THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

OPINION

CONCERNING

INORGANIC SULFITES AND BISULFITES

COLIPA n° P51

Adopted by the SCCNFP during the 23rd plenary meeting
of 18 March 2003

1. Terms of Reference

1.1 Context of the question

The Cosmetic Directive 76/768/EEC, adopted on 27 July 1976 (published OJEC on 27 September 1976) has been amended six times and at present covers more than 25 adaptations to technical progress. Technical Annexes (II – VII) are a set of lists, qualifying the use of certain ingredients for the safety of the final preparation.

In Annex VI, a positive list of preservatives which may be used is laid down. Besides the intended use as a preservative some of these substances are also destined "for other uses", mostly in other concentrations as for preservative purposes. These substances are marked by (+). At present 26 different substances are listed in Annex VI with(+) "other uses" in addition.

In order to guarantee consumer's health protection and safety of the respective products and following the 6th amendment to the Cosmetic Directive 76/768/EEC - document 93/35/EEC (14 June 1993) - those already evaluated substances have to be judged concerning the "other uses" (+) separately, especially related to the toxicological characteristics of the probably different concentration applied in the other uses than as listed as preservative (Annex VI).

In order to fulfil these demands a special submission (I) for inorganic sulfites and bisulfites describing appropriate investigations and their results has been presented for an evaluation.

1.2 Request to the SCCNFP

Scientific evaluation and opinion on the "other uses" (+) of inorganic sulfites and bisulfites; see also Annex VI, part 1 – n° 9.

* Are inorganic sulfites and bisulfites, at concentrations up to 0.67 % in oxidative hair dye products, up to 6.7 % in hair waving/straightening products, up to 0.45 % in self-tanning products for the face and up to 0.40 % in self-tanning products for the body safe for use in cosmetic products (all expressed as SO_2)?

* Does the SCCNFP propose any restrictions or condition of the use of inorganic sulfites and bisulfites in cosmetic products?

1.3 Statement on the toxicological evaluation

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers.

The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible. In this context, the SCCNFP has a specific working group on alternatives to animal testing which, in co-operation with other Commission services such as ECVAM (European Centre for Validation of Alternative Methods), evaluates these methods.

The extent to which these validated methods are applicable to cosmetic products and its ingredients is a matter of the SCCNFP.

SCCNFP opinions include evaluations of experiments using laboratory animals; such tests are conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available will such tests be evaluated and the resulting data accepted, in order to meet the fundamental requirements of the protection of consumer health.

2. Toxicological Evaluation and Characterisation

2.1. General

2.1.1. Primary name

sodium sulfite	potassium sulfite	ammonium sulfite
sodium bisulfite		ammonium bisulfite
sodium metabisulfite, potassium metabisulfite		

A bisulfate solution will become a solid metabisulfite after removal of water vice versa.

2.1.2. Synonyms

Sulfite and bisulfate, P 51, E 221

Natriumsulfit	Kaliumsulfit	Ammoniumsulfit
Natriumhydrogensulfit		Ammoniumhydrogensulfit
Natriummetabisulfit	Kaliummetabisulfit	
Sodium disulfite	Potassium disulfite	
disodium pentaoxodisulfate	Dipotassium pentaoxodisulfate	
sodium pyrosulfite	Potassium pyrosulfite	

2.1.3. Trade names and abbreviations

See point 2.1.2.

2.1.4. CAS no. / EINECS n°

CAS n°	:	7757-83-7	10117-38-1	10196-04-0
		7631-90-5		10192-30-0
EINECS	:	231-821-4	233-197-0	233-484-1
		231-548-0		233-469-7

2.1.5. Structural formula

/

2.1.6. Empirical formula

Emp. Formula : Na_2SO_3, K_2SO_3, $(NH_4)_2S0_3$, $NaHSO_3$, NH_4HS0_3, $Na_2S_2O_5$, $K_2S_2O_5$
Mol weight : 126.06, 158.26, 116.14, 104.07, 99.11, 190.13, 222.32

2.1.7. Purity, composition and substance codes

Fine inorganic chemicals or trade grade

2.1.8. Physical properties

Appearance : white crystals or powder, sometimes odour of sulphur dioxide
Melting point : /
Boiling point : /
Density : /
Rel. vap. dens. : /
Vapour Press. : /
Log P_{ow} : /

2.1.9. Solubility

Freely soluble in water; slightly soluble in alcohol.

2.2. Function and uses

Preservative	:	0.2 %	(expressed as SO_2)
Oxidative hair dye products	:	up to 0.67 %	(expressed as SO_2)
Hair waving/straightening products	:	up to 6.7 %	(expressed as SO_2)
Self-tanning products for the face	:	up to 0.45 %	(expressed as SO_2)
Certain self-tanning products	:	up to 0.40 %	(expressed as SO_2)

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

Guideline	:	/
Species	:	Wistar rat, male and female
Group size	:	20 rats (10 per sex) per dose group
Test substance	:	sodium sulfite and sodium bisulfite solution of 38 % in water
Dose	:	2000, 2500, 2800, 3100 and 5000 mg/kg bw respectively ml/kg bw for the sodium bisulfite solution
Observ period	:	2 weeks
GLP	:	/

The acute oral toxicity of sodium sulfite and sodium bisulfite solution (38 % in water) was investigated in healthy young albino rats with an average body weight of 171-172 g for females and 178-181 g for males. Twenty rats (ten per sex) per dose group were used in these studies.

The test compound sodium bisulfite solution was dissolved in water and aliquots of 20 ml/kg bw were administered orally by gavage. Sodium sulfite was administered orally as the neat test substance. The doses given were 2000, 2500, 2800, 3100 and 5000 mg/kg bw respectively ml/kg bw for the sodium bisulfite solution. During a two weeks observation period, mortalities and clinical-toxicological observations were recorded daily.

Results
The median acute lethal dose of sodium bisulfite, demonstrated by a LD_{50}-value, was found as 2.90 ml/kg bw for male rats and 3.85 ml/kg bw for females. The LD_{50}-value of sodium sulfite was found to be 3560 mg/kg bw for females and 3930 mg/kg bw for male rats.
The following clinical signs were noted in sodium sulfite treated animals: reduction of body weight, sedation, spasms and rough hair. After sodium bisulfite application the animals showed similar symptoms but additionally cyanoses and abnormal positions.

Ref. : 1, 2

2.3.2. Acute dermal toxicity

No data

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

No data

2.3.5. Repeated dose dermal toxicity

No data

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Sub-chronic oral toxicity

In 1982 sulfur dioxide, sodium sulfite, sodium and potassium bisulfite and sodium and potassium metabisulfite were classified GRAS *(generally recognized as* safe) by the US FDA. This status was supported by an evaluation of the Federation of American Societies for Experimental Biology. Within their evaluation they used animal studies to estimate a "no-observed-adverse effect level" (NOAEL) of 30 - 100 mg sulfur dioxide for humans'. In 1983, the Joint Expert Committee on Food Additives (JECFA) of the WHO established an ADI of 0.7 mg/kg bodyweight (expressed as sulfur dioxide). This ADI includes a 100-fold safety factor with regard to a NOAEL of 72 mg/kg/day (expressed as sulfur dioxide) observed in a three generation animal study.

2.3.8. Sub-chronic dermal toxicity

No data

2.3.9. Sub-chronic inhalation toxicity

No data

2.3.10. Chronic toxicity

No data

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

The acute dermal irritation properties of sodium sulfite and sodium bisulfite solution (38 % in water) were investigated in healthy adult male albino rabbits of New Zealand White strain. Three animals were used per test substance. Each animal served as its own control. Approximately 24 hours prior to the treatment, the dorsal fur was shaved, to expose an area of about 6 cm^2.

The test procedure followed the competent OECD guideline n° 404 (1981). Both test compounds were examined under semi-occlusive conditions.
An aliquot of 0.5 g (ml) of the moistened test substance was exposed to the intact shaved back skin of each animal. The patch was removed four hours after semiocclusive contact. Animals were examined for signs of erythema, eschar and oedema formation. The skin reactions were assessed approx. 1 hour, 24, 48 and 72 hours after termination of the exposure

and the effects were scored according to the Draize criteria[5] supporting the EEC general classification and labelling requirements for dangerous substances.

Results
Under the conditions of the study, both sodium sulfite and sodium bisulfite solution (38 % in water) were neither irritating nor corrosive when applied to the intact rabbit skin under semi-occlusive patch conditions.
The animals did not show any symptoms of systemic intoxication.

Ref. : 3,4

Potassium sulfite

The acute dermal irritation properties of potassium sulfite were investigated in healthy adult male albino rabbits of White Vienna strain. Three animals were used and each animal served as its own control. Approximately 24 hours prior to the treatment, the dorsal fur was shaved, to expose an area of about 6 cm^2.

The test procedure followed the competent OECD guideline n° 404 (1981). The test compound was examined under semi-occlusive conditions.
An aliquot of 0.5 g of the moistened test substance was exposed to the intact shaved back skin of each animal. The patch was removed four hours after semi-occlusive contact. Animals were examined for signs of erythema, eschar and oedema formation. The skin reactions were assessed approx. 1 hour, 24, 48 and 72 hours after termination of the exposure and the effects were scored according to the Draize criteria[5] supporting the EEC general classification and labelling requirements for dangerous substances.

Results
Under the conditions of the study, potassium sulfite was neither irritating nor corrosive when applied to the intact rabbit skin under semi-occlusive patch conditions.
The animals did not show any symptoms of systemic intoxication.

Ref. : 5

2.4.2. Irritation (mucous membranes)

Sodium sulfite and sodium bisulfite

The acute eye irritation properties of sodium sulfite and sodium bisulfite solution (38 % in water) were investigated each in three healthy male albino rabbits of New Zealand White strain.

The test procedure followed the competent OECD guideline n° 405 (1981).
A quantity of 100 mg (respectively 0.1 ml) of sodium sulfite and sodium bisulfite solution was instilled into the conjunctival sac of one eye of each test animal. The substances remained in permanent contact with the eyes until rinsing with a 1 % fluorescein solution 24 hours after instillation. The other eyes served as controls.
The eye irritation reactions were scored approx. 1 hour, 24, 48 and 72 hours and 7 days after instillation of the test substances according to the Draize criteria[5] supporting the EEC general classification and labelling requirements for dangerous substances.

Results
Instillation of sodium sulfite and sodium bisulfite solution (38 % in water) into the rabbits eye did not effect the cornea and the iris at any time. Only slight conjunctival effects presented as erythema and oedema were noticed at several animals up to 24 hours after instillation.
The animals did not show any symptoms of systemic intoxication.

Ref. : 6,7

Sodium sulfite and sodium disulfite

The acute eye irritation properties of sodium sulfite and sodium disulfite were investigated in three and six healthy male albino rabbits of White Vienna strain, respectively.

The test procedure followed the competent OECD guideline n° 405 (1981).

A quantity of 0.1 ml of a 38 % solution of sodium sulfite (without crystal water) and sodium disulfite was instilled into the conjunctival sac of one eye of each test animal. The substances remained in permanent contact with the eyes during the whole study. The other eyes served as controls.
The eye irritation reactions were scored approx. 1 hour, 24, 48 and 72 hours and 8 days after instillation of the test substances. For sodium sulfite (without crystal water) animals were additionally observed 15 days after instillation.

Results
Instillation of sodium sulfite (without crystal water) and sodium disulfite into the rabbits eye altered the cornea and the iris of most of the exposed animals. Slight up to severe effects persisted during the study period of 8 respectively 15 days. Additional slight to moderate conjunctival effects, presented as erythema and oedema, were noticed up to the study's end. Due to the persistency of effects, especially of increased cornea opacity, both test samples have to be evaluated as severe eye irritants.
The animals did not show any symptoms of systemic intoxication.

Ref. : 8, 9

2.5. Sensitisation

According to evaluation of test results in the corresponding CIR report [Cosmetic Ingredient Review - Tentative Report (2000), Safety Assessment of Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite, Sodium Bisulfite, Ammonium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite (including references therein)], which were officially submitted to the US governing bodies, neither sodium sulfite nor sodium metabisulfite were found to be a potent primary sensitiser.

Related to this data and by in use experience with millions of cosmetic products per year containing these sodium salts, it seems to be unlikely that a modification of the cation would increase the sensitisation potential seriously.

In conclusion, inorganic salts of sulfite, bisulfite and metabisulfite are rare contact allergens.
Ref. : 19

2.6. Teratogenicity

Embryo-toxicity including Teratogenicity

According to test results of several reprotoxicity studies in rats, mice and rabbits, oral doses of sodium sulfite, bisulfite and metabisulfite or potassium metabisulfite up to a dose of at least 100 mg/kg bw/d given during the period of gestation, were neither found to be embryotoxic nor teratogenic.

Ref. : 20

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Sulfite that enters the body by ingestion, inhalation or via injection is immediately oxidised by the sulfite oxidase to physiological sulfate. Such metabolism was demonstrated to be very fast in analysed species like dogs, monkeys, rats and rabbits. In all species, ≤ 10 % of the administered dose was excreted unchanged via urine. The dermal absorption/percutaneous penetration of inorganic sulfites bisulfites and metabisulfites has not been analysed in particular, but due to the high level of sulfite oxidase in the body and due to general limited bioavailability of charged chemicals topically applied, the above mentioned 10 % level was taken as the worst case.

Ref. : 20

2.8. Mutagenicity/Genotoxicity

2.8.1. Bacterial Reverse Mutation Test

Guideline	:	OECD 471 (1983)
Strains	:	TA1535, TA100, TA1537, TA98
Metabolic Activation	:	Rat liver homogenate (Aroclor induced)
Doses	:	20, 100, 500, 2500, 5,000 µg/plate
Substance	:	Sodium Sulfite Water-free A
Batch/Purity	:	88/639; 96-98%
Positive controls	:	+S9: 2AA -S9: NMNG; 4NOPD; AminoAcridine
No of experiments	:	2 (standard plate test; preincubation test)
Replicate	:	3 plates/dose/experiment
Results	:	No mutagenic effect was observed

Ref. : 10

Guideline	:	OECD 471 (1983)
Strains	:	TA1535, TA100, TA1537, TA98
Metabolic Activation	:	Rat liver homogenate (Aroclor induced)
Doses	:	20, 10, 500, 2500, 5000 µg/plate

Substance	:	Sodium bisulfite
Batch/Purity	:	89/380 ca -98%
Positive controls	:	+S9: 2AA -S9: NMNG; 4NOPD; AminoAcridine
No of experiments	:	2 (standard plate test; preincubation test)
Replicate	:	3 plates/dose/experiment
Results	:	No mutagenic effect was observed

Ref. : 12

Guideline	:	OECD 471(1983)
Strains	:	TA1535, TA100, TA1537, TA98
Metabolic Activation	:	Rat liver homogenate (Aroclor induced)
Doses	:	20, 10, 500, 2500, 5000 µg/plate
Substance	:	Potassium sulfite water-free
Batch/Purity	:	88/841; 97%
Positive controls	:	+S9: 2AA -S9: NMNG; 4NOPD; AminoAcridine
No of experiments	:	2 (standard plate test; preincubation test)
Replicate	:	3 plates/dose/experiment
Results	:	No mutagenic effect was observed

Ref. : 11

8.2.2. *In vivo* Unscheduled DNA Synthesis - UDS-Test (Rats, Hepatocytes)

Sodium bisulfite

Healthy young adult male rats of Wistar Hanlbm:WIST (SPF) strain were used. The mean initial body weight of the test animals (six to ten weeks old) was about 190 g. Four rats were used per dose and eight animals (two per dose) had been used in the range finding experiment. In the UDS-test the induction of unscheduled DNA synthesis in hepatocytes is based on DNA repair processes and taken as indicative for a mutation response.

The test procedure followed the OECD guideline n° 486 (1997) and was conducted to comply with the principles of GLP.
Sodium bisulfite (sodium hydrogensulfite), formulated in citrate/NaOH buffer at pH 5.0 was administered orally in a dose of 625 and 1250 mg/kg bw (based on pre-experiments). The volume administered was 10 ml/kg bw. Two and 16 hours after treatment the animals were sacrificed by liver perfusion. Primary hepatocytes were exposed to ^{3}HTdR (methyl 'H-thymidine) for four hours to show its incorporation if UDS occurs. Hepatocytes from three animals per group were assessed for UDS.
N,N'-dimethylhydrazine dihydrochloride (DMH) (40 mg/kg bw) and 2-acetylamino-fluorene (2-AAF) (100 mg/kg) served as positive controls.

Results
The rats showed no substantially affected hepatocytes after treatment. No dose level of the test item revealed UDS induction in the hepatocytes of the treated animals as compared to the current vehicle controls. The net gain values obtained after treatment with the test item were

consistently negative. In addition, no substantial shift to higher values was obtained in the percentage distribution of nuclear grain counts.
From the results obtained in this study, it was concluded that sodium bisulfite (sodium hydrogensulfite) failed to show any evidence of mutagenic potential in this *in vivo* test for unscheduled DNA synthesis when administered orally at pH 5.0.

Ref. : 13

2.8.3 *In vivo* Chromosome Mutation Assay - Micronucleus Test (Mice)

Sodium Bisulfite

Healthy young adult male and female mice of NMRI strain were used. The average body weight of the test animals (eight to ten weeks old) was about 34 g (females) and 41 g (males) respectively. Six mice were used per dose and sex. Additional 20 animals (two per dose and sex) had been used for the range finding.
In the micronucleus test, the induction of chromosome mutations is presented as the induction of micronuclei in bone-marrow erythrocytes of *in vivo* treated rodents.

Test Conditions:

The test procedure followed the OECD guideline n° 474 (1997) and was conducted to comply with the principles of GLP.
Sodium bisulfite (hydrogensulfite), formulated in citrate/NaOH buffer at pH 5.0 was administered in a total dose of 75, 150 and 300 mg/kg bw by intraperitoneal injection (15 ml/kg) to ensure bioavailability at target cells. Bone marrow of femora was prepared 24 and 48 hours after application. For each animal at least 2,000 polychromatic erythrocytes (PCE) obtained from femoral bone marrow were examined. The frequency of micronuclei was calculated for each animal and dose group.
Cyclophosphamide (CPA) (40 mg/kg bw) and the vehicle (citrate/NaOH buffer), respectively served as positive and negative controls.

Results
The treated mice exhibited normochromatic/polychromatic erythrocytes ratios which were higher than in negative controls, demonstrating the bioavailability of the test substance in the bone marrow. The bioavailability was also obvious on clinical effects seen in the treated animals especially in the high dose group and was supported specifically by performing the intraperitoneal application. The number of micronucleated PCE was similar to those seen in controls. From the results obtained in this study, it was concluded that sodium bisulfite (hydrogensulfite) failed to show any evidence of mutagenic potential in this *in vivo* test for chromosomal alterations when administered intraperitoneally at pH 5.0.

Ref. : 14

2.9. Carcinogenicity

Potassium metabisulfite was tested for carcinogenicity in one study in mice by oral administration. Three groups of 50 male and 50 female ICR/ICL mice were given 0, 1 or 2%

potassium metabisulfite [purity unspecified] in the drinking-water for 104 weeks. No increase in tumour incidence was observed.

Ref.: 15

Sodium metabisulfite was tested for carcinogenicity in one study in rats by oral administration. Six groups of 20 male and 20 female weanling Wistar rats were fed 0, 0.125, 0.25, 0.5, 1 or 2% sodium metabisulfite (95–99% pure [impurities unspecified]) in the diet for 104 weeks. More than 50% of controls and about 75% of each experimental group survived until the termination of experiment. Groups of five females and five males of the F1 generation were fed the same concentrations in the diet for 104 weeks. Data on tumour incidence are given for the F0 and F2 generations combined. The incidences of thyroid and pituitary tumours were increased in treated males, but no dose–response relationship was observed. The authors reported that the incidences of these tumours in the concurrent controls were exceptionally low compared to those in historical controls and that the incidences found in treated animals represent the numbers normally found in this strain of rat.

Ref.: 16

Sulphur dioxide was tested for carcinogenicity in one study in mice by inhalation. An experimental group of 35 male and 30 female LX mice and a control group of 41 males and 39 females were exposed to 0 or 500 ppm [1310 mg/m^3] sulphur dioxide [purity unspecified] for 5 min per day on five days a week for life. Female mice exposed to sulphur dioxide had an increased incidence of lung tumours: 13/30 adenomas and carcinomas versus 5/30 in controls [p = 0.02]; 4/30 lung carcinomas versus none in the controls. The incidence of lung neoplasias was higher in treated males (15/28 versus 11/35 in controls), but the difference was not significant; lung carcinomas occurred with equal frequency in treated and control males (2/28 and 2/35)

Ref.: 17

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

Ref.: 18

2.10. Special investigations

No data

2.11. Safety evaluation

Inorganic sulfites, bisulfites and metabisulfites (sodium, potassium and ammonium salts) are defined as substances that liberate sulphur dioxide under certain conditions. Therefore the officially tabled limits for these chemicals normally correspond to the yield of deliberated sulphur dioxide.
Due to the fact that bisulfites do not exist as solids, an aqueous solution of about 40 represents the maximum concentration of this chemical. Therefore a bisulfite solution will become a solid metabisulfite after removal of water vice versa. The conversion of sulphur dioxide to bisulfite and sulfite is strongly pH dependent in aqueous solutions.

Sulfites and bisulfites are listed as preservatives on Annex VI of the Cosmetics Directive with a maximum permitted concentration of 0.2 % (expressed as SO_2). They are also used for "other uses" as reducing agents in cosmetics, especially in hair dyeing formulations, in hair-waving- /straightening products and as stabilizers in certain self-tanning products. All three product types are characterised by an infrequent rather than daily use :

* In common practice, when used in hair dye formulations, approximately 100 ml are placed to the head for a time period of about 30 minutes not more often than once a month. The maximum sulfite content after mixing (expressed as free SO_2) is 0.67 %.
* Hair-waving- /straightening products are typically applied in quantities of about 75 ml and are placed to the hair for a duration of about 20 minutes, in a frequency typically not exceeding once a month. The maximum sulfite content (expressed as free SO_2) is 6.7 %.
* Self-tanning products are typically applied once a day for a maximum of three days to achieve an initial tan and are then reapplied every three days to maintain the tan. The maximum sulfite content (expressed as free SO_2) is 0.45 % for face products and 0.40 % for body-products.

Neither ammonium sulfite nor ammonium bisulfite are currently reported as used in cosmetic formulations. But some of the inorganic salts are also used as common antioxidants in foods.

The mentioned sulfites and bisulfites were found to be slightly toxic or non-toxic in rats and mice after single oral administration. The lethal dose of the test substances was demonstrated by LD_{50}-values in the range of 600 - >2,000 mg/kg in such rodents. In particular for the most frequently used sodium sulfite, the LD50-value exceeded 3,500 mg/kg for rats.

The mentioned test substances were found to be neither corrosive nor irritating to rabbit skin. For most of the undiluted salts only slight incompatibility effects were found when applied to rabbit eyes, resulting in a slight reversible erythema in combination with a slight temporary oedema. Sodium sulfite (without crystal water) and sodium metabisulfite were found to be severe eye irritants when tested neat and eye contact should be avoided.

Inorganic sulfites, bisulfites and metabisulfates failed to show any sensitisation properties in the common appropriate guinea pig tests for dermal sensitisation. The known sulfite-sensitivity, a rare anaphylactic hypersensitivity most times associated with asthmatics after oral intake of preserved food, is discussed elsewhere. This effect is not relevant for cosmetic applications and is excluded from this toxicological dossier.
Existing mutagenicity studies support information on different categories of biological endpoints. Gene mutations were analysed in a couple of *in vitro* tests (Ames-test with *Salmonella typhimurium* and E. *coli,* tests with yeasts). Chromosomal aberration and sister chromatid exchange (SCE) were tested *in vitro* (in human blood lymphocytes) as well as *in vivo* (micronucleus-test in mice and rats). The induction of unscheduled DNA synthesis (UDS) was analysed in rat hepatocytes after *in vivo* application. Dominant lethal assays in mice and rats completed the spectrum of different mutagenicity tests.

Neither sodium sulfite and sodium metabisulfite nor potassium metabisulfite were found to be genotoxic in the test battery. Some of the *in vitro* assays performed with sodium bisulfite showed positive results, especially in the Ames test and in the chromosome aberration

test. However, none of the *in vivo* tests have shown a genotoxic potential. To further investigate the above mentioned positive *in vitro* results found with sodium bisulfite, an UDS in rats and a micronucleus test in mice were added. To be sure that the bisulfite and not the disulfite was tested, an appropriate pH-value was maintained by a buffer system. Both additional tests were negative.

Summarising results of the available mutagenicity tests, genotoxic potential of the specified inorganic sulfites, bisulfites and metabisulfites seems to be very unlikely.

Sulfite that enters the body by ingestion, inhalation or via injection is immediately oxidised by the sulfite oxidase to physiological sulfate. Such metabolism was demonstrated to be very fast in analysed species like dogs, monkeys, rats and rabbits. In all species ≤ 10 % of the administered dose was excreted unchanged via urine. The dermal absorption/percutaneous penetration of inorganic sulfites bisulfites and metabisulfites has not been analysed in particular, but due to the high level of sulfite oxidase in the body and due to general limited bioavailability of charged chemicals topically applied, the above mentioned 10 % level is taken as the worst case.

Exposure

Inorganic sulfites are listed on Annex VI as a preservative with a maximum concentration of 0.2 % and are used for "other uses" at concentrations up to 0.67 % in oxidative hair dye products, up to 6.7 % in hair-waving- /straightening products, 0.45 % in self-tanning products for the face and up to 0.40 % in self-tanning products for the body (all expressed as SO_2).

a) Theoretical topical exposure from preservative use

In its most recent "Notes of Guidance for Testing of Cosmetic Ingredients for their Safety Evaluation" (Revision October 2000) the SCCNFP presents a worst-case scenario for the global consumer exposure to cosmetics. From this, a total topical exposure to cosmetics potentially containing sulfites estimated to be 17.79 g/day.

In the case of evaluation of a new preservative for the positive list, exposure is calculated based on the assumption that the substance is present in all cosmetic products at the maximum requested concentration. The SCCNFP Notes of Guidance acknowledge that this exposure scenario is exaggerated and such *"extreme values would not be reached in practice "*.

17.79 g * 0.2 % = 35.58 mg/day (expressed as SO_2)

b) Theoretical topical exposure from self-tanning use (#)

Face + Body : 0.8 g * 0.45 % + 8 * 0.40 % = 35.6 mg / application

Self-tanning products are typically applied every three days to maintain a tan. Converted to a daily base, topical exposure would be estimated to be 11.9 mg / day (expressed as SO_2).

c) Theoretical topical exposure from hair products, considering 1% retention after rinse-off and hair/scalp partition

Dyeing + Waving : 100 g* 0.67 %* 1 % + 75* 6.7 %* 1 % = 57 mg / application (expressed as SO2).

Hair waving and oxidative hair dyeing products are typically applied once every month. There is no standard method to convert such infrequent exposure to a daily base. In a highly conservative estimate, application of 57 mg/day is therefore assumed.

d) Combined Topical Exposure from Preservative use and „other uses"

35.58 mg/d + 11.9 mg/d + 57 mg/d = 104.5 mg/day

e) Systemic Exposure from combined Preservative use and „other uses"

Worst case systemic exposure would be estimated to be 0.17 mg/kg body weight/day, assuming worst-case bioavailability of 10 % and a consumer weighing 60 kg (104.5 mg/d * 10 % / 60 kg).

(#) Most consumers use these products on legs, arms and chest rather than on the whole body surface

SAFETY FACTOR CALCULATION

In 1982 sulfur dioxide, sodium sulfite, sodium and potassium bisulfite and sodium and potassium metabisulfite were classified GRAS *(generally recognized as* safe) by the US FDA.

This status was supported by evaluations of the Federation of American Societies for Experimental Biology. Within their evaluation they used animal studies to estimate a "no-observed-adverse effect level" (NOAEL) of 30 - 100 mg sulfur dioxide for humans. In 1983, the Joint Expert Committee on Food Additives (JECFA) of the WHO established an ADI of 0.7 mg/kg bodyweight (expressed as sulfur dioxide). This ADI includes a 100-fold safety factor with regard to a NOAEL of 72 mg/kg/day (expressed as sulfur dioxide) observed in a three generation animal study.

Exposure from cosmetic use from the combination of preservative use and "other uses (0.17 mg/kg body weight/day) provides a worst case safety factor of 176 - 588 (NOAEL of 30 - 100 mg/kg/day). Exposure remains well below the ADI recommended by WHO.
Real safety factors are likely to be substantially higher since a number of worst case assumptions have been made in the exposure assessment :

* 10 % of topically applied sulfites become bioavailable without prior conversion to sulfate;
* Daily consumer use of exclusively such products which contain sulfites at the maximum permitted preservative concentration.

* Daily consumer use of hair waving or straightening products and of oxidative hair dye products
* Additional use of self-tanning products for the face and body on every third day

NOT APPLICABLE

CALCULATION OF THE MARGIN OF SAFETY

(Name of substance)
(preservative)

Maximum absorption through the skin	A ($\mu g/cm^2$)	=	$\mu g/cm^2$
Typical body weight of human		=	60 kg
Skin Area surface	SAS (cm^2)	=	cm^2
Dermal absorption per treatment	SAS x A x 0.001	=	µg
Systemic exposure dose (SED)	SAS x A x 0.001/60	=	mg/kg
No observed adverse effect level (mg/kg) (rat, 90 day, oral)	NOAEL	=	mg/kg

Margin of Safety	NOAEL / SED	=

2.12. Conclusions

Based upon the shown test results and considering the calculated safety factors, it can be concluded that inorganic sulfites, bisulfites do not pose any unacceptable risk to human health for use in cosmetic formulations at the intended use concentrations.

2.13. Opinion

The SCCNFP is of the opinion that inorganic sulfites and bisulfites do not pose a health risk when used in cosmetic products at concentrations up to 0.67% in oxidative hair dye products, up to 6.7 % in hair waving/straightening products, up to 0.45 % in self-tanning products for the face and up to 0.40 % in self-tanning products for the body (all expressed as SO_2).

2.14. References

1. Loser, E. (1982) Natriumsulfit - Untersuchungen zur akuten oralen Toxizität an mannlichen/weiblichen Ratten. Bayer AG Institute of Toxicology, Germany, Study-no. T 3011344 coded at Henkel KGaA, Düsseldorf, Report No. R 0001241/R 0001242

2. Loser, E. (1982) Natriumbisulfitlauge - Untersuchungen zur akuten oralen Toxizität an mannlichen/weiblichen Ratten. Bayer AG Institute of Toxicology, Germany, Study-no. T 0011341 coded at Henkel KGaA, Düsseldorf, Report No. R 0001245/R 0001246
3. Suberg, H. (1983) Natriumsulfit - Untersuchung auf primäre Reiz-/Ärtzwirkung an der Kaninchenhaut. Bayer AG Institute of Toxicology, Germany, Study-no. T 6015235 coded at Henkel KGaA, Düsseldorf, Report No. R 0001243
4. Suberg, H. (1983) Natriumbisulfitlauge - Untersuchung auf primare Reiz-/Artzwirkung an der Kaninchenhaut. Bayer AG Institute of Toxicology, Germany, Study-no. T 3015124 coded at Henkel KGaA, Düsseldorf, Report No. R 0001247
5. Hildebrand, B; and Kirsch, P; (1989) Kaliumsulfit – Report on the Acute Dermal Irritation/Corrosivity to the Intact Dorsal Skin of the White Rabbit Based on OECD 404. BASF AG, Project : 18H0640/882393 coded at Henkel KgaA, Düsseldorf, Report n° R 0001263
6. Suberg, H. (1983) Natriumsulfit - Untersuchung auf Schleimhautreizwirkung am Kaninchenauge Bayer AG Institute of Toxicology, Germany, Study-no. T 7015236 coded at Henkel KGaA, Düsseldorf, Report No. R 0001244
7. Suberg, H. (1983) Natriumbisulfitlauge - Untersuchung auf Schleimhautreizwirkung am Kaninchenauge Bayer AG Institute of Toxicology, Germany, Study-no. T 4015125 coded at Henkel KGaA, Düsseldorf, Report No. R 0001248
8. Kirsch, P. and Grundler, O.J. (1981) Natriumsulfit wasserfrei Z - Prüfung auf Reizwirkung am Auge des Albino-Kaninchens in Anlehnung an Draize. BASF AG, Project: 81/311 coded at Henkel KGaA, Düsseldorf, Report No. R 0001260
9. Kirsch, P. and Kieczka, H. (1984) Natriumdisulfit Report on the Acute Irritation to the Eye of the White Rabbit Based on OECD 405. BASF AG, Project: 84/200 coded at Henkel KGaA, Düsseldorf, Report No. R 0001265
10. Hoffmann, H.D. and Engelhardt, G. (1989) Natriumsulfit wasserfrei A - Amest Test (Standard Plate and Preincubation Test with Salmonella typhimurium). BASF AG, Project No.: 40MO639/884492 coded at Henkel KGaA, Düsseldorf, Report No. R 0001262
11. Hoffmann, H.D. and Engelhardt, G. (1989) Kaliumsulfit wasserfrei - Amest Test (Standard Plate and Preincubation Test with Salmonella typhimurium). BASF AG, Project No.: 40MO841/884455 coded at Henkel KGaA, Düsseldorf, Report No. R 0001264
12. Hoffmann, H.D.and Engelhardt, G. (1989) Natriumdisulfit - Amest Test (Standard Plate and Preincubation Test with Salmonella typhimurium). BASF AG, Project No.: 40M0380/894265 coded at Henkel KGaA, Düsseldorf, Report No. R 0001266
13. Schulz, M. (2000) In vivo/in vitro DNA Synthesis in Rat Hepatocytes with Sodium Bisulfite (Sodium Hydrogensulfite) RCC-CCR, Rol3dorf/D, Project 672702 sponsored by Henkel KGaA, Düsseldorf, Report No. R 0001174
14. Honarvar, N. (2000) Micronucleus Assay in Bone Marrow Cells of the Mouse with Sodium Bisulfite (Sodium Hydrogensulfite). RCC-CCR, Rossdorf/D, Project 672701 sponsored by Henkel KGaA, Düsseldorf, Report No. R 0000956
15. Tanaka T, Fujii M, Mori H, and Hirono I (1979) Carcinogenicity test of potassium metabisulfite in mice. Ecotoxicol. environ. Saf., 3, 451–453.
16. Til HP, Feron VJ, and De Groot AP. (1972) The toxicity of sulfite. I. Long-term feeding and multigeneration studies in rats. Food Cosmet. Toxicol., 10, 291–310.
17. Peacock PR, and Spence JB. (1967) Incidence of lung tumours in LX mice exposed to (1) free radicals; (2) SO2. Br. J. Cancer, 21, 606–618.

18. IARC (1992) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 54, Occupational Exposures to mists and vapour from strong inorganic acids; and other industrial chemicals, Lyon, pp. 131-188.
19. Vestergaard L, Andersen KE. Allergic contact dermatitis from sodium metabisulfite in a topical preparation. Am J Contact Dermatitis 1995; 6 : 174-175.
20. Cosmetic Ingredient Review - Tentative Report (2000), Safety Assessment of Sodium Sulfite, Potassium Sulfite, Ammonium Sulfite, Sodium Bisulfite, Ammonium Bisulfite, Sodium Metabisulfite, and Potassium Metabisulfite (including references therein)