

# **Annex I to the CLH report**

## **Proposal for Harmonised Classification and Labelling**

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),  
Annex VI, Part 2**

### **International Chemical Identification:**

#### *Hydrogen Sulfide*

**EC Number:** 231-977-3  
**CAS Number:** 7783-06-4  
**Index Number:** 016-001-00-4

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## 1 PHYSICAL HAZARDS

*Not provided.*

## 2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

*Not provided*

## 3 HEALTH HAZARDS

### Acute toxicity

#### 3.1 Acute toxicity - oral route

#### 3.2 Acute toxicity - dermal route

#### 3.3 Acute toxicity - inhalation route

##### 3.3.1 Animal data

###### 3.3.1.1 Tansy et al. (1981)

###### *Study reference:*

Tansy M.F., Kendall F.M., Fantasia J., Landin W.E., Oberly R., and Sherman W. (1981): Acute and subchronic toxicity studies of rats exposed to vapors of methyl mercaptan and other reduced-sulfur compounds. *Journal of Toxicology and Environmental Health* 8 (1-2), 71-88.

###### *Detailed study summary and results:*

###### *Test type*

- Acute inhalation toxicity study, no guideline followed, no GLP

###### *Test substance*

- *Test substance:* hydrogen sulphide (EC: 231-977-3)
- *Degree of purity:* not specified
- *Impurities:* not specified
- *Batch number:* not specified

###### *Test animals*

- *Species/strain/sex:* rat / Sprague-Dawley / male and female (sexes were combined for treatment)
- *No. of animals per sex per dose:* 5
- *Age and weight at the study initiation:* age not specified, 90-100g (weight) at the study initiation

###### *Administration/exposure*

- *Type of inhalation exposure and test conditions:* gas inhalation (simultaneous whole body exposure in a glass chamber)
- *Duration of test/exposure period:* **4 h**
- *Doses/concentration levels:* 400, 440, 475, 500, 525, 554, and 600 ppm

- *Analytical verification of test atmosphere concentrations:* not specified
- *Post exposure observation period:* 14 days
- *Control group and treatment:* yes (sham-exposure to air)
- *Statistical methods:* LC50 values + 95 % confidence estimated according to Litchfield and Wilcoxon (1949)

**Results and reliability**

- *LC50:* 444 ppm, 95 % CL: 416 - 473
- *Number of deaths at each dose level:* following 4 h exposure

<b>Concentration (ppm)</b>	<b>Mortality</b>
Control	0/10
400	3/10
440	3/10
475	7/10
500	8/10
525	8/10
554	9/10
600	10/10

**Additional information that may be needed to adequately assess data for reliability:**

- *Time of death:* all animals that survived 24 h post-exposure, survived till the end of the 14-day post-exposure observation period
- *Clinical signs:* not specified
- *Necropsy findings, including doses affected, severity and number of animals affected:* not specified
- *Potential target organs (if identified in the report):* not specified
- *Other findings:* altered behaviour (exploring, huddling, preening, and obvious distress)
- *If both sexes tested, results should be compared:* not specified

**3.3.1.2 Prior et al. (1988)**

**Study reference:**

Prior M.G., Sharma A.K., Yong S., and Lopez A. (1988): Concentration-time interactions in hydrogen sulphide toxicity in rats. *Canadian Journal of Veterinary Research* 52 (3), 375-379.

**Detailed study summary and results:**

**Test type**

- Acute inhalation toxicity study, no guideline followed, no GLP

**Test substance**

- *Test substance:* hydrogen sulphide (EC: 231-977-3)
- *Degree of purity:* 99.5 %
- *Impurities:* not specified
- *Batch number:* not specified

**Test animals**

- *Species/strain/sex:* Rat / Long Evans, Sprague Dawley and Fischer 344 / male and female
- *No. of animals per sex per dose:* 9-12
- *Age and weight at the study initiation:* age: 7-8 weeks + 10 days acclimatization, weight: not specified

**Administration/exposure**

- *Type of inhalation exposure and test conditions:* gas inhalation (whole body exposure in a gas exposure chamber)
- *Duration of test/exposure period:* **2 h, 4 h and 6 h**
- *Doses/concentration levels:* various concentrations (details not given)
- *Analytical verification of test atmosphere concentrations:* yes (H<sub>2</sub>S concentration was determined four times / h using gas chromatography)
- *Post exposure observation period:* 14 days
- *Control group and treatment:* yes
- *Statistical methods:*
  - Estimation of the effects of hydrogen sulphide concentration, sex, hours of exposure and strain on the weight loss: standard regression and analysis of covariance techniques
  - Estimation of lethal concentration (LC10 and LC50) values: probit analysis using a maximum likelihood iteration technique
  - Additional statistics: standard deviation, 95% confidence intervals, and chi-square goodness of fit

**Results and reliability**

- *LC50 and LC10:*

<b>Duration of exposure</b>	<b>LC50</b>	<b>LC10</b>	<b>Number of animals (n)</b>
2 h	587 ppm	549 ppm	156
4 h	501 ppm	422 ppm	144
6 h	335 ppm	299 ppm	156

- *Number of deaths at each dose level:* not specified

**Additional information that may be needed to adequately assess data for reliability:**

- *Time of death:* not specified
- *Clinical signs:* weight loss due to toxic gas

- *Necropsy findings*: mouth, nose, trachea, and bronchi contained foamy fluid; severe pulmonary edema (most likely cause for death); histology: proteinaceous fluids in conductive airways, alveoli and in perivascular space of major blood vessels
- *Potential target organs (if identified in the report)*: whole respiratory tract
- *If both sexes tested, results should be compared*:
  - weight loss due to toxic gas: higher in males vs. females
  - mortality: 30 % in males vs. 20 % in females

### 3.3.1.3 Zwart et al. (1990)

#### *Study reference:*

Zwart A., Arts J.H.E., Klokmán-Houweling J.M., and Schoen E.D. (1990): Determination of concentration-time-mortality relationships to replace LC50 values. *Inhalation Toxicology* 2 (2), 105-117.

#### *Detailed study summary and results:*

##### *Test type*

- Acute inhalation toxicity study, no guideline followed, no GLP

##### *Test substance*

- *Test substance*: hydrogen sulphide (EC: 231-977-3)
- *Degree of purity*: not specified
- *Impurities*: not specified
- *Batch number*: not specified

##### *Test animals*

- *Species/strain/sex*:
  - *rat*: Wistar / male and female
  - *mouse*: Swiss / male and female
- *No. of animals per sex per dose*: 5
- *Age and weight at the study initiation*:
  - *rats*: 5-6 weeks + 5 days acclimatization (age); 150-170 g (weight males), 130-140 g (weight females)
  - *mice*: 7-8 weeks + 5 days acclimatization (age); 23-34 g (weight males), 23-27 g (weight females)

##### *Administration/exposure*

- *Type of inhalation exposure and test conditions*: gas inhalation (whole body exposure in a gas exposure chamber)
- *Duration of test/exposure period*: **5, 10, 30 and 60 min**
- *Doses/concentration levels*: concentrations are given in mg/m<sup>3</sup>
  - 5 min: 931, 1196, 1831
  - 10 min: 931, 1199, 1821

- 30 min: 449, 706, 813, 833, 881, 935, 972, 1032
- 60 min: 448, 703, 774, 806, 826, 939, 972
- *Analytical verification of test atmosphere concentrations:* yes
- *Post exposure observation period:* 14 days
- *Control group and treatment:* not specified
- *Statistical methods:* stepwise forward selection procedure based on maximum likelihood regression methods

**Results and reliability**

- *LC50:*

	Rats (♀/♂)		Mice (♀/♂)	
	mg/m <sup>3</sup> air	ppm <sup>1</sup>	mg/m <sup>3</sup> air	ppm <sup>1</sup>
<b>LC50 (10 min)</b>	1160	~ 824	1610	~ 1143
<b>LC50 (30 min)</b>	1010	~ 717	1110	~ 788
<b>LC50 (60 min)</b>	950	~ 675	940	~ 667

- *Number of deaths at each dose level:*

Exposure Concentration		Duration (min)	Number of animals/sex	Mortality rats		Mortality mice	
(mg/m <sup>3</sup> )	ppm <sup>1</sup>			Males	Females	Males	Females
931	661	5	5	0	0	0	0
1196	849	5	5	2	0	0	0
1831	1300	5	5	5	5	1	2
931	661	10	5	0	0	0	0
1199	851	10	5	3	5	0	0
1821	1293	10	5	5	5	4	5
449	319	30	5	0	0	0	0
706	501	30	5	0	0	0	0
813	577	30	5	0	0	0	0
833	591	30	5	0	0	-	-
881	626	30	5	4	5	1	1
935	664	30	5	0	1	0	1
972	690	30	5	2	0	1	2
1032	733	30	5	2	1	0	0
448	318	60	5	0	0	0	0
703	499	60	5	0	0	0	3
774	550	60	5	0	0	0	2
806	572	60	5	0	0	2	1
826	586	60	5	0	0	-	-

<sup>1</sup> Calculated according to: 1 mg/m<sup>3</sup> = 0.71 ppm (WHO (CICAD) (2003))

939	667	60	5	3	4	3	4
972	690	60	5	3	4	4	2

***Additional information that may be needed to adequately assess data for reliability:***

- *Time of death:* not specified
- *Clinical signs:* not specified
- *Necropsy findings:* not specified
- *Potential target organs:* not specified
- *If both sexes tested, results should be compared:* not specified

**3.3.1.4 THRU Laboratories (1972)**

***Study reference:***

THRU Laboratories (1972): Toxic hazards research unit annual technical report: 1972. Report No. AMRL TR. Aerospace Medical Research Laboratory, Air Force Systems Command. Transportation U.D.o., Wright Patterson Air Force Base, Ohio, study report

***Detailed study summary and results:***

***Test type***

- Acute inhalation toxicity study, guideline not specified, no GLP

***Test substance***

- *Test substance:* hydrogen sulphide (EC: 231-977-3)
- *Degree of purity:* not specified
- *Impurities:* not specified
- *Batch number:* not specified

***Test animals***

- *Species/strain/sex:*
  - *rat:* Sprague-Dawley / male
  - *mouse:* CF-1 / male
- *No. of animals per sex per dose:* 10
- *Age and weight at the study initiation:*
  - *rats:* age not specified; 200-300 g (weight)
  - *mice:* age not specified; 20-30 g (weight)

***Administration/exposure***

- *Type of inhalation exposure and test conditions:* gas inhalation (whole body exposure)
- *Duration of test/exposure period:* **1 h**
- *Doses/concentration levels:* 400, 504, 635, or 800 ppm



- *Analytical verification of test atmosphere concentrations:* yes (using an ion specific sulphide electrode)
- *Post exposure observation period:* 14 days
- *Control group and treatment:* no
- *Statistical methods:* not specified

**Results and reliability**

- *LC50:*
  - *rat:* 712 ppm (1 h)
  - *mouse:* 634 ppm (1 h)
- *Number of deaths at each dose level:*

Concentration (ppm)	Mortality	
	Rat	Mouse
400	0/10	2/10
504	0/10	0/10
635	1/10	5/10
800	9/10	8/10

**Additional information that may be needed to adequately assess data for reliability:**

- *Time of death:* not specified
- *Clinical signs:*
  - *rat:* gasping
  - *mouse:* gasping + convulsions during exposure
- *Necropsy findings:*
  - *rat:* congestion and mottling of kidney and liver with moderate to severe fatty changes in the liver observed in animals that survived the exposure
  - *mouse:* one mouse in the 635 ppm-group and one mouse in the 800 ppm-group had a blocked urethral opening due to encrustation of the external orifice, and a distended bladder, but both survived until end of the 14-day-observation period
- *Potential target organs:* not specified
- *Other findings:* normal weight gain (surviving rats/mice during post-exposure period)
- *If both sexes tested, results should be compared:* not specified

**3.3.1.5 Lopez et al. (1989)**

**Study reference:**

Lopez A., Prior M.G., Reiffenstein R.J., and Goodwin L.R. (1989): Peracute toxic effects of inhaled hydrogen sulfide and injected sodium hydrosulfide on the lungs of rats. *Fundamental and Applied Toxicology : Official Journal of the Society of Toxicology*. 12 (2), 367-373.

**Detailed study summary and results:**

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### ***Test type***

- Acute inhalation toxicity study, no guideline followed, no GLP

### ***Test substance***

- *Test substance*: hydrogen sulphide (EC: 231-977-3)
- *Degree of purity*: not indicated
- *Impurities*: not indicated
- *Batch number*: not indicated

### ***Test animals***

- *Species/strain/sex*: rat / Sprague-Dawley / male
- *No. of animals per sex per dose*: 5
- *Age and weight at the study initiation*: appr. 6 month (age), 485.7 +/- 50.1 g (weight)

### ***Administration/exposure***

- *Type of inhalation exposure and test conditions*: gas inhalation (whole body exposure in a glass)
- *Duration of test/exposure period*: 5 min
- *Doses/concentration levels*: mean concentration 1655.4 +/- 390.9 ppm (2317.6 +/- 547 mg/m<sup>3</sup>)
- *Analytical verification of test atmosphere concentrations*: yes (using gas chromatography)
- *Post exposure observation period*: all exposed animals died within 3 min, control animals all survived
- *Control group and treatment*: yes
- *Statistical methods*: not specified

### ***Results and reliability***

- *LC100*: 1655 +/- 390.9 ppm (2317 mg/m<sup>3</sup> +/- 547 mg/m<sup>3</sup>); all exposed animals died within 3 min, all control animals survived

### ***Additional information that may be needed to adequately assess data for reliability:***

- *Time of death*: 3 min
- *Clinical signs*: dyspnea, exaggerated abnormal audible respiration, frothy fluid from the nose and mouth
- *Necropsy findings*: pulmonary edema
- *Potential target organs*: not specified
- *Other findings*: not specified
- *If both sexes tested, results should be compared*: not specified

### **3.3.1.6 Khan et al. (1990)**

#### ***Study reference:***

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Khan A.A., Schuler M.M., Prior M.G., Yong S., Coppock R.W., Florence L.Z., and Lillie L.E. (1990): Effects of hydrogen sulfide exposure on lung mitochondrial respiratory chain enzymes in rats. *Toxicology and Applied Pharmacology* 103 (3), 482-490.

**Detailed study summary and results:**

**Test type**

- Acute inhalation toxicity study, no guideline followed, no GLP

**Test substance**

- *Test substance:* hydrogen sulphide (EC: 231-977-3)
- *Degree of purity:* not specified
- *Impurities:* not specified
- *Batch number:* not specified

**Test animals**

- *Species/strain/sex:* rat / Fischer 344 / male
- *No. of animals per sex per dose:* 4-6
- *Age and weight at the study initiation:* 8-10 weeks (age) + 2 weeks acclimatization, weight not specified

**Administration/exposure**

- *Type of inhalation exposure and test conditions:* gas inhalation (whole body exposure)
- *Duration of test/exposure period:* **4 h**
- *Doses/concentration levels:* 0, 10, 50, 200, 400, or 500-700 ppm
- *Analytical verification of test atmosphere concentrations:* yes (using gas chromatography)
- *Post exposure observation period:*
  - 0-400 ppm-exposed rats: 1, 24 and 48 h
- *Control group and treatment:* yes
- *Statistical methods:* effects of H<sub>2</sub>S exposures compared to control treatments on the activities mitochondrial enzymes: GLM procedures contained in version 6.03 of SAS for microcomputers (SAS Institute, Inc., 1988)

Postexposure effects of H<sub>2</sub>S: Means of treatment combinations were compared by orthogonal contrasts

**Results and reliability**

- *LC50:* not specified
- *Number of deaths at each dose level:*

Concentration (ppm)	Mortality
0	0
10	0
50	0
200	0
400	0

500-700	All animals died
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**Additional information that may be needed to adequately assess data for reliability:**

- *Time of death:* not specified
- *Clinical signs:* no symptoms in rats exposed to 10, 50, 200 ppm; transient lethargy in animals exposed to 400 ppm
- *Necropsy findings:* not specified
- *Potential target organs:* not specified
- *Other findings:* inhibition of the mitochondrial respiratory chain enzymes in lung mitochondria
- *If both sexes tested, results should be compared:* not specified

**3.3.1.7 Lund and Wieland (1966)**

**Study reference:**

Lund O.E. and Wieland H. (1966): [Pathologic-anatomic findings in experimental hydrogen sulfide poisoning (H<sub>2</sub>S). A study on rhesus monkeys]. Int Arch Arbeitsmed 22 (1), 46-54. (Publication in German)

**Detailed study summary and results:**

**Test type**

- Acute inhalation toxicity study, no guideline followed, no GLP

**Test substance**

- *Test substance:* hydrogen sulphide (EC: 231-977-3)
- *Degree of purity:* not specified
- *Impurities:* not specified
- *Batch number:* not specified

**Test animals**

- *Species/strain/sex:* monkey / rhesus / not specified
- *No. of animals per sex per dose:* 3
- *Age and weight at the study initiation:* not specified

**Administration/exposure**

- *Type of inhalation exposure and test conditions:* gas inhalation (method of exposure not specified)
- *Duration of test/exposure period:*

Animal	Duration of exposure	Post-exposure observation period
1	35 min	none
2	25 min and 3 days later 17 min	5 days
3	22 min	10 days

- *Doses/concentration levels:* 500 ppm

- *Analytical verification of test atmosphere concentrations:* not specified
- *Post exposure observation period:* 0-10 days
- *Control group and treatment:* no
- *Statistical methods:* not specified

**Results and reliability**

- *LC50:* not specified
- *Number of deaths at each dose level:* One out of three animals died after 35 min of exposure to 500 ppm H<sub>2</sub>S

**Additional information that may be needed to adequately assess data for reliability:**

- *Time of death:* animal 1 died after 35 min of exposure to 500 ppm H<sub>2</sub>S
- *Clinical signs:* conjunctival irritation

Animal	Clinical signs + mortality
1	35 min: sudden unconsciousness, apnea, acute respiratory and cardiac arrest with lethality
2	25 min (1 <sup>st</sup> exposure): sudden unconsciousness + apnea (nearly lethal) during first exposure but successful reanimation 17 min (2 <sup>nd</sup> exposure 3 days later): sudden unconsciousness
3	22 min: sudden unconsciousness but no apnea, in post-exposure observation period animal was somnolent, low locomotor activity, anorexia

- *Necropsy findings:*

Animal	Histopathological findings
1	No marked findings
2	Necrotic alterations of the cerebral cortex and basal ganglia, no changes in kidney, adrenal glands and heart
3	Necrotic alterations of the cerebral cortex, no changes in kidney, adrenal glands and heart

- *Potential target organs:* not specified
- *Other findings:* not specified
- *If both sexes tested, results should be compared:* not specified

**3.3.1.8 Kage et al. (1992)**

**Study reference:**

Kage S., Nagata T., Kimura K., Kudo K., and Imamura T. (1992): Usefulness of thiosulfate as an indicator of hydrogen sulfide poisoning in forensic toxicological examination - a study with animal experiments. Japanese Journal of Forensic Toxicology 10, 223-227.

**Detailed study summary and results:**

**Test type**

- *Acute inhalation toxicity study, no guideline followed, no GLP*

**Test substance**

- 
- *Test substance:* hydrogen sulphide (EC: 231-977-3)
  - *Degree of purity:* not specified
  - *Impurities:* not specified
  - *Batch number:* not specified

#### ***Test animals***

- *Species/strain/sex:* rabbit / Japanese White / not specified
- *No. of animals per sex per dose:* 5
- *Age and weight at the study initiation:* not specified

#### ***Administration/exposure***

- *Type of inhalation exposure and test conditions:* not specified
- *Duration of test/exposure period:* 14-30 min
- *Doses/concentration levels:* 100-200 ppm and 500-1000 ppm
- *Analytical verification of test atmosphere concentrations:* not specified
- *Post exposure observation period:* 2 h and 24 h
- *Control group and treatment:* not specified
- *Statistical methods:* not specified

#### ***Results and reliability***

- *LD50 or LC50 value with confidence limits if calculated:* not specified
- *Number of deaths at each dose level:* all animals (5/5) died at 500-1000 ppm

#### ***Additional information that may be needed to adequately assess data for reliability:***

- *Time of death:* not specified
- *Clinical signs:* not specified
- *Necropsy findings:* not specified
- *Potential target organs (if identified in the report):* blood, lung, brain
- *Other findings:* not specified
- *If both sexes tested, results should be compared:* not specified

- 
- 3.4 Skin corrosion/irritation**
  - 3.5 Serious eye damage/eye irritation**
  - 3.6 Respiratory sensitisation**
  - 3.7 Skin sensitisation**
  - 3.8 Germ cell mutagenicity**
  - 3.9 Carcinogenicity**
  - 3.10 Reproductive toxicity**
  - 3.11 Specific target organ toxicity – single exposure**
  - 3.12 Specific target organ toxicity – repeated exposure**
  - 3.13 Aspiration hazard**

## **4 ENVIRONMENTAL HAZARDS**

*Not provided*

Kage S., Nagata T., Kimura K., Kudo K., and Imamura T. (1992): Usefulness of thiosulfate as an indicator of hydrogen sulfide poisoning in forensic toxicological examination - a study with animal experiments. Japanese Journal of Forensic Toxicology 10, 223-227.

[https://hero.epa.gov/hero/index.cfm/reference/details/reference\\_id/12026](https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/12026)

Khan A.A., Schuler M.M., Prior M.G., Yong S., Coppock R.W., Florence L.Z., and Lillie L.E. (1990): Effects of hydrogen sulfide exposure on lung mitochondrial respiratory chain enzymes in rats. Toxicol Appl Pharmacol 103 (3), 482-490. <https://www.ncbi.nlm.nih.gov/pubmed/2160136>

Lopez A., Prior M.G., Reiffenstein R.J., and Goodwin L.R. (1989): Peracute toxic effects of inhaled hydrogen sulfide and injected sodium hydrosulfide on the lungs of rats. Fundamental and Applied Toxicology 12 (2), 367-373. DOI: 10.1016/0272-0590(89)90053-5

Lund O.E. and Wieland H. (1966): [Pathologic-anatomic findings in experimental hydrogen sulfide poisoning (H<sub>2</sub>S). A study on rhesus monkeys]. Int Arch Arbeitsmed 22 (1), 46-54.

<https://www.ncbi.nlm.nih.gov/pubmed/5957909>

Prior M.G., Sharma A.K., Yong S., and Lopez A. (1988): Concentration-time interactions in hydrogen sulphide toxicity in rats. Canadian Journal of Veterinary Research 52 (3), 375-379.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1255467/>

Tansy M.F., Kendall F.M., Fantasia J., Landin W.E., Oberly R., and Sherman W. (1981): Acute and subchronic toxicity studies of rats exposed to vapors of methyl mercaptan and other reduced-sulfur compounds. Journal of Toxicology and Environmental Health 8 (1-2), 71-88. DOI: 10.1080/15287398109530051

THRU Laboratories (1972): Toxic hazards research unit annual technical report: 1972. Report No. AMRL TR. Aerospace Medical Research Laboratory, Air Force Systems Command. Transportation U.D.o., Wright Patterson Air Force Base, Ohio, study report

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WHO (CICAD) (2003): HYDROGEN SULFIDE: HUMAN HEALTH ASPECTS

Zwart A., Arts J.H.E., Klokman-houweling J.M., and Schoen E.D. (1990): Determination of Concentration-Time-Mortality Relationships to Replace LC50 Values. *Inhalation Toxicology* 2 (2), 105-117. DOI: 10.3109/08958379009145248