Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

sulfur

EC Number: 231-722-6
CAS Number: 7704-34-9

CLH-O-0000007107-77-01/F

Adopted

18 March 2022

18 March 2022

CLH-O-0000007107-77-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **sulfur**

EC Number: **231-722-6**

CAS Number: **7704-34-9**

The proposal was submitted by **France** and **Slovenia** and received by RAC on **16 June 2021.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

France and Slovenia have co-submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **5 July 2021**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **3 September 2021**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Lea Stine Tobiassen**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **18 March 2022** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	016-094-00-1	sulfur	231-722-6	7704-34-9	Skin Irrit. 2,	H315	GHS07 Wng	H315			
Dossier submitters proposal	016-094-00-1	sulfur	231-722-6	7704-34-9	**Retain** Skin Irrit. 2 **Add** Eye Irrit. 2, STOT SE 3	**Retain** H315 **Add** H319 H335	**Retain** GHS07 Wng	**Retain** H315 **Add** H319 H335			
RAC opinion	016-094-00-1	sulfur	231-722-6	7704-34-9	**Retain** Skin Irrit. 2	**Retain** H315	**Retain** GHS07 Wng	**Retain** H315			
Resulting Annex VI entry if agreed by COM	016-094-00-1	sulfur	231-722-6	7704-34-9	Skin Irrit. 2	H315	GHS07 Wng	H315			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Sulfur (or sulphur) is regulated under the Plant Protection Products, Classification, Labelling and Packaging and REACH regulations. The present opinion is based on the information provided in the classification proposal prepared in relation to the pesticide re-evaluation under regulation 1107/2009.

Sulfur is used as fungicide against mildew in wine and in cereal crops. It also has an acaricidal function.

The pesticide active substance sulfur (pure and technical grade) is a yellow solid with a purity of 990 mg/kg. Formulations on the market include powders, granules and flakes. The classification proposal is based on studies carried out on sulfur technical or on a formulation called Sulphur Dust, which contains 985 mg/kg sulfur, which is used as a representative formulation by one of the two applicant groups under PPPR. The other applicant group has included an 80% Wettable Granule in their re-evaluation dossier.

Sulfur is registered in the EU Observatory for nanomaterials (EUON) as the substance is included in the French nano-inventory. Sulfur is not registered under REACH as a nanomaterial, and the substance is not included in the Belgian nanomaterials inventory. No information specific to the nanoform of sulfur is available in the classification dossier or in the REACH registration. During the RAC evaluation process, the applicant under the PPPR argued in a submitted document that pesticide formulations of sulfur would not fall under the definition of nanoparticles according to EU nanomaterial definition, as it did not meet the condition for 50% or more of the particles being in the size range of 1-100 nm.

The substance has a solubility in water of 16 µg/L and the solubility in organic solvents ranges from 0.17 g/L in methanol to ~14 g/L in toluene and dichloromethane.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) proposed no classification for all physical hazards, based on test results and the results of the screening procedure relevant for each hazard class. Sulfur does not contain any molecular structures associated with self-reactive properties and no peroxide or acidic moieties and has a melting point above 55°C. Thus, it does not fulfil criteria for self-reactive substances, organic peroxides, or corrosive to metals. According to a UN RTDG N.4 test, sulfur is not a self-heating substance. Based on long-term handling experience, sulfur is not a pyrophoric solid, it doesn't emit flammable gases upon contact and does not react with water. According to a UN RTDG O.1 test, sulfur does not fulfil the criteria for an oxidising solid.

Comments received during consultation

No comments were received during the consultation.

Assessment and comparison with the classification criteria

Sulfur is a solid, hence hazard classes for gases and liquids do not apply. A test according to EEC method A.14 showed sulfur not to be explosive, in addition the DS stated that the substance does not contain structural features indicative of explosive properties as per table A6.1 of Annex 6 of the UN RTDG. A negative EEC method A.10 was included in the dossier. When negative, this test is equivalent to UN RTDG N.1 test.

RAC agrees with the assessment of the DS on the physical hazards and proposes **no classification.**

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

ORAL ROUTE

Summary of the Dossier Submitter's proposal

Three GLP and OECD test guideline compliant acute oral toxicity studies conducted in rats with Sulphur Dust or technical sulfur were included in the dossier. No mortalities were observed in any study, thus the oral LD_{50} for sulfur was concluded to be over 2000 mg/kg bw. In a limit dose LD_{50} test from 1994 using 2000 mg/kg bw technical grade sulphur in corn oil administered to female and male rats, clinical signs including laboured breathing and piloerection were noted in all animals, and nose staining and vocalisation were reported in one female and 2 males. No toxicological effects were noted in either of two studies conducted in 2005 and 2009, respectively, with Sulphur Dust, using peanut oil as vehicle. In first study, one dose of 2000 mg/kg bw was administered to 3 female rats, whilst the most recent study used an extreme regime of 7 administrations of 5000 mg/kg bw within 24 hours to 3 female rats. Based on the available data, the DS proposed not to classify the substance for acute oral toxicity.

Comments received during consultation

One MSCA supported the proposal for no classification. The commenter pointed to a published case of a man surviving ingestion of 60 g sulfur as supporting evidence. No further details were provided in the reference.

Assessment and comparison with the classification criteria

The criteria for classification for acute oral toxicity in category 4 was not met, as the LD_{50} values reported were all above 2000 mg/kg bw/day.

In agreement with the DS, RAC concludes that **sulphur does not warrant classification for acute oral toxicity.**

DERMAL ROUTE

Summary of the Dossier Submitter's proposal

Acute dermal toxicity data were from two GLP and OECD test guideline compliant studies published in 1994 and 2005, respectively, conducted in rats with sulfur technical and Sulphur

Dust, respectively. No mortalities were observed in either study. In the first study that used technical grade sulfur in corn oil, erythema and/or scaling was reported in 3 females and 5 males. No clinical signs were seen in the second study using deionised water as the vehicle. As the LD_{50} values were above 2000 mg/kg bw in the available studies, no classification for acute dermal toxicity was proposed by the DS.

Comments received during consultation

One MSCA supported the proposal for no classification for acute dermal toxicity.

Assessment and comparison with the classification criteria

The LD_{50} values reported were above 2000 mg/kg bw/day, thus above the criteria for classification for acute dermal toxicity in category 4. RAC noted that the use of water as the vehicle in the second study may have impacted on the reliability of this study, as sufficient contact with the skin may not have been ensured as specified in OECD TG 402.

However, based on the study using corn oil as vehicle, RAC concludes, in agreement with the DS, that **sulfur does not warrant classification for acute dermal toxicity.**

INHALATION ROUTE

Summary of the Dossier Submitter's proposal

Two GLP and OECD TG 403 compliant studies on acute inhalation toxicity in rats were available.

The first study (report published 1994), used sulfur technical applied by nose-only application as particles with a MMAD of 3.8 µm at a mean measured concentration of 5.43 mg/L. Two males out of five died whilst all females survived. Clinical signs were recorded in all animals and included affected breathing and partly closed eyes during exposure, and blepharospasms, nasal encrustations and dirty fur post-exposure.

The second study (report published 2005) used Sulphur Dust as a dust aerosol at 4.5 mg/L, the highest aerosol concentration achievable, using nose-only application. The MMAD was 4.2 µm. No mortalities or clinical signs were reported.

The DS proposed not to classify for acute inhalation toxicity.

Comments received during consultation

One MSCA supported the proposal for no classification.

Assessment and comparison with the classification criteria

In the acute inhalation toxicity studies sulfur was tested up to the highest concentration achievable, 5.43 and 4.5 mg/L, respectively. The mortality rate was below 50% of the animals in the first study, whilst the second study did not cause any mortality at 4.5 mg/L. Thus, the classification criteria for acute inhalation toxicity for dusts and mists (5 mg/L)are not met in either study.

Based on these data, RAC concludes that **sulfur does not warrant classification for acute inhalation toxicity.**

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The acute toxicity animal data described for oral and dermal routes showed no evidence of specific target organ toxicity. With respect to the inhalation route, an acute inhalation study in rats reported choking breathing from the first hour and decreased breathing frequency in all rats from the second hour of the 4-hour exposure to 5.43 mg/L technical sulfur. No clinical signs were reported in the acute inhalation toxicity study conducted with Sulphur Dust at 4.55 mg/L air.

A number of reports from epidemiological studies and toxicovigilance programmes show respiratory tract effects and chronic bronchitis.

The US-EPA RED (1991) concluded in their summary that handling of Sulphur Dust can cause eye and skin irritation in handling the pesticides or when in contact with treated foliage.

In 1996, the California Department of Food and agriculture reported an incident from 1986 in six vineyards workers exposed to sulfur dust applied by aerial spraying leading to signs of irritation to the respiratory tract amongst other throat irritation and cough in the workers.

Incidences in different American databases reviewed in the US EPA pesticide review of sulfur in 2009 showed that the effects related to exposure to sulfur were mostly related to the irritant properties of the substance to the eyes, the skin and the respiratory tract whilst toxicity of sulfur was low.

The DS further referred to the French governmental toxicovigilance programme of farmers that reported 13 cases of slight to severe irritation to skin, eyes and respiratory tract between 1997 and 2006, excluding cases of concomitant exposure to other pesticides. One worker with a medical history of asthma had bronchospasm requiring hospital admission. Amongst 24 cases reported from 1997 to 2012, there were 13 cases of respiratory findings, 5 of which were caused by wettable formulations and 8 were due to exposure to a dust formulation of sulfur. Findings of nasal irritation symptoms occurred in 6 workers exposed to dust formulations and 2 exposed to wettable formulations.

The dosser submitter proposed classification as STOT SE in category 3; H335 based on the irritation effects to the respiratory tract reported consistently in occupational exposure to sulfur, supported by the effect in one animal study.

Comments received during consultation

An industrial organisation disputed in a comment and an attached expert statement that the severity of the effects reported in humans are insufficient to support classification, and stressed that there were both animal and human data not showing irritation to the respiratory tract. In their response, the DS referred to their analysis on the animal and human data, which in a weight of evidence approach led to the conclusion that sulfur should be classified for respiratory tract irritation.

One MSCA supported classification as STOT SE, category 3; H335, pointing to the decreased breathing frequency seen in rats exposed to 5.43 mg/L and the signs of respiratory tract irritation reported in incident databases from occupational exposure to sulfur.

Assessment and comparison with the classification criteria

Substances should be classified for STOT SE in categories 1 respectively 2 if they produce significant toxicity or can be presumed to be harmful to humans from a single exposure. Guidance values for classification on the basis of animal data are specified in the classification criteria.

Classification as STOT SE in Category 3 is attributed to substances causing narcotic effects or causing respiratory tract irritation after single exposure.

Sulfur did not show signs of significant target organ toxicity in animals exposed to concentrations within the guidance values for classification in category 2 from either route of exposure. The available human data do not report significant organ toxicity from a single exposure. Thus, classification in categories 1 and 2 are not relevant.

Results from American and French human reports from incidences of exposure of workers show varying degrees of respiratory tract irritation including rhinitis, cough, and breathing difficulties. The US EPA refers to irritation as a well-known irritating property of sulfur. Symptoms of respiratory tract irritation (irregular and chocking breathing) were also seen in a study of acute inhalation toxicity in rats at a concentration 5.43 mg/L.

The DS considered the reports of respiratory tract irritation in humans exposed to sulfur, supported by the effects seen in one animal study in their proposal for classification for respiratory tract irritation STOT SE Category 3; H335.

RAC assessed that the effects on the respiratory tract reported in the acute inhalation study should not be considered for classification for STOT SE, as they occurred at a dose also leading to death in two animals, and therefore these findings are regarded as an unspecific, sublethal toxicity reaction.

RAC notes the human cases of respiratory tract effects from exposure to sulfur from American and French databases. However, the reports include few details on the severity of the effects. Considering the extensive use of sulfur through several decades, the number of cases reported are low. RAC concludes that the severity of the effects on the respiratory tract are low and outside the scope of classification for respiratory irritation.

Based on the available animal and human data, RAC concludes, contrary to the proposal from the DS, that **classification for STOT SE is not warranted for sulfur**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

Two skin irritation studies in rabbits as well as reports from human experience are presented in the dossier.

The animal studies from 1994 and 2005, respectively, were conducted according to OECD test guidelines and GLP and were deemed acceptable by the DS.

In the first study, application of technical sulfur mixed with vaseline for 4 hours led to mean erythema scores of 2.3 in 3 animals, and 3 in another 3 animals over 24, 48 and 72 hours, and oedema scores of between 1 and 2 in the 6 animals. The effects were reversible by 7 days in all animals.

In the second study, Sulphur Dust was applied as a paste with deionized water and did not lead to erythema, eschar or oedema of the skin up to 72 hrs after a 4 hour-application.

Further, the acute dermal toxicity study performed on the technical grade substance as described above (section on acute toxicity) reported minimal to mild and reversible irritation in some of the animals for 2-6 days.

In humans, skin and eye irritation in field workers in contact with sulfur dust or treated foliage were reported in the US-EPA RED[1] in 1991.

A publication from the Californian Department of Food and Agriculture reporting several symptoms, including itching in six Californian vineyard field workers exposed from helicopter application of sulfur.

Medical surveillance of French farmers by the governmental toxicovigilance body "Mutualité Sociale Agricole" in the period 1997-2012 identified 24 cases of various irritative symptoms from exposure to sulfur with no concomitant exposure. Skin findings observed were moderate to severe skin irritation in 14 workers out of 15 workers exposed to sulfur as wettable powder and in 4 out of 9 workers exposure to dust formulations.

No adverse findings were reported in most reports of occupational medical surveillance of factories, but several cases of eye and skin irritation and malaise were reported at one sulfur formulation site.

Also, incidences of skin irritation from the medical use of sulfur as a keratolytic agent were reported in a pharmacopeia (Reynolds, 1996).

The DS proposed to retain the existing classification for sulfur as Skin irritant Category 2 H315 based on a weight of evidence approach based on the animal and human data available.

Comments received during consultation

One MSCA provided the study report for the 1994 study and supported the classification proposal as Skin irritant Category 2 H315 based on the findings of that study and the available human evidence.

Assessment and comparison with the classification criteria

The CLP criteria for classification as a skin irritant includes reference to animal data as well as human evidence to be considered in a weight of evidence approach.

The results of the skin irritation study in rabbits from 1994 using technical sulfur in vaseline meet the criteria for classification as skin irritant in category 2, as all 6 animals showed mean skin erythema score ≥2.3 and ≤4.0. The DS points to the possible influence of the use of vaseline as a vehicle on the positive results of one of the animal tests. RAC considers that the possible enhancing irritative effect of vaseline on the response seen in rabbits cannot be qualified or quantified based on the presented data, and thus considers that the study from 1994 should be included in the weight of evidence evaluation of the endpoint for classification purposes.

[1] *US-EPA RED: US-EPA RE-registration Eligibility Document*

The second rabbit study did not cause any skin reaction and thus did not indicate a need for classification. However, the use of water as a moistening agent gives uncertainty as to the validity of this study.

The CLP criteria stipulate that human data should also be considered in the weight of evidence approach of all available data. Thus, occupational data should also be considered when deemed adequate and reliable. RAC considers that the reports of skin irritation from occupational exposure to sulfur from American and French governmental occupational health databases as well as information from one Industrial health and safety department constitute a robust and consistent evidence of the skin irritation potential of sulfur.

Based on the animal and human data available, RAC concludes that **the current classification of sulfur as Skin irritant Category 2 H315: Causes skin irritation should be maintained**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

Two OECD TG 405 and GLP compliant studies in rabbits included in the dossier resulted in slight to moderate eye irritation from sulfur application. In the first study, from 1994, instillation of 100 mg technical sulfur (powder) to the eye of 6 rabbits led to mean scores of 0 for corneal opacity, iritis and conjunctival chemosis, and scores of a maximum of 1 (one out of 6 animals) for redness of the conjunctiva. All effects were reversible within one to seven days.

A second study from 2005, used 0.1 mL (84g) grounded Sulphur Dust. The eyes were rinsed with deionised water after 24 hrs. Two out of the 3 animals reacted with mean conjunctiva redness scores of 1, whilst one animal had a score of 0.7. Chemosis scores were all less than one, whilst corneal opacity and iritis scores were 0 in all animals. Reversibility occurred within two or three days.

Eye irritation was reported by the US-EPA RED in field workers (incidences not available in the dossier) after handling sulfur pesticide or sulfur treated foliage.

The Californian Department of Food and Agriculture also reported eye irritation in six vineyard field workers exposed after helicopter application of sulfur.

In US-EPA (2009[1]), results from a number of American incidence databases on residential and occupational cases from the mid 1990's up to around 2006 related to exposure to sulfur confirmed the dermal ocular and respiratory irritative properties of sulfur. The incidence numbers generally were low, and most of them were of low severity, but also cases of moderate and a few cases of high severity were reported.

Medical surveillance of French farmers by the governmental toxicovigilance body "Mutualité Sociale Agricole" in the period 1997-2012 identified 24 cases of various irritative symptoms from exposure to sulfur with no concomitant exposure. Eye irritation was reported in 7 out of 15 workers exposed to sulfur as wettable formulations and in 6 out of 9 workers exposure to dust formulations. The severity of the effects varied from conjunctival irritation to corneal ulceration.

[1] *US-EPA (2009) Sulfur. Human Health Risk Scoping Document in Support of Registration Review - Addendum*

Medical surveillance at one industrial formulation site also reported cases of eye and skin irritation, whilst the other applicant's factories did not report any cases.

In a pharmacopeia (Reynolds, 1996), it is recommended to avoid contact with eyes and mucous membranes when using sulfur in pharmaceutical applications due to the keratolytic effect of sulfur.

Based on the consistent information from databases on occupational and residential exposure that sulfur causes irritation to the eyes in humans, supported by the animal data showing effects meeting the classification criteria and the caution recommendation for using sulfur as a pharmaceutical agent, the DS proposes to classify sulfur as an Eye irritant in Category 2 H319.

Comments received during consultation

One MSCA supported the proposed classification as eye irritant, stressing the consistent reporting of effects in humans ranging from conjunctival effects to corneal ulceration.

An industry group supported by an expert statement disputed that the eye irritant effects in humans are sufficient for classification. Further, they stressed that the results from the animal data did not meet the classification criteria. The DS maintained that the human data showed eye irritancy and their conclusion to classify with H319.

Assessment and comparison with the classification criteria

The effects reported of the two animal studies in the classification proposal as well as the additional study from the US registration process were insufficient for classification as an eye irritant in category 2, as no effects were reported to the cornea or iris, and the conjunctiva scores were below 2 in all studies, and the effects are reversible.

Incidents of eye irritation in workers and residents from exposure to sulfur in governmental databases in the US and in France an incident in an industrial formulation site and handbook information point to potential for transient eye irritation. However, the numbers reported are low when considering the extensive use of sulfur over several decades, and the effects are reversible.

Therefore, RAC concludes that based on the available data **sulfur does not fulfil the criteria for classification for serious eye damage/irritation**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Three Guinea pig studies with sulfur were included by the DS. All studies were stated to be GLP and OECD 406 compliant, and were therefore accepted in the pesticides dossier. However, the conduct and results of the Guineapig Maximisation Test (GPMT) and the Buehler test from 1994 were concluded to be of low reliability.

A GPMT from 2005 using Sulphur Dust was considered to be reliable by the DS. The study was conducted at concentrations of 1% Sulphur Dust in paraffin oil for intradermal induction and 100% of the substance, moistened with water, for topical induction and topical challenge. None of the 20 animals reacted at 24 hr or 48 hr after challenge.

The GPMT from 1994 conducted with sulfur technical used 1% test substance in paraffin oil and FCA for the intradermal induction phase. Strong skin reactions (oedema, then necrosis, encrustations) were seen after the intradermal induction with FCA/saline in control and treated groups. Reactions to epidermal induction were seen after use of SLS in paraffin oil of 25% sulfur in vaseline. First challenge concentrations of 15 and 25% sulfur in vaseline resulted in positive reactions in respectively 16 and 18 out of 19 animals, dropping to 2 and 5 animals at 48 hrs. After the second challenge, 2, 6 and 9 animals reacted to 10, 15 and 25% vaseline at 24 hrs, respectively. At 48 hrs, 5, 8 and 11 treated animals showed "signs of allergic skin reactions". Scar formation was reported in 5, 2 and 6 animals, in the 10, 15 and 25% sulfur treated groups at this time point and necrotic skin was reported in one animal of the 10% group at both time points. No positive reactions were observed in animals challenged with vaseline alone. The DS pointed to the limitations of the study due to the strong skin reactions to intradermal induction and the possible enhancing effect of vaseline to conclude that this GPMT study is therefore not suited for classification purposes.

A Buehler test from 1994 with technical sulfur was also available. Induction was conducted with 25% sulfur in vaseline. Skin irritation was reported in 3/20, 15/20 and 16/20 animals at the first, second and third topical induction treatments. The dossier submitter noted that 15 and 25% sulfur in vaseline had shown to be skin irritating in a preliminary test. The challenge and rechallenge used 15 or 25% sulfur in vaseline. The study also included application of 15% of an 80% sulfur formulation using water and vaseline at the rechallenge phase. Positive responses after the first and second challenge to 15% or 25% sulfur in vaseline were seen in 4/20 to 9/20 animals. No reactions were recorded with the formulation containing 12% sulfur (15% of 80%) using water as the vehicle, whilst 10 respectively 9 out of 10 animals reacted to at 24 respectively 48 hrs after challenge in the group treated with the formulation when using vaseline as vehicle. Therefore, the DS also regards this Buehler test as being inconclusive, and considered the skin reactions to instead reflect irritative properties of sulfur in vaseline.

Further animal studies conducted with sulfur pesticide products containing up to 80% sulfur using water as moistening agent were considered reliable. None of them resulted in skin sensitisation.

In humans, there are no reports of skin sensitising effects of sulfur.

Based on the above data, the DS proposed not to classify of skin sensitisation.

Comments received during consultation

One MSCA supported the proposal of no classification, and pointed to the limitations and unclarities in the Buehler and the GPMT with sulfur technical from 1994.

Assessment and comparison with the classification criteria

Classification for skin sensitisation can be based on results from animal studies and or human evidence. With respect to animal data, the classification criteria specifically refer to the GPMT, Buehler test and/or LLNA test.

The OECD TG 406 on the GPMT gives no specific recommendations for topical application (induction or challenge). For insoluble substances, in guidance given in that part of the TG, 80% ethanol/water is preferred for induction and acetone for challenge in the TG for the Buehler test.

The results from skin sensitising studies with sulfur technical using vaseline as the vehicle were regarded to be equivocal as the vehicle may have enhanced the skin irritation reactions. Also, reactions declined at rechallenge, supporting the conclusion that the effects were due to skin

irritation rather than to sensitisation. Therefore, RAC considers that the GPMT and the Buehler test conducted with sulfur (from 1994) were equivocal.

Furthermore, severe skin effects are seen with the use of FCA in the GPMT test with sulfur technical, further compromising the validity of that study.

In the most recent GPMT, using water as the vehicle, RAC considers that sufficient contact with the skin was not obtained and the study is therefore not regarded to be adequate.

Therefore, RAC concludes that **classification for skin sensitisation is not warranted due to inconclusive data**.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The evidence available on the potential repeated dose toxicity of sulfur to specific organs included animal studies and human data. The available animal studies included one 28-day and two 90-day studies in rats by the oral route and two 28-day dermal toxicity studies in rats, all conducted according to OECD TG between 2005 and 2009, using either technical sulfur or Sulphur Dust as the test substance.

In a 90-day study with Sulphur Dust, where animals were dosed with 0, 100, 400 and 1000 mg/kg bw by gavage, decreased body weights were seen in males at the high dose (7%, increasing to 10% in the subsequent 28-day recovery period). Increased relative testis and epididymides weights in the high dose group were reported in the recovery period but were considered to be due to decreased body weights in that dose group. Small changes in haematological and biochemistry parameters were not considered treatment-related. Small, non dose-related changes in haematology and clinical chemistry were also reported in the 28-day oral gavage study with Sulphur Dust using the same dose levels.

In the 90-day oral toxicity-study conducted with technical sulfur, the only effects reported were changes in haematological and biochemistry parameters with no other corresponding findings.

In the dermal 28-day study with no recovery period, doses of 0, 100, 400 and 1000 mg/kg bw/day technical sulfur caused no systemic effect, but hyperkeratosis was reported at the high dose at the treated sites in both sexes and in females also at untreated sites.

With Sulphur Dust applied under a gauze patch moistened with corn oil using the same doses, no local or systemic effects were reported at any dose level.

In the US-EPA databases, occupational cases of chronic bronchitis, chronic sinusal effects and respiratory effects were reported following exposure to sulfur, but in co-exposure to other pesticides. In the French Toxicological Programme, one case of bronchospasm occurred in a farmer with a medical history of asthma.

An epidemiological study of respiratory symptoms and spirometry was performed in 237 7-year old children living in the Salinas Valley in California, within 0.5 km and 1 km of agricultural areas treated with sulfur at one week, month and year after the applications. The study reported higher odds ratios for respiratory symptoms and asthma medication and poorer lung function in the children, the symptoms decreasing with time. The study had some limitations e.g. the reliability of the questionnaire used for symptoms and medication recording, uncertainty in the determination of exposure levels to sulfur, possible co-exposure to other pesticides and/or to

smoke, difficulties of performing spirometry in young age children. The DS assessed the study to constitute a "signal" and encouraged further studies to potentially confirm the findings.

The DS proposed no classification for STOT RE for any routes of exposure.

Comments received during consultation

An MSCA supported the DS proposal to not classify for STOT RE based on the available information. With respect to repeated dose by inhalation, the MSCA pointed to two publication on human experience provided in the dossier, commented on the requirement for an additional animal study and noted that testing requirements are not relevant under CLP.

A group of industrial companies disputed the need for requiring an additional sub-chronic inhalation study, pointing to the already existing database not supporting an effect of sulfur following repeated exposure.

The DS in their response maintained that the lack of animal data on toxicity to inhalation following repeated exposure led to the conclusion that data are inconclusive for classification for STOT RE.

In their specific response to this comment, RAC confirmed that classification is to be performed with the available data. Whilst agreeing with the DS that further information would strengthen the evaluation of this end-point, RAC emphasised that discussion of requirements for further data is not relevant under CLP.

Assessment and comparison with the classification criteria

The criteria for classification as STOT RE require significant functional disturbance or morphological changes or severe effects with a serious adverse impact on health. Guidance values are provided to placing substances in category 1 or 2 or to decide to not classify when evaluating animal data.

Effects on body weights reported in one 28-day study in rats at the highest dose of 1000 mg/kg/day was not considered to be of sufficient severity to warrant classification. The slight effects on clinical biochemistry and haematology of rats reported in the oral studies are insufficient for classification as they lack a dose-response relationship and statistical significance.

Therefore, no classification was warranted for STOT RE by the oral route.

In the dermal 28-day repeated dose toxicity study, hyperkeratosis occurred in the high dose group of 1000 mg/kg bw/day only. The findings were considered borderline with respect to their severity. When extrapolated to a 90-day duration, the dose-level corresponds to 333 mg/kg bw, which is above the guidance value for classification as STOT RE 2 of 200 mg/kg bw/day, and no classification for STOT RE by the dermal route is proposed.

No repeated or long-term inhalation toxicity studies in animals are available. In humans the restricted number of reports from occupational settings of chronic effects by the inhalation route related to sulfur exposure also reported co-exposure to other pesticides. One epidemiological study of 7-year old children residing near fields treated with sulfur raised concern. However, RAC concludes that the study is not sufficiently robust due to a number of uncertainties in its conduct to support classification on its own.

Therefore, RAC agrees with the DS that **no classification for STOT RE can be applied due to inconclusive data.**

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The CLH dossier included reference to two *in vitro* tests: an Ames assay and an *in vitro* chromosome aberration assay, both from 2005, conducted with Sulphur Dust. Both tests followed OECD TG applicable at the time and they were deemed acceptable by the DS. The Ames test included 4 *salmonella typhimurium* strains and one *E.coli* strain and used up to 5000 µg/plate. The results were negative with and without S9 metabolic activation in all 5 bacterial strains. The chromosomal aberration test that used Chinese hamster ovary cells up to 64 µg/mL, the maximal possible concentration due to cytotoxicity, also yielded negative results with and without S9 metabolic activation.

Furthermore, the dossier mentioned that an *in vitro* mammalian gene mutation assay is expected in October 2020. The study report was provided during the consultation of the CLH report (see below).

The DS also included in the dossier two negative GLP and OECD TG 474 compliant *in vivo* micronucleus assays conducted in mice: one with sulfur technical by the oral route and one with Sulphur Dust by intraperitoneal injection. Neither of the studies showed increased numbers of micronuclei at the limit dose of 2000 mg/kg bw. The DS pointed to the fact that it was not demonstrated that the substance had indeed reached the bone marrow, as no systemic toxicity was reported. Furthermore, no information on the toxicokinetics of sulfur was available in the application. The results of the *in vivo* tests were therefore questionable.

Based on the available negative *in vitro* data, although still pending the results of the *in vitro* mammalian cell gene mutation assay, on the low systemic toxicity of sulfur, on the lack of reports of genotoxicity from the use of the substance in food and as a pharmaceutical agent and on the exposure to the substances due to its nature as an essential element the DS concludes the genotoxic potential of sulfur is very low.

The DS quotes the co-RMS in the DAR that they were of the view that the genotoxicity at the first site of contact could not be totally excluded as no confirmatory *in vivo* test was available.

The DS concluded that the available data do not support classification for mutagenicity.

Comments received during consultation

One comment from a group of industrial companies supplied the report of the announced *in vitro* gene mutation test in Chinese Hamster V79 cells conducted according to OECD TG 476 with Sulphur 98.5 DP (dustable powder formulation). The comment and a separate document from an expert on the assessment of the genotoxicity of sulfur further argued that no further testing is necessary to confirm the lack of genotoxicity of sulfur at a site of contact. The DS responded that the new study is valid and that the negative result with and without metabolic activation confirmed the conclusion that sulfur is not mutagenic.

Another comment, from an MSCA, supported the proposal to not classify sulfur for mutagenicity, and proposed to include three publicly available reports of mutagenicity testing in the overall evaluation of mutagenicity. The DS noted that one of the studies was not considered acceptable by the DS, and although some limitations were identified in the OECD TG compliant studies, they are sufficient to conclude on the endpoint.

Assessment and comparison with the classification criteria

Considering that the *in vitro* mammalian cell gene mutation test confirmed the negative results from the other *in vitro* tests and having regard to the knowledge on the low systemic toxicity of the substance, the DS concluded that sulfur does not have a genotoxic potential and thus should not be classified.

RAC agrees the with the DS conclusion not to classify sulfur for Germ cell mutagenicity.

RAC agrees that sulfur is unlikely to have a systemic mutagenic effect given its nature as an essential element, and as its widespread use in pharmaceutical products and in food has not led to reporting of concern for genotoxic effects, and given that the *in vitro* assays were negative. RAC notes that the negative *in vivo* micronucleus tests are unreliable as none of them were demonstrated to have reached the bone marrow, and no other organs were investigated. RAC considers that a slight potential for sulfur to be capable of inducing a site-of contact genotoxic effect exists, but that this potential is low, given the consistently negative results of the *in vitro* studies.

Therefore, RAC concludes that based on the data available, including the recent *in vitro* mammalian cell gene mutation test, **sulfur does not warrant classification for Germ cell mutagenicity**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

No information from animal studies or human data were available on this endpoint for sulfur, and no classification is therefore proposed.

Comments received during consultation

One MSCA supported the proposal not to classify sulfur for carcinogenicity, as no data were available.

Assessment and comparison with the classification criteria

No classification is warranted based on a complete lack of information in the dossier[1].

[1] *As agreed for the first approval of the active substance (EFSA, 2008), sulfur is generally regarded as safe for human exposure given the wide range of background exposure, its low acute and short-term toxicity and its non-genotoxic potential. In addition, it is an essential element needed at a high dose level. Therefore, it was considered unnecessary to require long-term and carcinogenicity studies with sulfur.*

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

No animal studies providing information on sexual function and fertility, developmental toxicity or lactation were available. There was also no information in the open literature on human health effects of sulfur. Therefore, no classification is proposed for the endpoints related to reproductive toxicity.

Comments received during consultation

One MSCA supported the proposal not to classify for endpoints under reproductive toxicity due to lack of data.

Assessment and comparison with the classification criteria

RAC agrees with the DS that no classification should be applied to sulfur for sexual function and fertility, developmental toxicity or effect on or via lactation **based on a complete lack of information in the dossier[1].**

Additional references

Study report for Gene Mutation Assay in Chinese Hamster V79 Cells in vitro (V79/HPRT), Report/Study Number: 1992500, 2020

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).

[1] *As agreed for the first approval of the active substance (EFSA, 2008), sulfur is generally regarded as safe for human exposure given the wide range of background exposure, its low acute and short-term toxicity and its non-genotoxic potential. In addition, it is an essential element needed at a high dose level. Therefore, it was considered unnecessary to require reproductive toxicity studies with sulfur.*