schwartz HJ, Gilbert IA, Lenner KA, Sher TH, McFadden ER Jr. (1989). Metabisulfite sensitivity and local dental anesthesia. Ann Allergy. 1989 Feb; 62(2):83-6.

Schwartz HJ, Sher TH. (1985). Bisulfite sensitivity manifesting as allergy to local dental anesthesia. J Allergy Clin Immunol. 1985 Apr; 75(4):525-7.

Scientific Committee for Food (1997). Report on adverse reactions to foods and food ingredients. Report fof the Scientific Committee for Food 37th series, published by the European commission, directorate general for industry.

Seitz CS, Bröcker EB, Trautmann A.Eyelid dermatitis due to sodium metabisulfite. Contact Dermatitis. 2006 Oct; 55(4):249-50.

Seravalli, E.; Lear, E. (1987). Toxicity of chloroprocaine and sodium bisulfite on human neuroblastoma cells. Anesth. Analg. 66, 954-958.

Shtenberg, A. J.; Ignatev, A. D. (1970). Toxicological Evaluation of some combinations of Fd Cosmet. Toxicol. 8, 369-380.

Simon RA, Green L, Stevenson DD (1982). The incidence of ingested metabisulfite sensitivity in an asthmatic population. J. Allergy Clin. Immunol. 69, 118

Sokol WN, Hydick IB (1990). Nasal congestion, urticaria, and angioedema caused by an IgE-mediated reaction to sodium metabisulfite. Ann Allergy. 1990 Sep; 65(3):233-8.

Steiner M, Scaife A, Semple S, Hulks G, Ayres JG (2008). Sodium metabisulphite induced airways disease in the fishing and fish-processing industry. Occup Med (Lond). 2008 Dec; 58(8):545-50.

Stingeni L, Bianchi L, Lisi P (2009). Occupational airborne allergic contact dermatitis from potassium metabisulfite. Contact Dermatitis. 2009 Jan; 60(1):52-3.

Takahashi, M.; et al. (1986). Effects of Ethanol, Potassium Metabisulfite, Formaldehyde and Hydrogen Peroxide on Gastric Carcinogenesis in Rats after Initiation with N-Methyl-N`- Nitrosoguanidine. Jap. J. Cancer Res. 77, 118-124.

Takenaka, S.; et al. (1994). Morphologic effects of a sulfur (IV) aerosol on the nasal cavity of beagle dogs. Toxicology Letters, 72, 145-150.

Tanaka, T., Fujii, M., Mori, H., & Hirono, I. (1979). Carcinogenicity test of potassium metabisulfite in mice. Ecotoxicology and environmental safety, 3(4), 451-453.

Taylor SL, Higley NA, Bush RK (1986). Sulfites in Foods: Uses, Analytical Methods, Residues, Fate, Exposure Assessment, Metabolism, Toxicity, and Hypersensitivity. Advances in Food Research Volume 30, 1986, Pages 1–76

Til, H. P., Feron, V. J., de Groot, A. P. (1972). The Toxicity of Sulfite. I. Long-term Feeding and Multigeneration Studies in Rats. Fd. Cosmet. Toxicol. 10, 291-310.

Til, H. P., et al. (1972a). The Toxicity of Sulfite. I. Long-term feeding and multigeneration studies in rats. Food and cosmetics toxicology, 10, 291-310.

Til, H. P., et al. (1972b). The Toxicity of Sulfite. Long-term feeding and multigeneration studies in rats. Food and cosmetics toxicology, 10, 291-310.

Til, H. P., et al. (1972). The toxicity of sulfite. II. Short- and long-term feeding studies in pigs. Fd. Cosmet. Toxicol. 10, 463-473.

Towns SJ, Mellis CM (1984). Role of acetyl salicylic acid and sodium metabisulfite in chronic childhood asthma. Pediatrics. 1984 May; 73(5):631-7.

Tucker SC, Yell JA, Beck MH. (1999). Allergic contact dermatitis from sodium metabisulfite in Trimovate cream. Contact Dermatitis. 1999 Mar; 40(3):164.

Ueda K, Morita J, Yamashita K, Komano T (1980). Inactivation of Bacteriophage phiX174 by Mytomycin C in the presence of Sodium hydrosulfite and Cupric ions. Chem. -Biol. Interactions 29, 145-158.

Unpublished study report (1972a). Teratologic evaluation of FDA 71-35 (sodium thiosulfate).

Unpublished study report (1972b). Teratologic evaluation of FDA 71-20 (sodium bisulfite).

Unpublished study report (1974a). Teratologic Evaluation of Compound FDA 71-22, Sodium Metabisulfite in Rabbit.

Unpublished study report (1974b). Teratologic evaluation of sodium thiosulfate in rabbits.

Unpublished study report (1974c). Teratological evaluation of FDA 71-20 (sodium bisulfite) in rabbits.

Unpublished study report (1975). Teratological evaluation of FDA 71-21 (potassium metabisulfite) in mice and rats.

Unpublished study report (1978). Microbial mutagenesis testing of substances: compound report: sodium meta-bisulfite.

Unpublished study report (1981). Pruefung auf Reizwirkung an der intakten Ruekenhaut des Albino-Kaninchens (Kurzzeit-Test).

Unpublished study report (1982). Akute Inhalationstoxizitaet LC50, 4 Stunden (Ratte) Staub-Aerosol-Versuch von "Natriumsulfit wasserfrei Z"

Unpublished study report (1989a). Report on the acute dermal irritation/corrosivity to the intact dorsal skin of the white rabbit based on OECD.

Unpublished study report (1989b). Report on the Study of Hydrosulfit konz. in the Ames Test (Standard Plate Test and Preincubation Test with Salmonella typhimurium).

Unpublished study report (2001) In Vitro Mammalian Chromosome Aberration Test: Ammonium Thiosulfate.

Unpublished study report (2003a). Details on an Acute Oral Toxicity Study in the Rat.

Unpublished study report (2003b). Hydrosulfit Konz. BASF - Acute eye irritation in rabbits.

Unpublished study report (2006). Hydrosulfit PF - Acute eye irritation in rabbits.

Unpublished study report (2008). Natriumsulfit wasserfrei food grade (E 221) / (PRD 30042389) - Micronucleus test in bone marrow cells of the mouse.

Unpublished study report (2009). Natriumsulfit wasserfrei food grade: Acute dermal toxicity study in rats.

Unpublished study report (2010). Mutation at the hprt locus of mouse lymphoma L5178Y cells using the Microtitre fluctuation technique: Sodium metabisulfite.

Unpublished study report. Report on the study of the acute toxicity. (1982).

Unpublished study report. Determination of the inhibitory effect on the cell multiplication of the bacterium Pseudomonas putida following Bringmann Kuehn (1988).

Unpublished study report. Bestimmung der akuten Wirkung von Hydrosulfite F gegenüber dem Wasserfloh Daphnia magna Straus (1989a).

Unpublished study report. Determination of the acute toxicity to the algae (1989b).

Unpublished study report. Determination of the chronic toxicity of Hydrosulfit konz. to the water flea Daphnia magna STRAUS (1994).

Unpublished study report. Koc (estimated) (2001).

Unpublished study report. Recalculation of effect values (2004).

Unpublished study report. Sodium dithionite: a study on the respiration inhibition of activated sludge (2010a)

Unpublished study report. Sodium sulfite: a study on the toxicity to Early-Life stages of zebrafish (2010b).

Unpublished study report (2010c). Sodium dithionite: Skin sensitisation: Local lymph node assay in mice.

Unpublished study report. Report on the study of the acute toxicity. (1982).

Unpublished study report. Determination of the inhibitory effect on the cell multiplication of the bacterium Pseudomonas putida following Bringmann Kuehn (1988).

Unpublished study report. Bestimmung der akuten Wirkung von Hydrosulfite F gegenüber dem Wasserfloh Daphnia magna Straus (1989a).

Unpublished study report. Determination of the acute toxicity to the algae (1989b).

Unpublished study report. Determination of the chronic toxicity of Hydrosulfit konz. to the water flea Daphnia magna STRAUS (1994).

Unpublished study report. Koc (estimated) (2001).

Unpublished study report. Recalculation of effect values (2004).

Unpublished study report. Sodium dithionite: a study on the respiration inhibition of activated sludge (2010a)

Unpublished study report. Sodium sulfite: a study on the toxicity to Early-Life stages of zebrafish (2010b).

Unpublished study report (2010c). Sodium dithionite: Skin sensitisation: Local lymph node assay in mice.

Valero AL, Bescos M, Amat P, Malet A (1993). [Bronchial asthma caused by occupational sulfite exposure]. Allergol Immunopathol (Madr). 1993 Nov-Dec;21(6):221-4. Spanish.

Vally H, Misso NL (2012). Adverse reactions to the sulphite additives. Gastroenterol Hepatol Bed Bench. 2012 Winter;5(1):16-23. Review.

Vena GA, Foti C, Angelini G (1994). Sulfite contact allergy. Contact Dermatitis. 1994 Sep;31(3):172-5.

Vitaliti G, Guglielmo F, Giunta L, Pavone P, Falsaperla R. (2015). Sodium metabisulphite allergy with multiple food and drug hypersensitivities in a five-year-old child: a case report and literature review. Allergol Immunopathol (Madr). 2015 Jan-Feb;43(1):106-8.

Vissers-Croughs KJ, van der Kley AM, Vulto AG, Hulsmans RF (1988). Allergic contact dermatitis from sodium sulfite. Contact Dermatitis. 1988 Apr;18(4):252-3.

WHO (2000): Air Quality Guidelines, Second Edition. WHO Regional Office for Europe, Copenhagen, Denmark, 2000.

WHO (2000a) Chapter 7.4 Sulfur dioxide http://www.euro.who.int/ data/assets/pdf file/0020/123086/AQG2ndEd 7 4Sulfurdioxide.pdf

WHO (2006). EHC 235 Dermal absorption. http://www.who.int/ipcs/publications/ehc/ehc235.pdf

WHO (2006): WHO Air quality guidelines for particulate matter, ozone, nitrogendioxide and sulfur dioxide. Global update 2005. Summary of risk assessment. WHO/SDE/PHE/OEH/06.02. World Health Organization, Geneva.

Wirth, P. J. et al. (1986). Altered polypeptide expression associated with neoplastic transformation of Syrian hamster cells by bisulfite. Cancer Research 46, 390-339.

Wright W, Zhang YG, Salome CM, Woolcock AJ.(1990). Effect of inhaled preservatives on asthmatic subjects. I. Sodium metabisulfite. Am Rev Respir Dis. 1990 Jun;141(6):1400-4.

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

Yadav JS and Kaushik VK, 1996. Effects of sulphur dioxide exposure on human chromosomes. Mutation Research/Environmental Mutagenesis and Related Subjects 359, 25–29.

Yang WH, Purchase EC, Rivington RN (1986). Positive skin tests and Prausnitz-Küstner reactions in metabisulfite-sensitive subjects. J Allergy Clin Immunol. 1986 Sep;78(3 Pt 1):443-9.

Yao L, Bloomberg G (2007). Urticaria – a rare presentation of sulfite allergy. Annual meeting Am. Acad. Allergy Asthma Immunol., Pres. No. 811.

Yoshikawa T, Fujiuchi S, Inaba S, Nagai T, Terai T (1990). A case of asthma exacerbated by sulfite contained in betamethasone. Nihon Kyobu Shikkan Gakkai Zasshi. 1990 Jun;28(6):895-9.

Zhang, X.; et al. (2005). A mechanism of sulfite neurotoxicity. J. Biol. Chem. 279, 43035-43045.

7.15. Abbreviations

CI	Confidence interval
CLH	Harmonised classification and labelling
ECVAM	European Centre for the Validation of Alternative Methods
EFSA	European Food Safety Authority
ELISA	Enzyme-linked Immunosorbent Assay
eMSCA	Evaluating Member State Competent Authority
FEV1	Forced expiratory volume (first second)
IgE	Immunglobulin-E
LLNA	Local Lymph Node Assay
LNCC	Lymph Node Cell Count
MoA	Mode of Action
OR	odds ratio
SI	stimulation indices
WHO	World Health Organization