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TOXICOLOGICAL REVIEW

OF

HYDROGEN SULFIDE

(CAS No. 7783-06-4)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

February 2002

(DRAFT)

NOTICE

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U.S. Environmental Protection Agency
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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to hydrogen sulfide. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of hydrogen sulfide.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's Risk Information Hotline at 301-345-2870.

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1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC), and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious, noncancer effects during a lifetime. The inhalation RfC is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg/day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $µg/m^3$ air breathed. Another form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000; 1 in 100,000; or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for hydrogen sulfide has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA,1986a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c), *Guidelines for Developmental Toxicity*

Risk Assessment (U.S. EPA, 1991), *Proposed Guidelines for Carcinogen Risk Assessment* (1996a), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996b), and *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998); *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988); (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a); *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b); *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c); *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995b); *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2000a); memorandum from EPA Administrator, Carol Browner, dated March 21, 1995, Subject: Guidance on Risk Characterization; and *Science Policy Council Handbook: Risk Characterization* (U.S. EPA, 2000b).

Literature search strategies employed for this compound were based on the CAS Registry Number and at least one common name. At a minimum, the following databases were searched: HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLINE, MEDLINE, and MEDLINE backfiles. Pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. Information was contributed by the Hydrogen Sulfide Panel of the Chemical Manufacturers Association.

Previously, the RfD for H_2S was listed as 3×10^{-3} mg/kg/day based on a pig oral study in which the critical endpoint was GI disturbance (Wetterau et al, 1964). This figure is based on a NOAEL of 3.1 mg/kg/day and a LOAEL of 15 mg/kg/day with an uncertainty factor of 1000. The RfC for H_2S was previously listed as 1×10^{-3} mg/m^3 based on the mouse subchronic inhalation study by CIIT (1983a) using inflammation of the nasal mucosa as the critical endpoint. The derivation of the RfC used the $LOAEL_{HEC}$ of1.01 mg/m^3 and the $NOAEL_{HEC}$ of 2.6 mg/m^3, as well as an uncertainty factor of 1000.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Hydrogen sulfide is known as dihydrogen monosulfide, dihydrogen sulfide, hydrosulfuric acid, sewer gas, stink damp, sulfureted hydrogen, and sulfur hydride. Some relevant chemical and physical properties of hydrogen sulfide are listed below (Hazardous Substances Data Bank [HSDB] 1998).

CAS registry number:	7783-06-4
Molecular formula:	H_2S
Molecular weight:	34.08
Vapor pressure:	15,600 mm Hg at 25°C
Density:	1.5392 g/L at 0°C, 760 mm Hg
Boiling point:	–60.33°C
Water solubility:	3980 mg/L at 20°C
Log K_{ow}	Not applicable
Dissociation constants:	pKa1 = 7.04; pKa2 = 11.96
Conversion factor:	1 ppm = 1.39 mg/m^3

Hydrogen sulfide (H_2S) is a colorless gas and has a strong odor of rotten eggs (HSDB 1998). H_2S is soluble in certain polar organic solvents, notably methanol, acetone, propylene carbonate, sulfolane, tributyl phosphate, various glycols, and glycol ethers (HSDB 1998). It is also soluble in glycerol, gasoline, kerosene, carbon disulfide, and crude oil. Aqueous solutions of hydrogen sulfide are not stable; absorbed oxygen causes the formation of elemental sulfur and the solutions become turbid rapidly (HSDB 1998).

The primary uses of H_2S include the production of elemental sulfur and sulfuric acid, the manufacture of heavy water and other chemicals; in metallurgy; and as an analytical reagent (HSDB 1998). In agriculture, it is used as a disinfectant. Formulations include a technical grade (98.5%) and a purified grade (99.5% min.). Occupational exposure to hydrogen sulfide occurs primarily from its presence in petroleum, natural gas, soil, sewer gas and as a byproduct of chemical reactions, e.g., viscose rayon and certain leather tanning processes.

Hydrogen sulfide gas is a natural product of decay. In a residential setting it is most commonly the result of decomposition in septic or sewer systems

3. TOXICOKINETICS RELATIVE TO ASSESSMENTS

3.1. ABSORPTION

No studies were identified which quantitatively determined the absorption of H_2S in humans or animals. However, inhalation of high concentrations of H_2S (greater than 2000 ppm; 2780 mg/m^3) are fatal within seconds or minutes in both humans and animals, suggesting that it is absorbed rapidly through the lungs. Studies in pigs and rats suggested that H_2S is absorbed following ingestion. Since H_2S exists as a gas, oral exposure is not likely to occur. At physiological pH 7.4 approximately one third of the H_2S exists in the undissociated form and the remainder largely as the hydrosulfide anion (Beauchamp et al., 1984).

3.2. METABOLISM

H_2S is metabolized by three pathways: oxidation, methylation, and reactions with metalloproteins. However, the major pathway is oxidation in the liver with methylation being a very minor pathway. The major oxidation product of H_2S metabolism is thiosulfate.
At physiological pH (pH-7.4), H_2S is approximately 69% ionized (HS^-). The metabolic pathway for H_2S is depicted in Figure 1.

The major metabolic pathway for H_2S in the body is oxidation of sulfide to sulfate. Bartholomew et al. (1980) perfused heparinized blood containing sodium [S^{35}]sulfide through isolated rat lungs, liver, and kidney and determined the rate of oxidation for each organ. In the presence of water, sodium sulfide is rapidly catalyzed to H_2S (Haggard, 1921). Therefore, administration of sodium sulfide in the isolated perfused system would be similar to inhalation exposure to H_2S. In the isolated perfused liver, approximately 70% sulfide was rapidly metabolized to sulfate within 15 minutes, and 82% of the detected radioactivity was sulfate after 2 hours perfusion. When unlabeled thiosulfate was added to the liver perfusion system, 54% of the radioactivity was thiosulfate and 22% was sulfate after 15 minutes of perfusion. After 2 hours, these proportions reversed. This would suggest that sulfide is oxidized to thiosulfate with further oxidation to sulfate.

In the isolated perfused lung, 32% of the administered radioactivity was lost from the blood as volatile ^{35}S after 15 minutes. However, after 15 minutes no further radioactivity was lost. Sulfide was oxidized slowly to thiosulfate, and only trace amounts of sulfate were present after 2 hours of perfusion. The small amount of detectable sulfate in the isolated perfused lung was thought to be due to the absence of sulfide oxidase in the lung. In the kidney, sulfide was slowly oxidized to sulfate possibly through thiosulfate as an intermediate metabolite. The investigators also reported that the rat liver mitochondria catalyzed the oxidation of sulfide to thiosulfate *in vitro* through an unknown mechanism. This oxidation was inhibited at high concentrations of sulfide (60µM) because of reduced oxygen consumption by the mitochondria due to cytochrome *c* oxidase inhibition. When glutathione (GSH) was added to the rat liver mitochondria *in vitro*, thiosulfate was further metabolized to sulfate.

Kage and coworkers (1992) exposed adult male Japanese white rabbits to 100-200 ppm (139-278 mg/m^3) for 60 minutes and to 500-1000 ppm (695-1390 mg/m^3) until fatal (average of 22 minutes). In the low-level exposure group, blood and urine were collected immediately after exposure and 1, 2, 4, 6, and 24 hours following exposure. Blood and organs were collected immediately after death in high-exposure animals. In the high-exposure animals, thiosulfate levels in the blood, lung, and brain were 2-7 times higher than sulfide levels. Thiosulfate and sulfide were absent or present only in trace amounts in the liver, kidney, and muscle. In the low-exposure animals, blood thiosulfate decreased rapidly to trace levels 2 hours after exposure and was not detected 4 hours after exposure. Urinary thiosulfate levels exhibited a peak at one to two hours followed by a decline, but were still detectable 24 hours after exposure. Sulfide was not detected in the blood or urine of low-exposure animals.

It appears that oxidation is also the major metabolic pathway for H_2S in humans. Thiosulfate was found in the urine of volunteers exposed to 8, 18, or 30 ppm (11, 25, or 42 mg/m^3) H_2S for 30-45 minutes (Kangas and Savolainen, 1987). Kangas and Savolainen (1987) also determined thiosulfate levels in maintenance workers in a pelt processing plant with known exposures to H_2S, which were compared with thiosulfate levels in unexposed workers. Urinary thiosulfate concentration increased according to the gas concentration and exposure time. The highest concentration of urinary thiosulfate in the exposed workers was observed 15 hours after

exposure. At 17 hours, urinary levels of thiosulfate had returned to control levels suggesting that all of the absorbed sulfide had been oxidized within the 15 hours after exposure.

The relationship between sulfide concentration and cytochrome oxidase in tissues was explored by exposing male CD rats (6/group) to H_2S at 0, 10, 30, 80, 200, or 400 ppm (0, 14, 42, 111, 278, or 556 mg/m^3) for 3 hours (Dorman et al., 2002). The only target organ that demonstrated a clear dose-response relationship was the lung with statistical significance achieved at 80 ppm (111 mg/m^3) and above. Sulfide concentrations in the lung decreased to preexposure levels within 15 minutes of the end of exposure. Hindbrain sulfide concentrations were unaffected by exposure. Cytochrome oxidase concentrations were decreased ($p \leq 0.05$) in both the nasal respiratory and olfactory epithelium at levels of 30 ppm (42 mg/m^3) and above. In animals exposed to 400 ppm (556 mg/m^3) lung sulfite, sulfate, and thiosulfate concentrations ($p \leq 0.05$) increased 15 minutes after the end of the 3-hour exposure, then decreased to preexposure levels rapidly. Rats exposed to $\geq$10 ppm (14 mg/m^3) H_2S for 3 hr had significantly elevated hepatic cytochrome oxidase activity. When animals were exposed 3 hr/day for 5 consecutive days, also resulted in significant cytochrome oxidase inhibition in the olfactory epithelium. In animals (6/group) exposed 6 hr/day for 70 consecutive days to 0, 10, 30, or 80 ppm (0, 14, 42, or 111 mg/m^3), there was no effect of exposure on sulfide or cytochrome oxidase concentrations in either lung or hindbrain. Cytochrome oxidase appears to be a sensitive biomarker of exposure to H_2S.

Methylation has also been reported as a possible metabolic pathway following exposure to H_2S. Hydrogen sulfide was reported to be methylated to methanethiol *in vitro* by the intestinal mucosa of Sprague Dawley rats (Weisiger et al., 1980). Methanethiol can be further methylated, although much more slowly, to carbon disulfide. Thiol *S*-methyltransferase was reported to catalyze the two reactions. It is a ubiquitous enzyme, and the investigators reported the highest activity in the colonic and cecal mucosa (10^{-13} mol/min/mg of protein). High activities were also reported in the liver, lung, and kidney.

Levitt and coworkers (1999) also studied the metabolism of H_2S in the cecal mucosa of Sprague Dawley rats and concluded that oxidation, rather than methylation, was the primary metabolic route in the cecal mucosa. Cecal tissues when incubated with H_2S did not produce methanethiol or dimethylsulfide even when a methyl donor, *S*-adenosylmethionine, was added to

the reaction mixture. The rates of metabolism for cecal and liver homogenates were calculated to be 21 and 2.5 nmol/min/mg of protein, respectively, and the metabolic products of the cecal and liver homogenate were reported to be sulfate and thiosulfate, the primary metabolite. This oxidation rate is approximately 10,000 times the methylation rate reported by Weisiger et al. (1980), which would suggest that methylation is not an important pathway for H_2S detoxification.

Another possible pathway of H_2S metabolism is conjugation with GSH. Smith and Abbanat (1966) reported that an exogenous oxidized, but not reduced, GSH protected mice from lethal intraperitoneal (i.p.) injections of sodium sulfide. They also stated that endogenous oxidized GSH, and other endogenous compounds containing disulfide bridges might provide an important detoxification process following H_2S exposure.

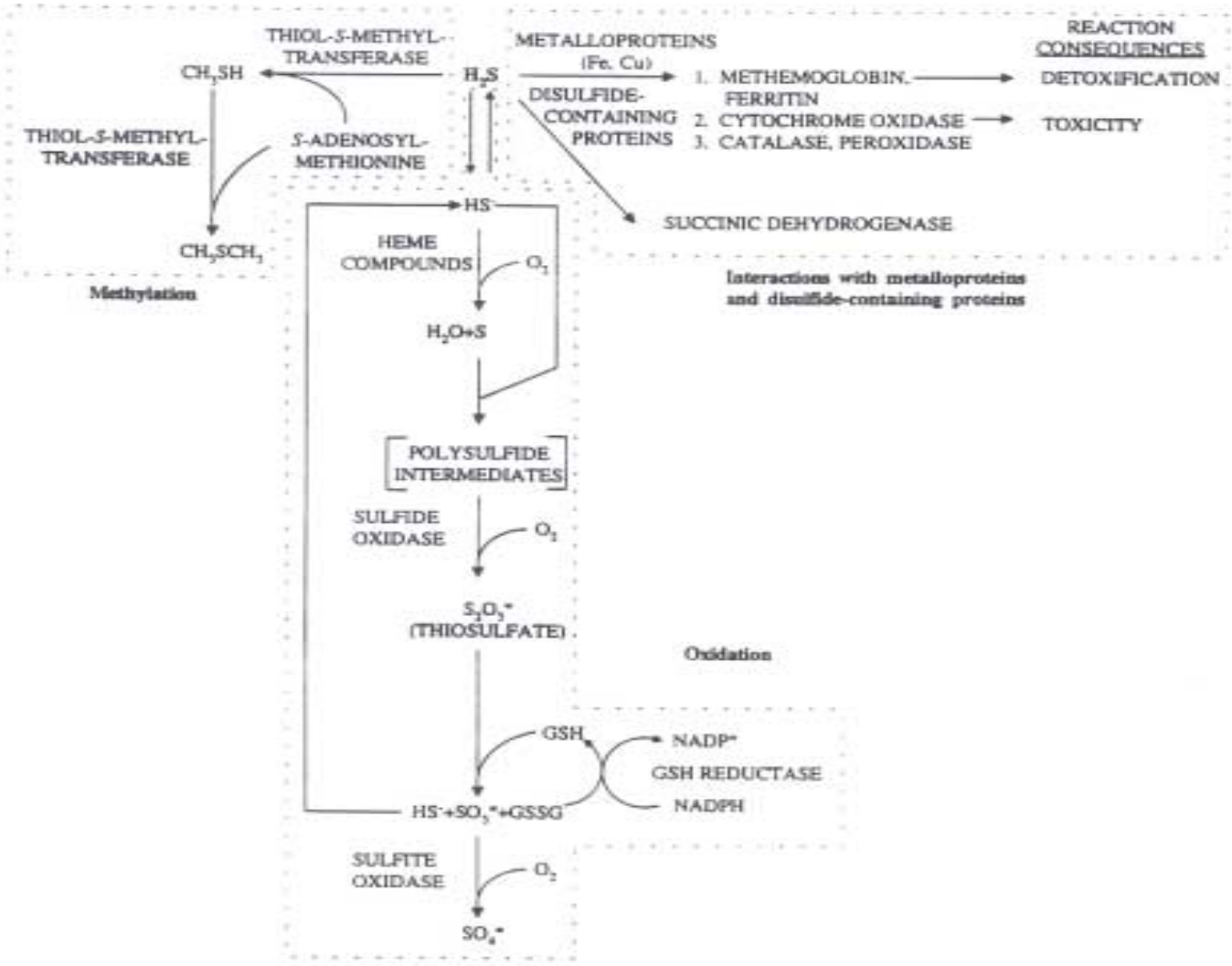
Hydrogen sulfide has also been reported to bind to metalloproteins such as cytochrome oxidase (CytOx) and methemoglobin. While H_2S interaction with the former has been implicated in its toxicity, the interaction between methemoglobin and H_2S is a possible detoxification pathway. Smith and Gosselin (1964) pretreated rabbits and armadillos with sodium nitrite, an inducer of methemoglobin, and reported increased survival following intracardiac and intravenous (i.v.) injection with lethal doses of sodium sulfide. The investigators also reported that female Charles River CD1 mice pretreated with sodium nitrite and *p*-aminopropiophenone, also an inducer of methemoglobin, had increased survival following intraperitoneal injection with sodium sulfide (60 mg/kg). Survival time also significantly increased in mice exposed to 722 and 985 ppm (1003 and 1369 mg/m^3) H_2S and pretreated with sodium nitrite and *p*-aminopropiophenone.

3.3. DISTRIBUTION

Forensic autopsies of 3 men who were overcome by H_2S gas (estimated concentration 767-906 mg/m^3) demonstrated that the gas is widely distributed in exposed humans (Kimura et al., 1994). Blood sulfide concentrations of the three victims were 0.1, 0.2, and 0.8 µg/g 2-3 hours after death. Twenty-four hours after death, blood sulfide was measured at 0.5 µg/g, 0.23 µg/g, and not detected, respectively. Sulfide was also detected in the brain (0.2-1.06 µg/g), lung (0.21-0.68 µg/g), liver (1.30-1.56 µg/g), kidney (0.47-1.50 µg/g), and spleen

Figure 1. Metabolic Pathways of Hydrogen Sulfide*

***Source: ATSDR, 1997 (as adapted from Beauchamp et al., 1984).**



(0.32-0.62 µg/g) 24 hours after exposure. The investigators state that postmortem generation of H_2S by the blood, liver, and kidney was extremely remarkable making the values from these three victims unreliable. However, sulfide is not normally detected in the brain and lung of control postmortem specimens, and sulfide detected in these tissues is considered a reliable indicator of exposure. Imamura and coworkers (1996) also reported detectable concentrations of sulfide in the blood, lung, brain, liver, and kidney of a worker overcome by H_2S gas. The investigators also reported that sulfide was detected in the skeletal muscles of the abdomen and leg.

Distribution of H_2S in animals is very similar to humans. Kohno and colleagues (1991) exposed male Wistar rats to 75 ppm (104 mg/m^3) for 20, 40, or 60 minutes. Following exposure, animals were sacrificed and blood and tissues (brain, lung, heart, liver, spleen, and kidney) were collected within five minutes of sacrifice. After 20 minutes of exposure, the concentration of sulfide in the blood, brain, lung, heart, liver, spleen, and kidney was approximately 10, 25, 22, 38, 23, 27, and 30 µg/g of tissue, respectively. The concentration of sulfide in these tissues was relatively constant regardless of the duration of exposure.

There is no information on distribution following ingestion of H_2S.

3.4. ELIMINATION

Urine is the primary route of elimination following hydrogen sulfide exposure. Gunina (1959) reported that following exposure to sodium sulfide via i.v. and subcutaneous (s.c.) routes or exposure to H_2S by inhalation routes in dogs and rats the majority of the dose (70-99%) was eliminated in the urine by 24 hours post-exposure. Kage and coworkers (1992) detected thiosulfate but not sulfide in the urine of rabbits exposed to 100-200 ppm (139-278 mg/m^3) H_2S by inhalation up to 24 hr after exposure with the highest levels being detected 2 hr after exposure. Thiosulfate was also found in the urine of volunteers exposed to 8, 18, or 30 ppm (11, 25, or 42 mg/m^3) H_2S for 30-45 minutes and maintenance workers in a pelt processing plant with known exposures to H_2S (Kangas and Savolainen, 1987).

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

A review of the occupational effects of exposure to H_2S and its treatment was provided by Guidotti (1996). Exposure to H_2S has been reported to be an important cause of morbidity and mortality in the workplace (Snyder et al., 1995) and olfactory dysfunction (Hirsch and Zavala, 1999). Another review of the health effects was provided by Glass (1990) and Mehlman (1994).

These reviews indicate that the typical "rotten-egg odor" of H_2S is an inadequate warning indicator of exposure since at levels perhaps around 100 ppm can paralyze olfactory nerves making it impossible to detect the presence of dangerous levels of H_2S.

Case Studies: A 19-year old oil rig worker was exposed to unspecified concentrations of H_2S (Burnett et al., 1977) rendering him unconscious for an indeterminate amount of time. Upon resuscitation, he exhibited malaise, anterior chest pain, shortness of breath, headache, nausea and vomiting, tearing of the eyes and photophobia and coughed up blood. Upon arrival at the hospital for further treatment, his vital signs were normal and he was no longer in respiratory distress. He had severe photophobia and blepharospasms, but no signs of conjunctivitis. He also possessed a cough and some motor weakness of his right arm and leg. A neurologic examination and a chest x-ray revealed no abnormalities. After a 3 day stay in the hospital, he was discharged.

Two fatalities were due to massive aspiration of liquid manure and 1 fatality was due to severe pulmonary edema with no aspiration of the manure (Osbern and Crapo, 1981). Another 41-year old individual fell unconscious into liquid manure during a rescue attempt. After resuscitation, he had difficulty breathing and was agitated, but he exhibited no focal neurologic deficits. His initial chest radiograph showed a five-lobed alveolar infiltrate. After 2 weeks in the hospital, his chest radiograph showed improvement, and lung function was normal except for a slightly reduced maximum mid-expiratory flow rate. Six months after the accident, he had a normal chest radiograph and was asymptomatic. Blood sulfide levels of the 2 individuals who had fatally aspirated manure were 5.0 mg/L and 3.6 mg/L. The individual who had died by pulmonary edema had a blood sulfide level of 0.8 mg/L. Control blood samples exhibited sulfide

levels below 0.05 mg/L, and blood samples from random autopsy cases of badly decomposed subjects do not exceed 0.4 mg/L. No blood sulfide measurements were performed on the 41-year old survivor. Eight days following the accident, analyzed air from the liquid manure storage tank contained 6360 ppm methane, 400 ppm carbon monoxide, 1.5 ppm ammonia, 2 % carbon dioxide, 18% oxygen, and 76 ppm (106 mg/m^3) H_2S.

Another case study illustrates the possible long-term sequelae of H_2S exposure. A 30-year-old man displayed dyspnea, chest tightness, and haemoptysis following exposure to a toxic as in a lavatory facility (Parra et al., 1991). The facility was connected to a manure pit; no measurements of H_2S were performed. Physical examination and routine laboratory studies revealed no abnormalities. However, a chest radiograph detected a mild bilateral interstitial pattern. Bronchoscopy showed a widespread reddish mucosa. Pulmonary function tests showed a mild restrictive disease. After five months, the patient possessed residual exertion dyspnea but was otherwise asymptomatic. The diagnosis was pneumonitis caused by the inhalation of a toxic gas. Other exposed workers exhibited nausea, vomiting, dizziness, dyspnea, and eye and nose irritation. One of the exposed workers died a few hours after exposure. An autopsy revealed haemorrhagic bronchitis and the cause of death was asphyxia due to inhalation of a toxic gas.

Several case studies indicate the rapid toxicity following exposure to high levels of H_2S. A 14-year-old boy found a discarded cylinder containing H_2S in a rubbish dump and immediately died when he opened the tank (Allyn, 1931). His father died during a rescue attempt. Both bodies were deeply cyanosed.

At a poultry feather fertilizer plant, a worker was exposed to H_2S while attempting to repair a leak and was killed (Breysse, 1961). H_2S was created as a by-product of the putrefaction of the feathers, and was eliminated through a pipe leading to a saw-mill log pond where it was discarded. In the lungs of the victim, the alveolar spaces were filled with edema fluid and numerous pigment-filled macrophages. The diagnosis was pulmonary edema, and the cause of death was H_2S inhalation. Measurements of H_2S concentrations at various locations in the fertilizer plant revealed levels as high as 4000 ppm (5,560 mg/m^3) during cooking and putrefaction of the feathers.

A 16-year old boy suffered fatal H_2S exposure during transport of liquid manure (Hagley and South, 1983). He was found at the bottom of the manure tank, pale, unconscious and apneic.

There was no evidence that he had aspirated any manure. The boy began breathing following resuscitation and his color returned to normal. His heart rate and blood pressure were normal though he did not respond to painful stimuli. Over the following hour, he became responsive to stimuli, but then developed extensor spasms and began to hyperventilate. A chest radiograph and a CT scan of the head were normal. The patient developed pneumonia. However, his neurological condition deteriorated, and he suffered partial seizures and exhibited a decerebrate response to painful stimuli. The patient died five days after the accident with clinical signs of brain stem damage. Post-mortem examination revealed that he had cerebral edema. A week later, H_2S was measured 30 cm below the man-hole of the tank and was found to exceed the upper limit of detection of the equipment at 150 ppm (208 mg/m^3).

By contrast, Milby (1962) has reported two cases of severe but non-fatal poisoning with H_2S. In the process of the disposal of an H_2S gas cylinder and release of the gas under pressure, two men exposed to H_2S collapsed immediately and, unconscious, underwent convulsions. Upon being rescued from the gas, the men required artificial respiration in order to begin breathing on their own. Both men were hospitalized in an unconscious state and were revived and administered oxygen treatment. At the conclusion of their hospital stay, both men were without symptoms, and the author states that they "remained well to the present", but it is unclear how long an interval that represents.

A mass exposure to H_2S took place among workers laying the foundation for a municipal sewage pumping station, leading to the death of a police officer attempting to rescue an unconscious worker and causing neurological sequelae in others (Snyder et al., 1995).

A 20-month-old child developed intermittent paroxysmal tonic deviation of the eyes (Gaitonde et al., 1987). After a few months the abnormal eye movements resolved, progressive involuntary movements of the entire body developed, and the child fell frequently. The child was admitted to the hospital with gross truncal ataxia, choreoathetosis, dystonia, and an inability to stand. The child was dysarthic but had normal eye movements. Computer tomography revealed bilateral areas of low attenuation in the basal ganglia and some of the surrounding white matter. Virology was negative and there was no evidence of streptococcal infection. The brain scan suggested toxic encephalopathy. Shortly after admission, the child's condition improved spontaneously. Ten weeks after admission, the ataxia had resolved and choreathetoid

movements were reduced. A repeat brain scan was normal. The child's illness was attributed to H_2S exposure. The family lived next to a coal mine where a burning tip emitted H_2S for nearly one year. Three months prior to the child's admission, H_2S emissions were monitored for 4 months, and the maximum recorded level in family's housing scheme was 0.6 ppm (0.8 mg/m^3) . However, local authorities admitted that H_2S levels may have been many times higher prior to monitoring. The burning tip had been extinguished just prior to the child's admission to the hospital.

Clinical Studies: Jappinen et al. (1990) exposed 10 asthmatic subjects to 2 ppm (2.8 mg/m^3) H_2S for 30 minutes. Three out of 10 subjects complained of headache immediately following exposure, and all subjects described the odor of H_2S as very unpleasant but became accustomed to it. All subjects also complained of nasal and pharyngeal dryness at the beginning of exposure. Airway resistance (Raw) was increased by 26.3% (-5.95% to +137.8%), but no clinical respiratory symptoms were observed. Specific airway conductance (Sgaw) was decreased by 8.4% (-57.7% to +28.9%). The changes, however, were not statistically significant because of the high amount of variability between subjects. Forced vital capacity (FVC), forced expiratory volume after one second (FEV_1), and forced expiratory flow ($FEF_{25-75\%}$) did not change significantly following exposure.

They also investigated the possible effects of low concentrations of H_2S on respiratory function. Twenty-six pulp mill workers with daily exposure to < 10 ppm (14 mg/m^3) H_2S were given respiratory function tests. There were no statistically significant changes in FVC and FEV_1 at the end of work-shifts compared to those measured on weekends and days off. There was also no effect in bronchial responsiveness as evaluated by histamine challenge.

Twenty-one cases of H_2S poisoning were assessed for blood sulfide levels and activities of heme-synthesizing enzymes (Jappinen and Tenhunen, 1990). Subjects were exposed to H_2S for 1 minute to 3.5 hours. The subjects were assumed to have been exposed to several hundred ppm of the gas if the workers were unconscious, or 20-200 ppm (28-278 mg/m^3) if the workers were not unconscious and had prolonged exposures. Three blood samples were taken for analysis of heme-synthesizing enzymes immediately, 1 week, and 1 month after exposure. One blood sample was taken from 6 workers immediately (< 2 hours) after accidental exposure for analysis of blood sulfides. The average duration of exposure was 4.5 minutes and the mean

blood sulfide concentration was 75 µg/L (30-130 µg/L). The activity of δ-aminolevulinic acid (ALA) was significantly lower than that of control in the first blood sampling and one week after. The effect was more prominent in workers with blood sulfide levels ≥ 100 µg/L. There were no effects in heme synthase. Eleven blood samples were taken at least 2 hours after accidental exposure and in seven of the cases blood sulfide concentration was below the detection limit of the method used. Blood sulfide concentrations ranged from < 10-300 ppm, ALA was decreased in eight of the samples, and heme synthase was decreased in seven of the samples. The investigators note that there was a high probability of co-exposure to methyl mercaptan, which has similar effects on heme metabolism.

Epidemiological Studies: Chronic low-level health effects in two communities exposed to industrial sources of H_2S were assessed by means of a symptom survey using trained interviewers (Legator et al., 2001). The results were compared to health effects reported by residents in three reference communities not exposed to H_2S. Symptoms were elevated in both exposed communities compared to the referent populations in (1) CNS effects, (2) respiratory effects, and blood (e.g., anemia, clotting, etc.). Although the odds ratios was elevated over controls, they fell within the confidence intervals. In addition, the two communities from which respondents were surveyed had long-standing involvement with H_2S exposure issues which may have contributed to study bias. Subjects in one of the exposed communities were also assessed by means of several objective psychoneurological tests, the results of which were consistent with results of the symptom survey (Borda, 1997; cited by Legator et al., 2001).

A mortality study was undertaken among Finnish sulfate mill workers exposed to H_2S and organic sulfides (Jappinen and Tola, 1990). Workers had been employed for at least one year between 1945 and 1961 at three pulp and paper mills owned by the same company. No exposure data were presented. Deaths from all causes were not increased. However, workers exposed to H_2S and organic sulfides exhibited an increase in cardiovascular related deaths compared to national death rates (37 observed compared to 24.7 expected). Cardiovascular mortality was higher in workers employed for ≥ 5 years compared to workers exposed for 1-4 years. The investigators state that increased mortality could not be explained by common risk factors and that differences in smoking habits did not explain the findings. They suggest that increased mortality may have been associated with H_2S and organic sulfide exposure.

A cross-sectional study investigated the effects of presumed H_2S exposure in sewer workers to determine if chronic exposure to the gas was associated with decreased lung function (Richardson, 1995). Sixty-eight sewer workers performed spirometric tests and results were compared to 60 non-exposed water treatment workers. Job titles were used to categorize sewer workers according to presumed H_2S exposure levels. There was a statistically significant decrease in mean FEV_1/FVC in sewer workers compared to water treatment workers. The effect was greater in sewer workers presumed to have higher exposures to H_2S and longer exposure histories. In non-smoking subjects, sewer workers were only able to attain 89% of the predicted FEV_1/FVC value compared to 98% in water treatment workers. The study author states that chronic low level exposure to H_2S may be associated with decreased lung function. However, no measurements of H_2S were made so any quantitative relationship between "low levels" of exposure and effect are speculative.

The pulmonary effects resulting from exposure to H_2S were assessed in 175 workers that extracted and processed oil and gas (Hessel et al., 1997). H_2S exposure was assessed via questionnaire in workers who were asked about sour gas exposures that caused symptoms or loss of consciousness. Thirty-four percent stated that they had exposures serious enough to cause symptoms, and 8% of workers stated they had experienced a loss of consciousness due to sour gas exposure. In workers that experienced symptoms, no decrease in spirometric values or excess symptoms were noted. Spirometric values were also not affected in workers that lost consciousness. However, these workers experienced shortness of breath while performing physical activity, wheezing with tightness in the chest, and attacks of wheezing. The investigators state that these symptoms are consistent with bronchial hyperresponsiveness.

A cross-sectional study on pulmonary function was performed in males that worked in viscose rayon plants (Higashi et al., 1983). A one-workday study was performed in which workers were monitored for H_2S exposure for 8 hr and had a forced expiratory flow-volume test performed. H_2S levels were determined using passive diffusion dosimeters worn by the workers ($n = 30$ in exposed and matched control workers). The rayon workers had been exposed to H_2S for an average of 12.3 years. The occupational exposure levels in exposed workers ranged between 0.3 and 7.8 ppm (2.9 ppm average; 4 mg/m^3) compared with < 0.1 ppm (0.1 mg/m^3) in matched control workers. No significant differences in pulmonary function tests were observed

in exposed compared to non-exposed workers. Also, no significant difference were noted in pulmonary function tests taken at the beginning and end of work shifts.

In addition to the one-workday study, a cross-sectional study was undertaken on 115 exposed workers and 209 non-exposed workers. The subjects underwent forced expiratory flow-volume tests, but were not monitored for exposure. No adverse pulmonary effect could be attributed to chronic H_2S exposure. The investigators (Higashi et al., 1983) conclude that chronic exposure to low levels of H_2S would not cause adverse pulmonary health effects.

A survey of 123 male viscose rayon workers exposed to H_2S and/or carbon disulfide (CS_2) and 67 non-exposed metal, plastic, and starch workers was conducted in which subjects were questioned about eye irritation complaints (Vanhoorne et al., 1995). Exposed and control workers had to be employed for at least one year by present employer. Exposure to H_2S and CS_2 was determined using personal monitoring pumps. Exposure to H_2S ranged between 0.2 and 8.9 mg/m^3, and workers exposed to H_2S tended to have high exposure to CS_2. No workers were exposed to H_2S only. Several types of eye complaints were reported by workers that were associated with CS_2 and H_2S exposure. These complaints included pain, tension, burning, hazy sight, photophobia, and irritation at work, and the percentage of complaints increased with exposure. In addition, the frequency of complaints was higher in workers exposed to H_2S and CS_2 compared to workers exposed to CS_2 alone. The investigators conclude, however, that deciding which of the two gases was responsible for eye irritation could not be determined, but argue that a priori evidence would tend to suggest that H_2S is responsible. Considering all of the evidence, the investigators suggested that CS_2 enhances the eye irritation properties of H_2S.

Several reports suggest that long-term follow-up after H_2S exposure may reveal effects not seen immediately (Tvedt et al., 1991a, b; Kilburn, 1993; Wasch et al., 1989). Tvedt et al. (1991b) described the follow-up of 6 H_2S-exposed patients which emphasized the long-term neurological sequelae of H_2S-induced unconsciousness. Immediate symptoms included cyanosis, pulmonary edema, seizures, and coma, and delayed symptoms (5-10 year follow-up) ranged from worker disability due to neurological symptoms to brain damage severe enough to qualify as dementia. No measures of H_2S in air, blood or urine were reported, and the authors suggest that it is the hypoxia caused by the H_2S that is important in neurological sequelae anyway. In Tvedt et al.'s other paper (1991a), effects of exposure to H_2S were also delayed, with follow-up

continued as long as five years. Kilburn (1993) reported a case of an oil well tester rendered semi-conscious by H_2S. Symptoms at follow-up at 39 and 49 months suggested damage to the brain stem, basal ganglia, vestibular apparatus, cortex, and other brain structures. Again, no measures of H_2S were made. In a report by Wasch et al. (1989), three cases of H_2S poisoning are described with 1 to 3 year follow-up and EEG evaluations. These authors also attribute the observed symptoms of brain damage to the anoxia that accompanied the H_2S exposure. No measure of H_2S was reported.

An additional report from Kilburn and Warshaw (1995) describes a study of 13 former workers and 22 near-plant residents of a desulfurization unit within a refinery processing "sour' crude oil in which data on the monitoring of H_2S (and other chemicals) in the air was included. Air monitoring at street level near the homes of the subjects during one week in July 1990 revealed that the H_2S concentration was 10 ppb (0.1 mg/m^3) with periodic peaks of 100 ppb (1 mg/m^3). In addition, dimethylsulfide, mercaptans, ethane, propane and vanadium (as vanadium pentoxide) and thiodiglycolic acid were detected in air. Reduced sulfur gases measured outside the facility showed 24-hr averages of up to 21 ppm mercaptans, 0 to 8.8 ppm (12 mg/m^3) H_2S, carbon oxide sulfide (2.6 to 52 ppm) and for total reduced sulfur 6 to 71 ppm.

Questionnaires were completed by subjects and controls. The ex-workers and near-plant residents were plaintiffs in a class-action lawsuit; controls were friend and relatives nominated by the exposed subjects. However, there was no evidence of bias because of the legal situation. Neurobehavioral functions and a profile of mood states were compared to 32 controls, matched for age and educational level. In addition neuropsychological functions were evaluated. These functions included two-choice reaction time, balance, color discrimination, digit symbol, trail-making A and B and immediate recall. The mean values of exposed subjects were statistically significantly abnormal compared to controls, for these functions. Mood state scores were much higher than those of controls. Alcohol use was excluded as a confounder. However, all ex-workers had been exposed to solvents. Although the authors concluded that long exposure to "low doses" of H_2S are a plausible cause of the neurological function deficits in the study, they acknowledged that other effects experienced by exposed subjects were attributable to other chemicals monitored (e.g., asthma and dermatitis in workers were attributed to sulfur dioxide and vanadium pentoxide. Given the complexity of the exposure environment in terms of the numbers

of chemicals monitored and the few numbers of individuals studied, it is premature to attribute the deficits cited with exposure to H_2S.

4.2. PRECHRONIC AND CHRONIC STUDIES IN ANIMALS

4.2.1. ORAL

Wetterau and coworkers (1964) examined the effects of H_2S in chickens and pigs that were fed dried green animal fodder. The investigators used green fodder that had been dried by sulfur-containing brown coal or fuels containing H_2S. The dried green fodder was analyzed for H_2S content by acidification with hydrochloric acid and iodometric determination prior to animal administration. The H_2S content in all of the experiments ranged from 0.035-0.121%.
In chickens (50 per group), no adverse effects were observed in feed intake, body weight, or survival in animals fed H_2S-treated alfalfa (2-12% of total feed) and H_2S-treated wheat bran (1-12% of total feed) daily for up to 70 days. Adult pigs (numbers not given) were fed diets containing 100, 200, or 400 grams of H_2S-treated dried alfalfa (4, 8, and 24% of the diet, respectively) daily for 105 days. Pigs fed 200 or 400 grams of H_2S-treated dried alfalfa had decreases in weight gain of 4 and 22%, respectively, compared with control. No statistical analysis was presented. Food intake was decreased by 33% in animals fed 400 grams of H_2S-treated dried feed per day. Diarrhea was reported in adult animals that consumed diets containing no treated dried feed that were suddenly changed to diets containing 24% treated dried feed.

In a separate experiment in the same paper, weaned pigs (numbers not provide) were fed diets containing 100, 200, or 400 grams of H_2S-treated dried alfalfa (4, 8, and 24% of the diet, respectively) daily for 105 days. In weaned animals previously exposed to treated dried feed, the animals did not develop diarrhea when the amount of treated dried feed was increased to 24% of total feed intake. The percent of H_2S in the diet containing 24% dried feed was 0.121%. The investigators concluded that there was no indication that diets containing a high amount of H_2S could cause adverse health effects in animals. The diarrhea observed in naive adult pigs might have been due to the sudden change in diet and not to the presence of H_2S, since increasing the feed containing H_2S in weaned pigs previously exposed to a similar diet had no adverse effects. The decreased weight gain observed in this second study is apparently due to a decrease in feed

intake. The pigs in this study did not develop diarrhea, but there was a decrease in food intake. The investigators reported that previous data had suggested that animals refused to eat food containing H_2S (Kling, 1928), which may explain the decreased feed intake and the subsequent decreased weight gain observed in this study.

Male and female Sprague-Dawley rats (20/sex/dose group) were administered 0, 1, 3.5, or 7 mg/kg/day H_2S by gavage daily for 89 days (Anderson, 1987). Dose-levels were determined after a 14-day range-finding study. H_2S doses were prepared by purging deoxygenated deionized water with pure H_2S gas. Prepared solutions of H_2S were stored in amber vials with Teflon seals and fresh vials were used at each dose administration. Dosing solutions were prepared weekly and solution stability was determined. Animals were examined for mortality at the beginning and end of each working day, and clinical signs were monitored immediately after dose administration. Food consumption of 10 rats per dose group was determined weekly. Animals were weighed weekly during the treatment period and at necropsy. Blood samples were collected from the suborbital sinus of animals prior to study initiation, days 27 to 31 of treatment, and before necropsy. Blood and platelet counts were determined, and clinical chemistry was performed. At study termination, animals were sacrificed by carbon dioxide asphyxiation. Sacrificed animals and animals that died during the treatment period were subjected to a full necropsy. In control and high-dose animals, liver, kidneys, spleen, gonads, brain, heart, adrenals, and gross lesions were examined microscopically. In the low- and mid-dose groups, only lung, kidney, liver, and gross lesions, were examined microscopically. Mortality was 50% in the high-dose males compared to 5% in control males. No deaths were observed in high-dose females. Mortality in mid-dose males and females was 10 and 5%, respectively, and no deaths were observed in low-dose animals or control females. Weekly mortality is reported in Table 1 below.

Compound-related neuromuscular and behavioral signs were observed in high-dose male and female animals, with the males displaying a higher incidence of these effects (Anderson, 1987). High dose-males exhibited abnormal posture, convulsions, fist clutching, sedation, unusual vocalization, piloerection, labored breathing, and increased respiratory rate. High-dose females exhibited convulsion, sedation, and labored respiration. Although restlessness was observed in all treated males, and increased salivation in all treated females, these clinical signs generally occurred sporadically, and the incidence of these findings did not show a clear dose-

TABLE 1. Cumulative Weekly Mortality in Male and Female Sprague-Dawley Rats Administered H_2S Orally

	Dose (mg/kg/day)	Weeks													
		1	2	3	4	5	6	7	8	9	10	11	12	13	%
Males	0	0	0	0	1	1	1	1	1	1	1	1	1	1	5
	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3.5	0	0	0	0	1	1	1	1	1	1	1	1	2	10
	7	0	0	0	0	2	2	4	4	6	6	7	9	10	50
Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3.5	0	0	0	0	0	0	0	1	1	1	1	1	1	5
	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0

response. Therefore, the toxicological significance of restlessness in treated males, and salivation in treated females, is not clear.

No significant differences in body weights were noted in any of the treated animals (Anderson, 1987). In addition, no differences in food consumption were noted between treated and control groups. There were no treatment-related effects in hematology or clinical chemistry. Ophthalmological findings prior to sacrifice were normal. The most common findings at necropsy were pink and red mottled lungs, which occurred in all groups. In high-dose males that died prior to sacrifice, pink-red discoloration of the lungs, nasal discharge, penile discharge, reddening of the scrotal sac, and darkening of the spleen were noted. Absolute organ weights were not affected by treatment. However, relative heart weights were increased ($p < 0.05$) in high-dose males, and relative kidney weights were increased ($p < 0.05$) in high-dose females. No gross lesions could be attributed to compound administration and all microscopic findings were considered incidental to compound administration.

It should be noted that there are a number of problems with this study that prevent its use in the derivation of the RfD. A 75 to 100% incidence of pneumonia was reported in the vehicle control and treated groups. However a 0% incidence of pneumonia was reported for the non-

gavaged, rack control group. This information indicates procedural problems with the dosing of the animals. Also, there was one group where mortality was 50%, the high dose male group. However, the cause of death was not determined. In addition, a malady diagnosed as ulcerative dermatitis was common across all groups. Good Laboratory Practice would preclude the acceptance of data from such diseased animals for the drawing of conclusions regarding the effects of H_2S.

4.2.2. INHALATION

The Chemical Industry Institute of Toxicology (CIIT, 1983a) performed a 90-day inhalation toxicity study in B6C3F1/CrlBr mice using H_2S. Animals were individually housed and exposed to 0, 10.1, 30.5, or 80 ppm (0, 14, 42, or 111 mg/m^3) H_2S for 6 hr/day, 5 days per week for 90 days (10 mice/sex/group). Animals were observed twice daily for mortality and clinical signs. Body weight and feed consumption were determined weekly beginning just prior to the first exposure. A final fasted body weight was determined for all animals just prior to necropsy. Each animal also received an ophthalmological exam before the first day of exposure and within 7 days of necropsy. A neurological exam was performed before necropsy in which posture, gait, facial muscle tone, and pupillary, palpebral, extensor thrust, and crossed-extensor thrust reflexes were assessed. Animals were housed in metabolism cages for 12 hr prior to necropsy. Urine samples were collected for all animals and volume, appearance, occult blood, specific gravity, protein, pH, ketone, and glucose were determined. On the day of necropsy, animals were anesthetized with ether and blood was drawn from the suborbital sinus (hematology) and abdominal aorta (chemistry). Animals were sacrificed on study day 91, 92, 93, 94, or 95, and 99 or 100 (rats only). The brain, kidney, spleen, liver, heart, and ovaries/testes were removed, weighed, and examined for gross and histopathologically observable abnormalities. In addition, the following tissues were examined microscopically: cerebellum, cerebrum, medulla, optic nerve, spinal cord, sciatic and anterior tibial nerves, eyes, pituitary, thyroid, parathyroid, salivary glands, heart, lungs, spleen, liver, pancreas, adrenals, mesenteric and mandibular lymph nodes, kidneys, bladder, lacrimal glands, ovaries, uterus, oviducts, vagina, cervix, stomach, duodenum, ileum, jejunum, large and small colon, caecum, skeletal muscle, skin, mammary glands (both sexes), femur, bone marrow, aorta, ear canal with zymbal gland,

nasal turbinates, trachea, testes, epididymis, esophagus, thymus, prostate, seminal vesicle, and gross lesions.

In mice, body weights were significantly depressed in both males and females in the high-exposure groups with sporadic significant decreases in the lower male exposure groups (CIIT, 1983a). Decreases in body weights tended to be more pronounced in the male and female high exposure groups beginning in the 6th week of exposure. Feed consumption was significantly reduced in the high-exposure animals. There were no toxicologically or statistically significant differences in hematology, serum chemistry, urinalysis, ophthalmology, or neurological function except for a 30.5 ppm (42 mg/m^3) female and 80 ppm (111 mg/m^3) male that did not respond to artificial light stimulus.

Two female controls and one 30.5 ppm (42 mg/m^3) male died prior to study termination. Both control animals exhibited multiple focal black discoloration of the glandular stomach. The 30.5 ppm (42 mg/m^3) male exhibited a skull fracture with red discolorations and trauma apparently due to a feeding accident. A female and male in the 80 ppm (111 mg/m^3) exposure group were sacrificed *in extremis* on study days 5 and 6, respectively. Both sacrificed animals exhibited prostration and/or hypoactivity prior to sacrifice. Also, both animals were observed to have the same black discoloration of the glandular stomach observed in the control animals. A low incidence of alopecia and emaciation was observed in control and exposed animals. In addition, a 10.1 ppm (14 mg/m^3) male lost use of anterior appendage with an appearance of paralysis and a 30.5 ppm (42 mg/m^3) female and 80 ppm (111 mg/m^3) female were observed to have a missing front appendage. The investigators stated that it was unknown how the male lost its appendage but that the female's loss was apparently congenital. The investigators considered none of the above findings to be compound-related. Gross pathology of surviving animals also revealed no gross lesions that were considered compound-related (CIIT, 1983a).

The mean absolute weights of the heart, liver, and spleen from male mice exposed to 80 ppm (111 mg/m^3) H_2S were significantly depressed. In addition, the mean absolute kidney weight of 80 ppm (111 mg/m^3) female mice was also significantly reduced by approximately 20%. However, relative weights were not significantly different and clinical pathology and histology were negative in both sexes. Histological examination of surviving animals revealed only one lesion that was considered compound-related. Male (8/9) and female (7/9) mice

exposed to 80 ppm (111 mg/m^3) H_2S exhibited minimal to mild inflammation of the anterior portion of the nasal mucosa (section I; section I and II in two mice). The lesion was primarily located in the squamous portion of the nasal mucosa but extended into the respiratory type epithelium (ciliated) in some animals. In one female, the lesion was suppurative and severe involving the entire nasal passage and associated structures. This lesion was also observed in the two 80 ppm (111 mg/m^3) animals that were exposed *in extremis*. No other histological findings were considered compound-related (CIIT, 1983a).

The critical effect in mice was inflammation of the nasal mucosa. This effect was present in male and female animals exposed to 80 ppm (111 mg/m^3) H_2S. Since H_2S is considered to be an irritant gas and other researchers (Brenneman et al 2000; Dorman et al 2000; Lopez et al 1987) have reported nasal inflammation following H_2S exposure, the critical effect in mice was considered to be inflammation of the nasal mucosa. The LOAEL for this effect in mice was 80 ppm (111 mg/m^3) and the NOAEL was 30.5 ppm (42 mg/m^3) (CIIT, 1983a).

With the following exception, the same methods described in the CIIT mouse study (1983a) were used in a similar study using Fischer-344 rats (CIIT, 1983b) . Following the 90-day exposure period in rats, 10 males and females from each group were selected for clinical pathology and histology, while the remaining 5 males and females from each group were used for special neuropathologic studies. For the neuropathologic studies, rats were anesthetized with sodium pentobarbital containing 200 units of heparin. Rats were perfused via the left ventricle with a 4% phosphate buffered glutaraldehyde solution. The perfused animals were then refrigerated at 4°C overnight. The following day the right and left sciatic nerves and cervical and lumbar portions of the spinal cord were dissected and placed in 4% glutaraldehyde. The left sural nerve and large muscle branch of the tibial nerve were oscimated and placed in cedarwood oil for two weeks. Nerve fibers in the oil were teased and individual fibers mounted to glass slides. Glutaraldehyde-stored specimens (sciatic nerves, cervical and lumbar spinal cord, tibial nerve and sural nerve) were oscimated, dehydrated, and embedded in Epon. Longitudinal and cross sections were stained with toluidine blue. Specimens were examined by light microscopy for pathological changes.

In Fischer 344 rats, no mortality was observed during the 90-day study (CIIT, 1983b). Body weights were significantly reduced from weeks 1 to 13 in males and females exposed to

80 ppm (111 mg/m^3) H_2S. Feed consumption was also significantly depressed in males and females exposed to 80 ppm (111 mg/m^3) H_2S. No abnormalities in ophthalmology, neurological function, serum chemistry, or urinalysis were reported. Sulfhemoglobin levels were significantly increased in males exposed to 80 ppm (111 mg/m^3) H_2S. Elevations in sulfhemoglobin levels were also observed in females exposed to 30.5 and 80 ppm (42 mg/m^3 and 111 mg/m^3) H_2S, but the levels were not statistically significant. The investigators state that assigning biological significance to the sulfhemoglobin values should be done with caution because of sensitivity of the method and the size of the groups investigated. Also, there were no H_2S-related lesions in animals selected for special neuropathologic studies. Gross pathology revealed a low incidence of lesions that were not compound-related. Organ weights were statistically similar between control and exposure weights except for the relative brain weight of males exposed to 80 ppm (111 mg/m^3) H_2S. Relative, but not absolute, brain weights were significantly increased in these animals. The investigators do not attribute any biological significance to this finding. Minimal multifocal peribronchial lymphocytic infiltrate was observed in the lungs of all rats. Minimal-to- mild mononuclear cellular infiltration of sections III and IV of nasal mucosa and nasolacrimal duct was observed in all rats including controls. There was also minimal to moderate inflammation of section I and II of the nasal mucosa, but this effect was not dependent on dose with 45, 40, 75, and 55% of animals effected in the 0, 10.1 (14 mg/m^3) , 30.5 (42 mg/m^3) and 80 ppm (111 mg/m^3) exposure groups, respectively. None of the histopathologic changes were considered treatment-related by the investigators. The critical effect was body weight gain depression observed in female rats. The LOAEL for this effect was 80 ppm (111 mg/m^3) and the NOAEL was 30.5 ppm (42 mg/m^3).

The same methods described above (CIIT, 1983a; 1983b), including the special neuropathologic studies (see above, CIIT, 1983b), were used in the next study which used Sprague Dawley rats (CIIT, 1983c). In the Sprague Dawley rats, 15 males and 15 females per group, no mortality was observed during the 90-day study. Statistically significant decreases in body weights were observed in males exposed to 80 ppm (111 mg/m^3) during weeks 1 through 3, and in 80 ppm-treated females throughout the study. Feed consumption was also significantly depressed in males and females exposed to 80 ppm (111 mg/m^3) H_2S. No abnormalities in ophthalmology, neurological function, hematology, serum chemistry, or urinalysis were reported.

Also, there were no H_2S-related lesions in animals selected for special neuropathologic studies. Gross pathology revealed a low incidence of lesions that were not compound-related. The black discoloration noted in the glandular stomach of mice was present in 5 treated rats. However, this effect was not dose-dependent. Organ weights were statistically similar between control and exposure weights except for the absolute brain weight of males exposed to 80 ppm (111 mg/m^3) H_2S. Absolute, but not relative, brain weights were significantly reduced in these animals. The investigators suggested the finding of decreased absolute brain weight to be biologically significant, in contrast to the lack of significance attached to changes seen in relative brain weight (CIIT, 1983b). The investigators also suggested that the observed decreased brain weight was due to the chemical nature of H_2S, i.e., low molecular weight and high degree of lipophilicity allowing passage through the blood brain barrier. Minimal to mild multifocal peribronchial lymphocytic infiltrate was observed in the lungs of all rats including controls. Minimal to mild mononuclear cellular infiltration of sections III and IV of nasal mucosa and nasolacrimal duct was observed in all rats including controls. There was also minimal to moderate inflammation of section I and II of the nasal mucosa, but this effect was not related to dose with 35, 35, 30, and 50% of animals affected in the 0, 10.1 (14 mg/m^3) , 30.5 (42.mg/m^3) and 80 ppm (111 mg/m^3) exposure groups, respectively. None of the histopathologic changes were considered treatment-related by the investigators. The critical effect was decreased absolute brain weight in males. The LOAEL for this effect was 80 ppm (111 mg/m^3) and the NOAEL was 30.5 ppm (14 mg/m^3).

The following study was actually performed in 1943 by Haskell Laboratory and submitted by Dupont to the Office of Pollution Prevention and Toxics, U.S. EPA (Haskell Laboratory, 1994). Four dogs (sex and species not reported) were exposed to 15 ppm (21 mg/m^3) H_2S 6 hours per day, 5 days per week for 7 weeks. At the start of the 8th week, dogs were co-exposed to 15 ppm (20.8 mg/m^3) H_2S and 10 ppm carbon disulfide. During the 9th week, dogs were only exposed to 15 ppm (20.8 mg/m^3) H_2S which was increased to 30 ppm (42 mg/m^3) for weeks 10-12. Blood pressure and heart rate were monitored in the morning and afternoon of exposure days. In addition, blood morphology (not described by the investigators), and arterial and venous CO_2 and O_2 were determined. The frequency with which these parameters were determined by the investigators was described as “time to time.” All measured endpoints were compared to control values which were established during a 4-week pre-exposure period. The dogs exhibited

normal health and behavior during the exposure period. Appetites and weights were maintained satisfactorily, and no blood abnormalities were noted. Abnormal pulse and blood pressure determinations were reported by the investigators which increased in frequency during the course of the experiment (weeks 1-7). The investigators did not state what constituted an abnormal blood pressure reading. However, the investigators suggested a cumulative effect from low exposure. Co-exposure with carbon disulfide increased the number of abnormal blood pressure readings. Increasing the concentration to 30 ppm (42 mg/m^3) H_2S doubled the number of abnormal blood pressure examinations when compared to a two week 15 ppm (21 mg/m^3) exposure.

Brenneman and coworkers (2000) exposed 10-week-old male CD rats (12/exposure group) to 0, 10, 30, and 80 ppm (0, 14, 427, and 111 mg/m^3) H_2S for 6 hr per day, 7 days per week, for 10 weeks. At the end of the 10- week exposure, animals were euthanized with CO_2 and the noses of the animals were dissected free. The nasal cavities were examined at 6 different cross-sectional levels for lesions. The lesions were graded in severity by a subjective scale: 0 = normal; 1 = mild; 2 = moderate; 3 = marked; and 4 = severe.

No effects were observed in the control or 10 ppm (14 mg/m^3) exposure animals that were considered treatment-related (Brenneman et al., 2000). Nasal lesions of the olfactory mucosa were observed in the 30 (427 mg/m^3) and 80 ppm (111 mg/m^3) exposure animals and consisted of multifocal, bilaterally symmetrical olfactory neuron loss and basal cell hyperplasia affecting the lining of the dorsal medial meatus and dorsal and medial region of the ethmoid recess. The incidence, mean severity, and distribution of the exposure-related lesions increased in a concentration-dependent manner as summarized in Table 2.

No effects were observed in the most rostral portions of the nasal cavity (levels 1 and 2). The severity of the observed lesions varied between mild and severe. At level 3 of the nose, the most rostral margin of the olfactory epithelium is integrated with the rostral portion of the respiratory epithelium. Therefore, this cross-section of the dorsal medial meatus consisted of olfactory epithelium, respiratory epithelium, or alternating regions of the two epithelium types. Olfactory neuron loss was only observed in 80 ppm (111 mg/m^3) exposure animals at this level of the nose. Level 4 of the nasal cavity had lesion distribution restricted to the dorsal medial meatus and the most rostral projection of the third ethmoturbinate. Olfactory neuron loss in the

TABLE 2. Incidence and severity of nasal lesions in male CD rats exposed to H_2S.

Lesion	Nasal Cavity Level	30 ppm		80 ppm	
		Incidence	Severity	Incidence	Severity
Olfactory Neuron Loss	3	0/6	—	8/8	2.4
	4	11/12	1.4	12/12	2.4
	5	9/12	1.1	11/12	1.5
	6	0/12	—	5/12	1.2
Basal Cell Hyperplasia	3	—	—	—	—
	4	10/12	1.8	12/12	1.2
	5	7/12	1.3	11/12	1.3
	6	0/12	—	6/12	1.0

30 ppm (42 mg/m^3) exposure animals was mild to moderate, but olfactory neuron loss in the 80 ppm (111 mg/m^3) exposure group increased in severity to moderate or severe. Basal cell hyperplasia was observed in both exposure groups at this level of the nasal cavity but was more pronounced in the 30 ppm (42 mg/m^3) exposure group.

At levels 5 and 6 of the nasal cavity, olfactory mucosa lined most of the nasal cavity. However, the ventral meatus and lateral walls of the nasal cavity were lined with respiratory epithelium. Although the olfactory mucosa was widely distributed, lesions to this tissue were found in select sites. At level 5, mild to moderate olfactory neuron loss and mild basal cell hyperplasia mainly affecting the nasal septum, dorsal nasal cavity, and marginal ethmoturbinate was observed in both exposure groups, except the nasal septum which was not affected in the 30 ppm (42 mg/m^3) exposure group. The same pattern and severity of lesions were observed at level 6 except only the 80 ppm (111 mg/m^3) exposure group was affected. The critical effects in this study are nasal lesions of the olfactory mucosa. The LOAEL and NOAEL for these effects are 30 (42 mg/m^3) and 10 ppm (14 mg/m^3), respectively (Brenneman et al., 2000).

While Brenneman et al. (2000) evaluated six levels of the rat nasal cavity, the mouse nasal cavity was divided into four sections in the CIIT study (1983a); it is not clear how each of the four sections in the mice of that study relate to the six levels in the rats described by Brenneman et al. (2000). In the mice the lesions were primarily in the squamous nasal mucosa

with some lesions extending into the respiratory epithelium. In Sprague-Dawley rats exposed to H_2S (Brenneman et al., 2000), the lesions were most severe in the olfactory mucosa. Effects on the nasal cavity in Sprague-Dawley rats reported by Brenneman et al. (2000) differ in severity and location (level) of incidence from those found in Sprague Dawley rats in an earlier study (CIIT, 1983c). Reasons for such discrepancies are unknown.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

Dorman et al. (2000) attempted to determine if perinatal exposure by inhalation to H_2S had an adverse effect on pregnancy outcomes. Virgin male and female Sprague-Dawley rats (12/sex/group) were exposed to 0, 10, 30, or 80 ppm (0, 14, 42, or 111 mg/m^3) H_2S 6 hr/day, 7 days/week for two weeks prior to breeding. Exposure was continued during a 2-week mating period and then throughout gestational days 0-19 (GD 0-19). Evidence of copulation (vaginal plug or sperm in vaginal lavage fluid) during the 2-week mating period was considered GD 0. On postnatal day (PND) 4, litters were randomly reduced to 4 animals per sex when possible. Remaining pups were euthanized and discarded without being examined. Dams and pups were then exposed PND 5-18. Non-pregnant adult females were exposed for an additional 23-24 days following the 2-week breeding period. Adult males were exposed to H_2S for 70 consecutive days.

Clinical examinations were performed on all animals before and after each exposure (Dorman et al., 2000). The body weights of the F_0 males and females were determined weekly throughout the study, except that female body weights were not determined weekly once evidence of mating was present. Presumed pregnant females were weighed on GD 0, 7, 14, and 20, and dams were weighed on PND 0, 4, 7, 14, and 21. Feed consumption was determined weekly in F_0 males and pre-breeding females. Feed consumption in presumed pregnant females was recorded on GD 0-7, 7-14, and 14-20. Dam feed consumption was recorded for PND 0-4, 4-7, 7-14, and 14-21. At the end of exposure, adult rats were euthanized and a complete necropsy was performed with emphasis on reproductive and accessory sex organs. Post-parturient animals were necropsied the day of or day after weaning. At necropsy, the right testis from each F_0 male was examined for sperm number, production, motility, and morphology.

No deaths or adverse clinical signs were observed in F_0 males and females for any exposure group. There was a statistically significant decrease in feed consumption in male rats exposed to 80 ppm (111 mg/m^3) H_2S during the first week of the study. There was a small, but not statistically significant, decrease in body weight (5-6%) observed in F_0 males and females exposed to 80 ppm H_2S that was present throughout entire exposure period. There were no statistically significant reproductive performance (mating index, fertility index, postimplantation loss per litter, and number of late resorptions or stillbirths) effects in F_0 animals. Also, the number of live pups, litter size, average length of gestation, and average number of implants per female was not affected (Dorman et al., 2000).

In F_0 males, there was no effect on sperm production or morphology. However, a large percentage of abnormal sperm was observed in one F_0 male from both the 30 (42 mg/m^3) and 80 ppm (111 mg/m^3) exposure groups (29 and 73%, respectively). The only significant difference in organ weights was an increase in absolute and relative adrenal gland weights observed in F_0 males exposed to 10 (14 mg/m^3) and 80 ppm (111 mg/m^3) H_2S and a decrease in the relative ovary weight observed in females in the 10 ppm (14 mg/m^3) exposure group. Male rats from all exposure groups displayed mild to marked sensory neuron loss and basal cell hyperplasia in the olfactory mucosa lining the dorsal medial meatus and the dorsal medial region of the ethmoid recess (only males were examined). However, the investigators do not state if there was a dose-response relationship for these observed effects. No other statistically significant histological effects were noted in either males or females. The investigators however noted a higher incidence of seminiferous tubular degeneration (intratubular sperm stasis, tubular mineralization, sperm granulomas, and multinucleated giant cells) and epididymal changes (degenerate sperm forms in the lumen, aspermia, and oligospermia) in the 80 ppm (111 mg/m^3) exposure group. However, in none of these cases was statistical significance reported. Also, one incidence each of sperm granuloma and unilateral necrosis of the cauda was present in the 80 ppm (111 mg/m^3) exposure group. Notable histological findings in the females included one incidence each of ovarian cysts in the 10 (14 mg/m^3) and 30 ppm (42 mg/m^3) exposure group. Also, one incidence each of squamous metaplasia of the endometrium was noted in the 30 (42 mg/m^3) and 80 ppm (111 mg/m^3) exposure group (Dorman et al., 2000).

All pups were counted, sexed, examined for external anomalies, and weighed on PND 0. Each pup was then monitored for developmental landmarks, i.e., pinnae detachment (PND 1), surface righting (PND 4), incisor eruption and negative geotaxis (PND 7), and eyelid separation (PND 14). Female pups were examined for vaginal patency starting on PND 27. Each male pup was examined for preputial separation starting on GD 35. Motor activity was assessed in one male and female pup from each litter prior to H_2S exposure on PND 13 and 17 and on PND 21 and 60 using photobeams that detected movement. Passive avoidance was evaluated for one male and female from each litter on PND 22 and 62 using a "step-through to darkness" model. In this model, animals were placed on the "the brightly lit side" of a two-sided box. After a 20-second acclimation period, animals were allowed access to darkened side of the box. Upon entry to the darkened side, animals were given an electrical shock. The procedure was repeated 24 hours later. The latency to enter the dark side of the box was measured for each trial day. If the latency was 240 seconds, the rat was removed from the box and assigned a latency score of 240. A functional observation battery (FOB) was performed on one male and female from each litter on PND 60. Also, acoustic startle was assessed in one male and female from each litter on PND 21 and 62. Neuropathology was examined in weanling rats tested for passive avoidance (PND 23) and in adult rats assessed for passive avoidance (PND 61). The remaining pups were euthanized and had a complete necropsy performed.

There were no statistically significant increases in structural malformations. Observed malformations included kinked tail, agenesis of the tail, anophthalmia, small rear legs and body, frontal bone defects, hypognathia, and skin lesion characterized by detachment of the skin and dermis. However, none of these effects were dose related. There was no significant difference in pup weight gain or development (pinnae detachment, surface righting, incisor eruption and negative geotaxis, vaginal patency, preputial separation, and eyelid separation). Surface righting was also equivalent across exposure groups. There were no treatment-related effects on motor activity, acoustic startle response, passive avoidance observed, or FOB. Terminal body and organ weights in all exposure group were comparable to controls. A wide variety of gross observations were noted but not considered treatment related by the investigators. Microscopic examination of nervous tissues failed to demonstrate any treatment-related effects. Seminiferous tubular degeneration in males is a critical effect. The LOAEL and NOAEL for seminiferous

tubular degeneration in male rats (Dorman et al 2000) were 80 and 30 ppm (111 and 4 mg/m^3), respectively.

Dorman et al. (2000) exposed 12 male and 12 female Sprague-Dawley rats/dose to H_2S at concentrations of 0, 14, 42, or 111 mg/m^3 (0, 10, 30, or 80 ppm), 6 hr/day, 7 days/week from two weeks prior to copulation until postnatal day 19. Exposure of dams and pups resumed between postnatal day 5 and 18. The offspring were evaluated for motor activity on postnatal days (PND) 13, 17, 21, and 60, passive avoidance on PNDs 21 and 62, functional observation battery on PND 60, acoustic startle response on PNDs 21 and 62, and neuropathology on PNDs 23 and 61. Exposure to H_2S did not affect pup growth, development, or performance on any of the behavioral tests.

Saillenfait and coworkers (1989) reported that exposure of pregnant Sprague-Dawley rats exposed to 0, 70, 140 or 210 mg/m^3 (0, 50, 100, or 150 ppm) H_2S 6 hr/day during gestational days 6-20 resulted in no signs of maternal toxicity or adverse effects on the developing fetus. There was a slight but significant decrease in fetal weight in all treated groups, but the biological significance of this finding is questionable since the dams also lost weight and there were greater numbers of live fetuses per litter in those groups.

Significant elevations in maternal blood glucose levels were observed in Sprague-Dawley rats exposed to 0, 28, 70, or 105 mg/m^3 (0, 20, 50, or 75 ppm) H_2S, 7 hr/day from gestation day 1 through postnatal day 21 (Hayden et al., 1990a). There were no changes in serum protein, LDH, SGOT, or alkaline phosphatase activities in the treated dams or pups. Pups exhibited decreased serum triglycerides in the 70 mg/m^3 dose group. In a similarly designed study using the same exposure levels (Hayden et al., 1990b), there was a dose-dependent increase in parturition time of approximately 10, 20, and 40% over matched controls at 28, 70, or 105 mg/m^3 (0, 20, 50, or 75 ppm), respectively. The biological significance of this latter effect is questionable since parturition time was quite variable among control groups and was not examined statistically. Maternal liver cholesterol content was significantly elevated on day 21 postpartum in the high-dose dams.

Although no studies have reported effects of prenatal exposure to H_2S on the adult nervous system, early postnatal measurements provide indications that adverse changes may occur. Hannah et al. (1989) exposed timed pregnant Sprague-Dawley rats (number not reported)

to 105 mg/m^3 (75 ppm) H_2S, 7 hours/day from day 5 postcoitus until day 21 postnatal. Groups of 8 offspring, randomly selected, were sacrificed on PND 7, 14, and 21. The neurotransmitters taurine, aspartate, glutamate, and gamma amino-butyric acid (GABA) were significantly altered in the cerebellum at least at one postnatal time point. Glycine was unaltered. In a similarly designed study, Skrajny et al. (1992) exposed groups of 20 pregnant Sprague-Dawley rats to 0, 28, or 105 mg/m^3 (0, 20, or 75 ppm) H_2S. Ten pups, randomly selected, were sacrificed on PND 7, 14, and 21. Significantly increased ($p<0.05$) serotonin levels were observed in the frontal cortex on day 21 postpartum in pups exposed to 28 mg/m^3 (20 ppm) H_2S, while increased ($p<0.01$) serotonin levels were observed in both the cerebellum and frontal cortex on postpartum days 14 and 21 in pups exposed to 105 mg/m^3 (75 ppm) H_2S. Norepinephrine levels were increased ($p<0.05$) at 105 mg/m^3 (75 ppm) in the cerebellum on postpartum days 7, 14, and 21, and in the frontal cortex on postpartum day 21. At 28 mg/m^3 (20 ppm), frontal cortex epinephrine levels were decreased compared to controls on days 14 and 21. In another series of studies designed to evaluate dendritic branching of cerebellar Purkinje cells, mean terminal path length, an indicator of the overall size of the dendritic field, was significantly larger in pups exposed to both 28 and 70 mg/m^3 H_2S (20 and 50 ppm) at PND day 21 (Hannah and Roth 1991). Ten treated and ten control litters were used at each concentration level. Dams were exposed for 7 hours per day from post-coital day 5 until PND 21. Pups were euthanized on day 21, and one pup was randomly selected from each litter for analysis.

The effect of H_2S on spermatogenesis in the rat was assessed by Andrew et al. (1979). A group of 10 14-week-old male Wistar rats were exposed to 220 ppm (306 mg/m^3) H_2S for 3 hr/day for 7 days and compared to a control group. Following last exposure each male was caged with two unexposed virgin females for 10 consecutive weeks. Four male rats were used as positive controls and administered triethylenemelamine. The females were sacrificed after 18 days cohabitation. Parameters evaluated included (1) fertility, (2) corpora lutea, (3) total implants, and (4) dead implants. There were no adverse effects of exposure on these parameters. Subsequently, these investigators evaluated exposure on prenatal development by exposing pregnant Wistar rats to 220 ppm (306 mg/m^3) for 3 hr/day, for 5 days either (1) days 1-18 of gestation or (2) days 7-11 or days 12-16, 5 days total. All animals were sacrificed at day 21 and

examined for (1) dead implants, (2) fetal malformations, and (3) growth retardation. There was no evidence of embryotoxicity or external malformations.

4.4. OTHER STUDIES

4.4.1. RESPIRATORY TOXICITY

Cytotoxic effects of H_2S exposure in the respiratory tract of rats was investigated by Lopez and colleagues (1987). Fischer 344 rats were exposed to 0, 10, 200, and 400 ppm (0, 14, 278, and 556 mg/m^3) H_2S for 4 hr and then sacrificed at 1, 20, and 44 hours post-exposure. Bronchoalveolar lavage (BAL) and nasal lavage (NL) were performed on all animals and lavage protein, lactate dehydrogenase (LDH), alkaline phosphatase (AP), cellular composition, γ-glutamyl transpeptidase (GGT), and cytopathology were determined. Animals exposed to 400 ppm (556 mg/m^3) H_2S exhibited a significant but transient increase (800%) in NL protein at 1 hr post-exposure which returned to normal at 20 hr post-exposure. There was also a transient increase (320%) in LDH from NL fluid in the 400 ppm (556 mg/m^3) exposure group. The cellularity in the NL fluid was increased by 139 and 483% in the 10 and 200 ppm (14 and 278 mg/m^3) 1 hour post-exposure groups, but these values returned to baseline by 20 hr post-exposure. Cellularity was increased by 817, 501, and 73% at 1, 20, and 44 hr post-exposure, respectively, for animals exposed to 400 ppm (556 mg/m^3) H_2S. Differential cell counts indicated the increase in cellularity was initially due to desquamation of nasal epithelial cells (1 hr post-exposure) followed by exudation of polymorphonuclear lymphocytes (PMN). Cytopathology revealed that the desquamated epithelial cells showed stages of ciliocytophthoria, cytoplasmic vacuolation, pyknosis, karyorrhexis, cytoplasmic constriction between the basal region and terminal plate, and separation of ciliated tufts. In the 10 (14 mg/m^3) and 200 (278 mg/m^3) ppm animals, no other effects in NL fluid were observed.

Similar to NL fluid, the BAL fluid of rats exposed to 400 ppm (556 mg/m^3) had an increase in protein content of approximately 3000, 1400, and 450% at 1, 20, and 44 hr post-exposure. There was no increase in BAL fluid proteins in the other exposure groups. In animals exposed to 400 ppm (556 mg/m^3) H_2S, LDH was significantly increased at all post-exposure times, but LDH levels were decreasing towards baseline at the 20 and 44 hr post-exposure times. In the 200 ppm (278 mg/m^3) exposure group, LDH activity in the BAL fluid was significantly

decreased in the 44 hr post-exposure group. In animals exposed to 400 ppm (556 mg/m^3) H_2S, AP was initially significantly increased in the 1 hr post-exposure group but significantly decreased below baseline at 20 and 44 hr post-exposure. Animals exposed to 200 ppm (278 mg/m^3) H_2S had AP levels below baseline at 20 and 44 hr post-exposure. GGT was elevated (900%) in the 400 ppm (556 mg/m^3) 1 hour post-exposure animals. Animals exposed to 400 ppm (556 mg/m^3) H_2S had a 42% reduction in nucleated cells in the BAL fluid at 1 hr post-exposure, but which returned to normal at 20 hr and increased to 30 percent over baseline at 44 hr post-exposure. Pulmonary alveolar macrophages were significantly reduced at 1 hr post-exposure in animals exposed to 400 ppm (556 mg/m^3) H_2S but returned to normal by 20 hr post-exposure. PMN were increased in all post-exposure times (highest at 20 hours) in animals exposed to 400 ppm (556 mg/m^3) H_2S. In the 10 (14 mg/m^3) and 200 ppm (278 mg/m^3) animals, no other effects in BAL were observed.

The investigators concluded that vacuolization, ciliocytophthoria, and nasal sloughing is evidence of cytotoxicity of the nasal epithelium. The investigators also cautioned that although cell count in the NL fluid was elevated in animals exposed to 10 ppm (14 mg/m^3) H_2S, dramatic changes can rapidly be restored due to the remarkable repair capacity of the respiratory epithelium. In addition, the cell counts in rats exposed to 10 ppm (14 mg/m^3) returned to baseline values by postexposure hour 20. Exudation of protein into the nasal passages is a sign of vascular permeability, and the investigators suggested that increased LDH in the NL fluid is a sign of damage to the epithelium. The investigators also suggested that increased LDH and AP observed 1 hr post-exposure in BAL fluid is suggestive of damage to the pulmonary epithelium. However, the investigators stressed that H_2S appears to be a weaker toxicant to the respiratory epithelium than other pneumotoxicants. The authors stated that the olfactory epithelium appears to be more sensitive to the toxic effects of H_2S than the respiratory epithelium. In addition, the investigators considered the large increase in protein content in the BAL to be consistent with the observed edematous properties of the gas. The LOAEL in this study is 200 ppm (278 mg/m^3). The NOAEL is 10 ppm (14 mg/m^3).

Histologic and ultrastructural alterations in the lungs of rats were reported in a similar study in which male Fischer 344 rats were exposed for 4 hr to either 82 or 440 ppm (116 or 613 mg/m^3; n = 12 rats per dose level) H_2S followed by sacrifice at 1, 18, or 42 hours post-

exposure (Lopez et al., 1988a). Histologic changes were transient and mainly present in rats exposed to 440 ppm (613 mg/m^3) H_2S. While some histologic changes were noted at 82 ppm (116 mg/m^3), no pathologic changes were reported at this exposure level. In rats exposed to 440 ppm (613 mg/m^3) H_2S, bronchiolar ciliated cells developed necrosis, but necrotic damage was rapidly repaired through mitosis. Examination of the lung demonstrated the notable edematogenic effect following exposure to 440 ppm (613 mg/m^3) H_2S. Based on findings from the post-exposure groups, the investigators suggested a chronology to the edematogenic effect in which fluid first accumulates around the blood vessels, then the interstitium, and finally in the alveoli. Perivascular edema without involvement of the alveoli in animals exposed to 82 ppm (116 mg/m^3) H_2S suggested that fluid that accumulated in the interstitium was reabsorbed before it entered the bronchoalveolar spaces. This may explain the finding by Lopez and colleagues (1987) that protein was detected in BAL fluid from animals exposed to 400 ppm (556 mg/m^3) H_2S, but not 200 ppm (278 mg/m^3) H_2S. The investigators also found that there was a lack of structural changes in the alveolar endothelium, basement membrane, or type I pneumocytes, which suggests that H_2S exposure to concentrations as high as 440 ppm (613 mg/m^3) does not compromise the air-blood barrier. In addition, there was no adverse effect on the mast cells. If the damage to the air-blood barrier and mast cell degranulation is not responsible for the observed pulmonary edema following H_2S exposure, then the investigators suggested that the edema may be due to an outflow of liquid from the peribronchovascular connective tissue into the lumen of small airways via high conductance pathways which then fill the alveolar spaces in a retrograde manner.

Lopez and coworkers (1988b) examined the effect of acute H_2S exposure on nasal epithelial cells. Male Fischer 344 rats were exposed to 0, 14, 280, or 560 mg/m^3 (0, 10, 200, or 400 ppm) H_2S for 4 hr. Animals were sacrificed at 1, 18, and 44 hr post-exposure. No animals died, but clinical signs of lethargy and epiphora were present in animals exposed to 560 mg/m^3 H_2S. Nasal lesions were present only in the high-dose group and manifested as necrosis and exfoliation of the respiratory and olfactory epithelium. Of the four different sections of the nasal cavity, sections two and three (mid-nasal cavity) were the most severely affected. The rostral section (section 1) was not affected, and section 4 was only slightly affected. These results are similar to those reported by Brenneman and coworkers (2000). At 44 hours post-exposure, the

respiratory mucosa was essentially repaired, but the olfactory mucosa continued to exfoliate. Effects of H_2S on nasal parameters were much less severe in the Sprague-Dawley rat study reported by CIIT (1983c).

Green and colleagues (1991) examined the effects of H_2S on surface properties of pulmonary surfactant. Male Fischer 344 rats were exposed to 0, 200, and 300 ppm (0, 278, and 417 mg/m^3) H_2S for 4 hours, and at 1 hr post-exposure, BAL fluid was obtained from animals. The lavage fluid from exposed animals contained dose-related elevations in protein concentration and LDH compared to non-exposed animals. Microscopic examination of the low-dose animals revealed perivascular edema and proteinaceous material in the alveoli. The lungs of high-dose animals showed areas of red atelectasis, patchy alveolar edema, and perivascular edema. In addition, the lavage fluid of high-dose animals had a marked increase in surface tension properties, which is suggestive of a threshold for this response.

The effects of H_2S on lung bacterial defense has been investigated. Rogers and Ferin (1981) exposed male Long-Evans rats to 45 ppm (636 mg/m^3) H_2S for 2, 4, or 6 hr followed by bacterial challenge to *Staphylococcus epidermidis*. In control animals, almost all of the bacteria was inactivated by the 6-hour post-challenge sacrifice time. Rats exposed to H_2S for 2 hr responded similarly to controls. However, rats exposed to H_2S for 4 and 6 hr had 6.5- and 52-fold greater percent bacteria remaining, respectively, compared to controls. The investigators suggest that an H_2S-induced absence of bacterial inactivation may explain secondary pneumonias in humans subsequent to acute or subacute H_2S exposure. The effect of bacterial inactivation was hypothesized by the investigators to be due to alveolar macrophage inactivation. This hypothesis is supported by Robinson (1982) who demonstrated that rabbit alveolar macrophages lost the phagocytic ability *in vitro* when exposed to 54 ppm (75 mg/m^3) H_2S for 24 hr.

Studies (Moulin et al., 2002) in adult CD rats (12/concentration**)** exposed to 0, 10, 30, or 80 ppm (0, 14, 42**,** or 111 mg/m^3) H_2S have shown that nasal regions of predicted high flux (by means of a computational fluid dynamics model) lined with olfactory epithelium show a close correlation with lesion incidence at $p<0.005$ for both the mid- and high-concentration groups**.** An extension of these studies is in progress (Brenneman et al., in press).

4.4.2. NEUROTOXICITY

Kombian et al. (1988) examined the acute effects of H_2S on amino acid levels of various brain regions in the rat following i.p. administration of 10 or 30 mg/kg doses. H_2S administration was associated with alterations in several amino acid transmitters, in particular, enhanced levels of aspartate, glutamate, glutamine, GABA, glycine, taurine and alanine in the brainstem. Amino acid transmitters may play a role in the neuronal control of breathing. Kombian et al. (1988) postulated that the observed changes in neurotransmitter amino acid levels in the brainstem may at least partially contribute to the acute toxicity of H_2S through mechanisms involving respiratory arrest.

Elovaara et al. (1978) demonstrated a marked reduction in the incorporation of labeled leucine in cerebral protein and myelin in adult female mice exposed to 100 ppm (139 mg/m^3) H_2S for 2 hr. The results indicate decreased brain protein synthesis. The authors suggested that the changes may have resulted from the inhibition of cerebral cytochrome oxidase activity. The authors also noted that the concentration of 100 ppm (139 mg/m^3) is 5 to 20 times lower than exposure levels associated with human fatalities.

Utilizing *in vitro* whole brain preparations, Roth et al. (1997) showed that addition of 0.13 µm Na_2S can inhibit CytOx and carbonic anydrase. Also, KCN, a specific inhibitor of cytochrome oxidase, altered the rhythmical activity of rat hippocampal neurons in a manner similar to Na_2S or H_2S. The authors speculated that the inhibition of the respiratory enzyme cytochrome oxidase may be involved in the sulfide-induced alteration of neuronal function in brain regions such as the hippocampus.

Skrajny et al. (1996) investigated the effects of low levels of H_2S on the hippocampus and neocortex of freely moving rats using electroencephalographic activity (EEG) as a measurement of neuronal function. The hippocampus was a focus of this study because this exposure of humans to high levels of H_2S (i.e., about 500 ppm; 695 mg/m^3) has been associated with memory loss and variable degrees of learning and perceptual deficits (Kilburn, 1993; Tvedt et al, 1991; Wasch et al., 1989). The hippocampus plays a role in processing between cortical structures involved in cognitive behavior, and is very susceptible to toxic insult. Rats were exposed to 25, 50, 75 or 100 ppm (35, 69, 104, or 139 mg/m^3) H_2S for 3 hours/day for 5 days. Results indicated that repeated exposure to low levels of H_2S can produce cumulative changes in hippocampal

function. The effect of H_2S on hippocampal EEG was dose dependent. The authors postulated that elevated catecholamine and/or serotonin levels resulting from the inhibition of MAO may be involved in the sulfide-induced alteration of hippocampal theta activity, since the time for reactivation of MAO activity correlated with the time required for the recovery of the total power of hippocampal EEG activity following exposure to H_2S. The authors also noted that exposure to 100 ppm (139 mg/m^3) H_2S produced no significant effects on rat EEG activity recorded from the neocortex.

Higuchi and Fukamachi (1977) examined the effects of inhalation exposure to H_2S on avoidance behavior in rats. Rats were exposed to 100–500 ppm (139-695 mg/m^3) H_2S. H_2S was generated from the reaction of iron sulfide and hydrochloric acid. The exposure duration was 2 hr. Both the Sidman-type conditioned avoidance and discriminated avoidance behavior were assessed. A rapid and significant decrease in discriminated avoidance response was observed at concentrations $\geq$ 200 ppm (278 mg/m^3) . At concentrations $\geq$300ppm (417 mg/m^3) , the Sidman-type conditioned avoidance response was also decreased. The behavioral effects were reversed following ventilation with clean air or when exposure was terminated.

In an attempt to show that the neurological sequelae following exposure to high concentrations of H_2S is due to neuronal necrosis, Baldelli and coworkers (1993) administered sodium sulfide (Na_2S) to unventilated and anesthetized ventilated male Wistar rats by intraperitoneal injection. Doses in unventilated rats ranged from 84 to 200 mg/kg, while ventilated rats were administered 120, 150, or 200 mg/kg. In unventilated animals, all animals administered doses greater than 120 mg/kg (n=11) sulfide died in $\leq$ 10 minutes. Doses of 120, 108, 100, 96, and 84 mg/kg were lethal to 7/10, 3/3, ½, 2/5, and 0/1 animals, respectively. An LD_{50} of 94 mg/kg was calculated for the 32 unventilated rats by the investigators. Of the 8 surviving unventilated animals, only one 120 mg/kg rat demonstrated histopathological signs of neuronal necrosis of the cerebral cortex. In ventilated animals (5 rats/group), an LD_{50} of 190 mg/kg was calculated. No 120 or 150 mg/kg group animals died during compound administration. However, 4/5 of the 200 mg/kg group animals died within minutes of compound administration, and the sole-surviving 200 mg/kg animal and 4/5 of the 150 mg/kg group animals died within 1 week of exposure. Blood pressure and arterial blood gases were monitored only in ventilated animals. None of the ventilated animals exhibited a decrease in arterial pO_2 or

alteration in pH or pCO_2. There were decreases in mean arterial blood pressure to 42.8, 34.2, and 16.8 mm Hg for ventilated animals within 2-5 minutes of compound administration. Mean arterial blood pressure recovered within 15- 20 for the 120 and 150 mg/kg dose groups, but blood pressure did not return to baseline for 50 minutes in the lone surviving 200 mg/kg ventilated animal. There were significant deceases in EEG activities that were also dose-dependent. After doses of ≥ 150 mg/kg, EEG activity decreased to near isoelectric levels. EEG activity returned to normal in the two lowest doses and showed some recovery at the highest dose. Neuronal necrosis of the cerebral cortex, caudate, and brain stem was observed in the surviving 200 mg/kg animals. The investigators concluded that neuronal necrosis is not directly related to H_2S exposure. Rather, neuronal necrosis is indirectly caused by hypotension induced by H_2S exposure. The profound hypotension observed in the surviving 200 mg/kg ventilated animal probably resulted in cerebral ischemia.

Two recent reports describe effects of H_2S on neurological function in rats (Partlo et al., 2001; Struve et al., 2001). In the first (Partlo et al., 2001), rats were exposed to 125 ppm (174 mg/m^3) H_2S for 4 hr per day for 5 days per week for 5 weeks. Controls were exposed to a nitrogen/air mixture. Testing of the animals revealed that this level of exposure did not affect memory retention or acquisition, but impaired the animals' performance during the re-acquisition of a reversed contingency radial arm maze task. The investigators concluded that treatment of this type produced mild brain dysfunction. Struve et al. (2001) exposed rats to the inhalation of 0, 30, 80, 200, or 400 ppm (0, 43, 111, 278, or 556 mg/m^3) H_2S for 3 hr per day for 5 consecutive days. When exposure levels equaled or exceeded 80 ppm (111 mg/m^3), the animals exhibited significant reductions in motor activity, water maze performance (spatial learning), and body temperature. While spontaneous motor activity was tested immediately after the fifth exposure, water maze performance and core body temperature were tested immediately following each daily H_2S exposure.

4.4.3. CARDIOVASCULAR

In a series of experiments, Kosmider et al. (1967) exposed 10 rabbits to 100 mg/m^3 (72 ppm) H_2S for 1.5 hours and 17 rabbits to 100 mg/m^3 for 0.5 hours for 5 days. Electrocardiograms were taken after exposure and histochemical studies of the heart were

performed. Ten rabbits served as controls for the histochemical studies and control electrocardiograms were made on all animals 10 days prior to exposure. Nine of ten animals exposed for a single 1.5 hour exposure exhibited mainly disorders in ventrical repolarization (T wave depression or inversion) without cardiac arrhythmia. However, animals exposed for 5 days exhibited arrhythmias such as ventricular extrasystoles and bigeminal rhythm or contractions elicited from several pacemakers. These effects were noted in 15 of 17 rabbits, and two of the affected animals exhibited atrial fibrillation. The arrhythmias lasted for several days after exposure. In addition, multiply exposed animals also exhibited T wave depression. Histochemical studies revealed a decrease in ATP phosphohydrolase and $NADPH_2$ oxidoreductase activity, which suggests a direct effect on myocardial cells. A direct effect on ATP phosphohydrolase could alter Na^+ and K^+ transport to the myocardial cell and could result in arrhythmia. Administration of sodium citrate, a calcium chelator, attenuated the arrhythmias.

Electrocardiogram effects have also been reported in rats. Kohno and colleagues (1991) exposed male Wistar rats to 75 ppm (105 mg/m^3) H_2S for 20, 40, or 60 minutes. Heart rate, blood pressure, and electrocardiogram were monitored during exposure and for one hour post-exposure. At 20, 40, and 60 minutes, heart rates decreased by 10, 17, and 27%, respectively, and continued to decrease by 29, 35, and 38%, respectively. Electrocardiograms revealed that the P-Q intervals were longer during the exposure and post-exposure periods, which is suggestive of some disorder in stimulus transmission. No changes were noted in blood pressure either during of after exposure.

4.4.4. OCULAR EFFECTS

H_2S has previously been reported to affect the eyes in a condition called “sore eye” or “gas eye”. Lefebvre and coworkers (1991) examined this effect by exposing male Fischer 344 rats to 560 mg/m^3 (403 ppm) H_2S for four hours or 2100 mg/m^3 (1511 ppm) H_2S for 4 minutes. Ocular lavages were performed and cells were examined. There was a significant increase (approximately 2 times) in exfoliated ocular cells associated with both H_2S exposures. In all animals, the majority of recovered cells were corneal epithelial cells. However, a greater percentage of conjuctival epithelial cells was detected in exposed animals. The study demonstrates that H_2S is an ocular irritant.

4.4.5. GENOTOXICITY

The mutagenic potential of H_2S was investigated using the Ames *Salmonella typhimurium* mutagenicity assay with and without Aroclor-induced hamster and rat liver S9 fractions. H_2S vapor (17-1750 µg/plate) was not mutagenic in *S. typhimurium* strains TA 97, TA 98, or TA 100 with and without metabolic activation (Hughes et al., 1984). Although H_2S is not mutagenic, Berglin and Carlsson (1986) reported that H_2S gas potentiated the mutagenicity of hydrogen peroxide in *S. typhimurium* strain T102. The investigators believed that the increased mutagenicity was due to the formation of iron sulfide, which converts hydrogen peroxide to hydroxyl radicals more efficiently than ferrous iron.

4.4.6. ACUTE STUDIES IN HUMANS

Bhambhani et al. (1991) exposed 16 male volunteers to stepwise increases in exercise levels during exposure to either 0, 0.5, 2.0 or 5.0 ppm (0, 0.7, 2.8, and 7.0 mg/m^3, respectively) H_2S. Measurements included power output, oxygen uptake (VO_2), CO_2 output (VCO_2), heart rate, minute volume, respiratory exchange ratio (RER), minute volume respiration (VE)/VO_2, and blood lactate. In the 5.0 ppm (7.0 mg/m^3) exposure group, lactate levels were significantly increased at the threshold for ventilatory increase, moderate exercise levels, and maximal exercise levels. Lactate levels were not increased in any other exposure group compared to controls. RER decreased significantly from 1.17 ± 0.11 at 0 ppm to 1.02 ± 0.05 at 5.0 ppm (7.0 mg/m^3) H_2S during moderate exercise. During maximal exercise, VO_2 increased significantly from 3.11 ± 0.44 L/min breathing clean air to 3.39 ± 0.50 breathing 5.0 ppm (7.0 mg/m^3) H_2S, but RER decreased from 1.34 ± 0.11 to 1.14 ± 0.09 during exposure to 5.0 ppm (7.0 mg/m^3) H_2S. The increase in lactate levels is considered to be related to an inhibition of CytOx by H_2S, which tends to increase the rate of lactate production at a given work rate because of a greater dependency on anaerobic metabolism. The rate of removal of lactate from the blood by the nonexercising tissues also tends to be lower because the primary fate of lactate is oxidative under these conditions. Despite the known relationship between lactate buildup and muscular fatigue, however, maximal power output was not decreased, indicating the ability to adapt to exposures up to 5.0 ppm (7.0 mg/m^3) H_2S.

Bhambhani et al. (1994, 1996b) conducted a clinical trial in which 25 healthy volunteers, 13 men and 12 women, completed two 30-minute submaximal exercise tests at 50% of their predetermined aerobic capacity (maximum oxygen uptake), breathing either clean air or 5.0 ppm (7.0 mg/m^3) H_2S. Immediately after exercise, biopsies were obtained from the vastus lateralis muscle under local anesthesia. They were subsequently analyzed for concentration of the following markers of anaerobic and aerobic metabolism: lactate (La), lactate dehydrogenase, (LDH), citrate synthetase (CS), and CytOx. CS decreased significantly in men (14.9 ± 4.3 in clean air versus 12.0 ± 4.1 mmol/g/min) following exposure to H_2S, but not women (12.6±5.0 in clean air versus 10.2 mmol/g/min after H_2S exposure). No other parameters were significantly altered in either sex as a result of exposure to H_2S at this concentration.

In a similarly designed study, Bhambhani et al. (1996a, 1997) exposed 15 men and 13 women for 30 minutes to either clean air or 10 ppm (14 mg/m^3) H_2S. Arterial and finger prick blood samples were obtained before exercise and during the final minute of exercise. Muscle biopsies were withdrawn from the right vastus lateralis immediately after exercise. Cardiorespiratory measurements were monitored using an automated metabolic cart interfaced with an electrocardiogram and blood pressure apparatus. Following exposure to H_2S, VO_2 decreased significantly in both sexes, while respiratory exchange ratios increased significantly. Blood lactate levels were also significantly increased following exposure in both sexes. Minute volume respiration, expired CO_2, systolic and diastolic blood pressure and heart rate were not significantly affected. Likewise muscle La, LDH, CS and CytOx were not significantly altered by exposure to 10 ppm (14 mg/m^3) H_2S during submaximal exercise. In summary, it appears that 10 ppm (14 mg/m^3) H_2S inhalation most likely reduced VO_2 during exercise by inhibiting the aerobic capacity of the exercising muscle.

The studies by Bhambhani et al. indicate that the primary effects were related to inhibition of aerobic metabolism. The authors did not consider these changes to be adverse to health because they were not of sufficient degree. The results, however, do suggest that 10 ppm (14 mg/m^3) H_2S is near, or at the threshold for the ability of anaerobic metabolism to compensate for inhibition of aerobic metabolism during physical activity.

4.4.7. ACUTE EFFECTS IN ANIMALS

Mortality has been observed in animals exposed to high concentrations of H_2S by inhalation. Thirteen studies were reported in which animals were exposed to lethal concentrations of H_2S of which 8 are summarized in Table 3. Pulmonary edema was frequently reported in dead animals (Lopez et al., 1989; Prior et al., 1988) and was considered to be sufficiently severe to account for lethality. Lung hemorrhage and collapse has also been reported (Weedon et al., 1940). Prior to death, cyanosis, hypostatic congestion of the lungs, and, muscle spasm were observed in pigs exposed to high concentrations of H_2S (O'Donoghue, 1961). Neurological effects have been observed in monkeys exposed to 700 mg/m^3 (504 ppm) H_2S for 22-35 minutes (Lund and Wieland 1966). Effects included ataxia, anorexia, parenchymal necrosis of the brain, sudden loss of consciousness, and extensive changes in gray matter. Moderate liver hyperemia was also observed (Lund and Wieland 1966). Lethargy and breathing difficulties have been reported in rats and mice that survived 16 hours of exposure to 88 mg/m^3 (63 ppm) H_2S (Weedon et al., 1940).

Table 3. Effects of Exposure to H_2S on Mortality

Species	Sex	Exposure (mg/m^3)	Duration (hours)	Endpoints	Results	Reference
Rat	Both	Various concentrations	246	Mortality	LC50s: 822, 701, 469 mg/m^3	Prior et al. (1988)
Rat	Male	2,317	0.08	Mortality	All died	Lopez et al. (1989)
Rat	Both	23-1,394	16	Mortality	0/8,1/8, 5/8, 8/8 died with exposure to 22, 88, 348, and 1394 mg/m^3	Weedon et al. (1940)
Mouse	Both	23-1,394	16	Mortality	88 mg/m^3 and above resulted in 100% mortality	Weedon et al. (1940)
Rat, Mouse	Both	448-1,821	0.083-1.0	Mortality	Wide range of mortalities	Zwart et al. (1990)
Rat	Both	556-834	4	Mortality	Most died	Tansy et al. (1980)
Rat	Male	560-1,120	1	Mortality	LC50 = 888 mg/m^3	MacEwen and Vernot, (1972)
Monkey	-----	700	0.37-0.58	Mortality	Two of 3 survived	Lund and Wieland, (1966)

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION—ORAL AND INHALATION

4.5.1. ORAL EXPOSURE

In a subchronic oral study in rats, the only one that was found, mortality and neuromuscular and behavioral signs have been reported in animals gavaged with 7 mg/kg/day H_2S in deionized/deoxygenated water for 89 days (Anderson, 1987). Effects included labored breathing, convulsions, and sedation. Mortality was also noted in some males and females exposed to 3.5 mg/kg/day H_2S for 89 days. Although restlessness was observed in all treated males, and increased salivation in all treated females, these clinical signs generally occurred sporadically, and the incidence of these findings did not show a clear dose-response. The toxicological significance of restlessness in treated males, and salivation in treated females, is not clear. No histopathological lesions attributable to H_2S were found. However, this study has serious problems with execution which prevent its being used for derivation of the RfD. These problems include a prevalence of pneumonia and ulcerative dermatitis in vehicle control and treated groups, that fact that the study is not peer-reviewed, and that although 50% of the high dose males died, the cause of death could not be determined.

4.5.2. INHALATION EXPOSURE

Limited epidemiological studies have described cardiovascular, pulmonary, and ocular effects. Jappinen and Tola (1990) reported an increase in cardiovascular mortality among sulfite mill workers when compared to national death rates (37 observed compared to 24.7 expected). However, no exposure data were available to determine if this effect was related to H_2S exposure. In another study in which exposure data were not presented, Richardson (1995) found that mean FEV_1/FVC was significantly decreased in sewer workers assumed to be exposed to H_2S. Pulmonary effects were also reported by Hessel and coworkers (1997) in workers that extracted and processed oil and gas. However, a cross-sectional study of viscose-rayon workers exposed to an average of 3 ppm (4 mg/m^3) H_2S displayed no adverse pulmonary effects following chronic exposure (Higashi et al., 1983). In another study (Vanhoorne et al., 1995), ocular effects were reported in workers chronically exposed to between 0.2 and 8.9 mg/m^3 (0.1 and 6.4 ppm) H_2S, but co-exposure to CS_2 occurred. Thus, exposure to high concentrations of H_2S, such as in

the Breysse (1961) report "as high as 4000 ppm H_2S", can result in pulmonary edema and death. Exposure to low concentrations on the order of 0.3 to 7.8 ppm (0.4 to 11 mg/m^3) on a chronic basis (Higashi et al., 1983) appears to result in no change in pulmonary function in humans.

Subchronic inhalation of H_2S mainly affected the nasal mucosa in animals. Dorman et al. (2000) reported that male Sprague-Dawley rats displayed mild to marked sensory neuron loss and basal cell hyperplasia of the olfactory mucosa when exposed to exposed 10, 30, or 80 ppm (14, 42, or 111 mg/m^3) H_2S 6 hours/day, 7 days/week for 70 consecutive days. However, the investigators do not state if there was a dose-response relationship for these observed effects. The effects in male rats described in Dorman et al. (2000) was assessed more extensively by Brenneman et al (2000). Brenneman and coworkers (2000), examining the same animals in Dorman et al. (2000), reported no effects in control or 10 ppm (14 mg/m^3) exposure animals that were considered treatment-related. Nasal lesions of the olfactory mucosa were observed in the 30 and 80 ppm (42 and 111 mg/m^3) exposure animals and consisted of olfactory neuron loss and basal cell hyperplasia affecting the lining of the dorsal medial meatus and dorsal and medial region of the ethmoid recess. A similar effect was reported in mice (CIIT, 1983a). CIIT reported that male and female B6C3F1/CrlBr mice exhibited minimal to mild inflammation of the anterior portion of the nasal mucosa when exposed to 80 ppm (111 mg/m^3) H_2S for 6 hr/day, 5 days per week for 90 days. Only minimal lesions were found in Sprague-Dawley or Fischer 344 rats (CIIT 1983 b,c). While strain and species differences may explain the discrepancy in response between the Sprague-Dawley rats and the Fischer-344 rats and B6C3F1 mice, it is not known why differing effects of H_2S on nasal lesions were seen by Dorman et al. (2000) vs. CIIT (1983c) since Sprague-Dawley rats were used in both cases.

Subchronic inhalation in Sprague-Dawley rats has also been reported to increase seminiferous tubular degeneration and epididymal changes in rats exposed to 80 ppm (111 mg/m^3) H_2S (Dorman et al., 2000). This study failed to demonstrate reproductive effects in female rats or developmental effects in offspring.

4.5.3. MODE-OF-ACTION

The primary cause of death induced by H_2S poisoning has been considered to be respiratory paralysis due to a toxic effect on the respiratory centers of the brain with pulmonary

edema being a secondary effect. However, some investigators believe that pulmonary edema might be a primary cause of death following exposure to high concentrations of H_2S. Lopez et al. (1989) exposed 5 male Sprague-Dawley rats to an atmosphere of 1660 ppm (2307 mg/m^3) H_2S or injected intraperitoneally animals with 30 mg/kg sodium hydrosulfide (NaHS, an alkali salt of H_2S). Animals from both groups died within 3 minutes of exposure with rats exposed via inhalation showing signs of severe respiratory distress. In contrast, animals exposed to NaHS exhibited no signs of respiratory distress. While the rats exposed via inhalation showed evidence of gross pulmonary edema, the injected rats did not, indicating death in the NaHS exposed animals occurred as a result of neurologic and cardiovascular effects.

Haggard and Henderson (1922) demonstrated that sodium sulfide injection in dogs induced hyperpnea followed by apnea. However, this response did not occur after cutting the vagi. The investigators suggested that sulfide was an irritant to the afferent endings of the pulmonary vagi, and that high amounts of sulfide paralyze the respiratory center. According Almeida and Guidotti (1999), this early observation appears accurate. The importance of the vagus nerve in the transmission of signals from the lung to the brain was demonstrated by administering Na_2S via the femoral vein or carotid artery and comparing the effects observed with the vagus paralyzed by lidocaine with those observed without vagal paralysis. The combination of vagal paralysis plus HS ion exposure prevented the apnea associated with H_2S. Experiments involving the injection of Na_2S via the carotid artery demonstrated also that HS ion does not act on the carotid body to trigger apnea as is generally believed. The authors conclude that the lung is the primary locus of HS ion action. The number of rats involved and the strain used were not reported.

The action of H_2S on a molecular level in the lung has been investigated. Following the exposure of Fischer-344 rats to 50- 400 ppm (70- 556 mg/m^3) H_2S, lung mitochondrial respiratory chain enzyme activities were examined (Khan et al., 1990). In addition, lung mitochondria were incubated in vitro with sulfide. There was inhibition of CytOx activity when animals exposed to greater than 50 ppm (70 mg/m$^{3)}$ H_2S were sacrificed immediately or 1 hour following exposure. Mitochondria incubated with H_2S exhibited a dose-dependent inhibition of CytOx activity. As a result of the findings of their investigation, Khan and coworkers (1990) concluded that under physiological conditions H_2S would block the mitochondrial respiratory

chain primarily by inhibition of CytOx. Since CytOx is the terminal step in aerobic oxidative metabolism, inhibition by H_2S would stop tissue respiration and lead to the development of hypoxia.

The potential mechanism of the neurotoxicity of H_2S has been investigated. Studies have included neurochemical, neuroelectrophysiological, and behavioral approaches to address this issue. Baldelli et al. (1993) concluded from studies using H_2S with or without simultaneous ventilation that the neuronal necrosis seen following H_2S exposure is not a direct result of H_2S but an effect of hypotension and ischemia. Warenycia et al. (1989a) demonstrated a selective uptake of sulfide by the brainstem of rats. Rats were exposed by i.p. injection with sodium hydrosulfide, an alkali salt of H_2S. The selective accumulation of sulfide by the brainstem may be due to the increased H_2S solubility in lipid-enriched brain regions (Warenycia et al., 1989b). Subcellular fractionation revealed sulfide content in the myelin, synaptosomes, and mitochondria. The selective uptake of sulfide in the brainstem may in part explain the lethal effect of sulfide on respiratory function as a consequence of an interaction with the brainstem respiratory center. Most of the catecholaminergic innervation of the brain originates from within the brainstem, and catecholamines and serotonin affect respiratory rhythm (Warenycia et al., 1989b). To explore this possibility further, experiments on monoamine oxidase (MAO) enzyme activity and neurotransmitter levels in various regions of the brain have been performed in rats. Administration of 10 or 30 mg/kg NaHS resulted in elevation of noradrenaline and adrenaline levels in the hippocampus, striatum and brainstem (Warencyia et al., 1989b). Also, brainstem dopamine and serotonin levels were increased. In addition, inhibition of monoamine oxidase activity (MAO) in whole brain preparations was demonstrated. The investigators concluded that inhibition of MAO activity and increases in neurotransmitter levels in the brainstem may be important in the loss of central respiratory drive after H_2S exposure.

Khan and coworkers (1990) exposed Fischer 344 rats (12/group) to various concentrations of H_2S for 4 hours to examine the effects of the gas on lung mitochondrial respiratory chain enzymes. Mitochondrial fractions were assayed for respiratory chain enzyme activity (CytOx, succinate oxidase, succinate-cytochrome *c* oxidase, and NADH-cytochrome *c* reductase) in animals that were sacrificed immediately, or 1, 24, or 48 hr after exposure. In animals that were sacrificed immediately after exposure, exposure to 10 ppm (14 mg/m^3) H_2S did

not significantly alter any of the respiratory chain enzymes. However, exposure to 50 ppm (70 mg/m^3) H_2S significantly reduced cytochrome *c* oxidase activity by 15%. Exposure to 200 and 400 ppm (278 and 556 mg/m^3) H_2S significantly reduced cytochrome *c* oxidase by 43 and 68%, respectively. Succinate oxidase was also inhibited following exposure to 200 and 400 ppm H_2S by 40 and 63%, respectively. Cytochrome *c* oxidase activity was decreased by > 90% in animals exposed to 500-700 ppm (695-973 mg/m^3) H_2S (other respiratory chain enzymes were not examined at these exposure levels).

In the 200-400 ppm (278 and 556 mg/m^3) animals that were sacrificed at 1, 24, and 48 hr post-exposure, no effects on NADH-cytochrome *c* reductase or succinate-cytochrome *c* oxidase were observed. However, cytochrome *c* oxidase was significantly reduced in delayed sacrifice exposure groups with the greatest inhibition observed in the 1 hr post-exposure sacrifice groups. The inhibition observed in 24- and 48-hr post-exposure groups exposed to 200 ppm (278 mg/m^3) was not statistically significant compared to control. Results are summarized in Table 4.

TABLE 4. Effects of H_2S Exposure on Cytochrome *c* Oxidase Activity.

Post-Exposure (hr)	Percent Inhibition Cytochrome *c* Oxidase	
	200 ppm	400 ppm
1	29%	55%
24	12%	26%
48	10%	29%

In vitro incubation of lung mitochondria with sulfide caused a dose-dependent inhibition of cytochrome *c* oxidase activity. Succinate oxidase was also inhibited by sulfide incubation, but inhibition was not as pronounced compared to cytochrome *c* oxidase (IC_{50} = 1.2 ± 0.3 µM for cytochrome *c* oxidase; IC_{50} = 58.3 ± 3.3 µM for succinate oxidase). However, the differences in IC_{50} were attributed to greater mitochondrial protein requirement (> 50 times) to conduct the succinate oxidase assay, which resulted in a similar increase in sulfide concentration. At low concentrations (10-50 µM), sulfide either had no effect or a 20-30% stimulation of NADH-

cytochrome *c* reductase and succinate-cytochrome *c* oxidase. The Lineweaver-Burk plot of cytochrome *c* oxidase activity with various concentrations of ferrocytochrome *c* demonstrated that sulfide inhibited the enzyme in a noncompetitive manner. It was also found that *in vitro* inhibition of cytochrome *c* oxidase by sulfide could be reversed with the addition of methemoglobin.

An investigation of the effects of H_2S exposure on pulmonary alveolar macrophages (PAM) by Khan and coworkers (1991) was performed because of the central role these cells have in scavenging particulates and their proximity to the alveolar surface readily exposes them to inhaled toxicants. Fischer 344 rats were exposed to 0, 50, 200, or 400 ppm (0, 70, 278, 556 mg/m^3) H_2S for 4 hr. PAM from the sacrificed animals were harvested and basal and zymosan-stimulated respiratory rates were measured. H_2S exposure had no effect on basal respiratory rates, but abolished zymosan-stimulated respiration to baseline levels. PAM viability was only significantly reduced (16% less survival compared to controls) in the animals exposed to 400 ppm (556 mg/m^3) H_2S. *In vitro* exposure of PAM to 30 µM sulfide, sulfite, or sulfate demonstrated a significant reduction in basal and zymosan-stimulated respiratory rates for PAM exposed to sulfide only. Zymosan-stimulated respiratory rates were also significantly inhibited when PAM were exposed to sulfite. The results from the *in vitro* studies would seem to imply that the ability to detoxify H_2S through oxidative mechanisms would play a prominent role on the *in vivo* alterations in the basal respiratory process (i.e., explaining why there was no reduction observed in basal respiratory processes *in vivo*). The investigators also suggested that the reason for the observed decrease in zymosan-stimulated respiratory rates could be due to H_2S and sulfide inhibition of other enzymes such as glucose-6-phosphate dehydrogenase or NADPH oxidase, which are also involved in the respiratory process.

Warenycia et al. (1989b) reported inhibition of brain monoamine oxidase (MAO) activity (whole brain homogenate) and increases in brain catecholamine and serotonin levels in rats following oral administration of 30 mg/kg (twice the LD50 dose) sodium hydrosulfide. Dopamine, serotonin, noradrenaline and adrenaline levels were increased in the brainstem. Warenycia et al. (1990) provided some evidence that persulfide formation may be involved in the sulfide-mediated inhibition of MAO.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION—SYNTHESIS OF HUMAN, ANIMAL, AND OTHER SUPPORTING EVIDENCE, CONCLUSIONS ABOUT HUMAN CARCINOGENICITY, AND LIKELY MODE OF ACTION

No human or animal studies assessing the potential carcinogenicity of H_2S were located.

4.7. SUSCEPTIBLE POPULATIONS

Based on data reported by Hannah and Roth (1991) in which in utero and perinatal exposure of Sprague-Dawley rats to 20 or 50 ppm (28 or 70 mg/m^3) H_2S resulted in abnormal growth and morphology of developing cerebellar Purkinje cells and changes in neurotransmitter levels, pregnant women might be considered s susceptible population. The hazard, if any, would apparently be to the offspring of these women.

4.7.1. POSSIBLE CHILDHOOD SUSCEPTIBILITY

There is limited evidence that children may be more susceptible to the adverse effects H_2S exposure than adults. A case study reported a 20-month-old child developed intermittent paroxysmal tonic deviation of the eyes and progressive involuntary movements of the entire body developed following exposure to at least 0.6 ppm (0.8 mg/m^3) H_2S (Gaitonde et al., 1987). Conclusions about childhood are extremely limited because of poor exposure data reported in this case report and lack of supporting data in the literature. Based on the findings of the effects of H_2S on developing brain cells in rats treated both pre- and postnatally (Hannah and Roth, 1991) and the fact that in humans the period of synaptogenesis extends from the sixth month of gestation to several years after birth (Ikonomidou et al., 2000), a possibility of children's vulnerability to H_2S may exist.

4.7.2. POSSIBLE GENDER DIFFERENCES

There is some evidence that male rats may be more sensitive to the lethal and neurotoxic effects of H_2S following oral exposure than females. Sprague-Dawley male rats orally exposed to 7 mg/kg/day H_2S for 89 days exhibited 50% mortality compared to no mortality in females exposed to the same dose and duration (Anderson, 1987). However, 10% mortality in males and 5% mortality in females were observed after administration of 3.5 mg/kg/day H_2S . Male

animals also displayed a higher incidence of neuromuscular and behavioral signs at 7 mg/kg compared to females.

Male reproductive effects have also been reported. Male rats exposed to 80 ppm (111 mg/m^3) H_2S for 70 consecutive days displayed seminiferous tubular degeneration and epididymal changes (Dorman et al., 2000).

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE

Relevant quantitative human subchronic and chronic oral toxicity data are not available. Only one subchronic oral animal study was found (Anderson, 1987). A number of problems with that study precluded the derivation of an oral RfD. Among the problems with that study are the following: (1) it is unpublished and therefore not peer-reviewed, (2) a 75 to 100% incidence of pneumonia was reported in vehicle controls and all treatment groups. However, a 0% incidence of pneumonia was reported for the rack control group (which was not gavaged). The study was obviously poorly executed, and the prevalence of pneumonia across all gavaged groups indicates a prevalence of disease that precludes drawing conclusions from this study regarding the effect of H_2S, (3) there was a 50% death rate in the high dose male group, the only group with significant mortality, but the cause of death "could not be determined". This also inspired a lack of confidence in the data, (4) alopecia was common in all groups, and the problem was diagnosed by a veterinarian as ulcerative dermatitis. This disease plus the pneumonia prevents one from calling this group "normal" and thus appropriate for the production of reliable data.

5.2. INHALATION REFERENCE DOSE

5.2.1. CHOICE OF PRINCIPAL STUDY AND CRITICAL EFFECT WITH RATIONALE AND JUSTIFICATION

Most of the few available studies in humans are judged not to be suitable for derivation of an inhalation RfC because of study limitations including lack of adequate exposure data, limited number of subjects examined, and co-exposure to other chemicals such as carbon disulfide. Neurotoxicity of H_2S m,ay be important because, in at least one study, effects on neurological were seen at concentrations almost as low (20 ppm or 28 mg/m^3) (Hannah and Roth, 1991) as

those on which the RfC is based using another endpoint. In support of this, several groups of investigators (Tvedt et al., 1991; Kilburn, 1993; Wasch et al., 1989) have reported long-term adverse neurological sequelae of H_2S-induced unconsciousness in humans during occupational exposure including psychological dysfunction, brain damage, and, sometimes, dementia.

Three subchronic animal studies were considered for derivation of an inhalation RfC: those by Dorman et al. (2000), CIIT (1983a), and Brenneman et al. (2000). Dorman et al. (2000) reported increased incidence of seminiferous tubular degeneration and epididymal changes in male rats exposed to 80 ppm (111 mg/mg^3) H_2S *in utero* and postnatally. In addition, male rats from all exposure groups (10, 30, and 80 ppm; 14, 42, and 111 mg/m^3) displayed mild to marked sensory neuron loss and basal cell hyperplasia in the olfactory mucosa, which may be the more sensitive effect. However, the investigators do not state if there was a dose-response relationship for these observed effects. CIIT (1983a) reported that male and female mice exposed to 80 ppm (111 mg/mg^3) H_2S, 6 hr per day, 5 days per week for 90 days exhibited minimal to mild inflammation of the anterior portion of the nasal mucosa. Only minimal lesions were observed in two species of rats (Sprague-Dawley and Fischer-344) examined by the same authors (CIIT, 1983b, 1983c) and in mice exposed to lower concentrations of H_2S. However, in male Sprague-Dawley rats exposed to 0, 10, 30, and 80 ppm (0, 14, 42, and 111 mg/m^3) H_2S for 6 hr per day, 7 days per week, for 10 weeks, Brenneman and coworkers (2000) reported nasal lesions of the olfactory mucosa in the 30 and 80 ppm (42, and 111 mg/m^3) exposure groups. No effects were observed in the control or 10 ppm (14 mg/m^3) exposure animals that were considered treatment-related. Nasal lesions consisted of multifocal, bilaterally symmetrical olfactory neuron loss and basal cell hyperplasia affecting the lining of the dorsal medial meatus and dorsal and medial region of the ethmoid recess. The difference in results between Brenneman and CIIT may indicate a variability in animal response.

The study by Brenneman was considered to be the best study for derivation of an inhalation RfC for several reasons. First, the critical effect (nasal lesions of the olfactory mucosa; summarized in Table 6 below) has been reported by other investigators (Dorman et al., 2000; CIIT 1983a; Lopez et al., 1988b), and the effect is consistent with the irritant properties of this gas. Secondly, the respiratory system has been reported be a target organ of H_2S toxicity by numerous researchers. Third, the critical effect was reported to occur at a LOAEL of 30 ppm

TABLE 6. Incidence of nasal lesions in male CD rats exposed to H_2S. (There were no effects in the 10 ppm or control groups.)

Lesion	Nasal Cavity Level	30 ppm Incidence	80 ppm Incidence
Olfactory Neuron Loss	3	0/6	8/8
	4	11/12	12/12
	5	9/12	11/12
	6	0/12	5/12
Basal Cell Hyperplasia	3	-	-
	4	10/12	12/12
	5	7/12	11/12
	6	0/12	6/12

(42 mg/m^3); the NOAEL is 10 ppm (14 mg/m^3). The LOAEL and NOAEL are at lower concentrations than those in the other subchronic studies. Therefore, the use of this study for RfC derivation would provide a more conservative RfC value compared to the other subchronic studies.

5.2.2. METHODS OF ANALYSIS—INCLUDING MODELS (PBPK, BMD, etc.)

The use of traditional RfC methodology yielded a NOAEL of 10 ppm (14 mg/m^3) and a LOAEL of 30 ppm (427 mg/m^3). Converting ppm to mg/m^3 resulted in a NOAEL of 14 mg/m^3 and a LOAEL of 42 mg/m^3 (1 ppm = 1.39 mg/m^3). The LOAEL and NOAEL were then converted to continuous exposure.

$$\text{LOAEL} = 42 \text{ mg/m}^3 \times 6/24 \text{ hours} = 10.5 \text{ mg/m}^3$$

$$\text{NOAEL} = 14 \text{ mg/m}^3 \times 6/24 \text{ hours} = 3.5 \text{ mg/m}^3$$

Thus, a LOAEL of 10.5 mg/m^3 and a NOAEL of 3.5 mg/m^3 were obtained.

The physicochemical characteristics of H_2S and its distribution in rodents after inhalation exposure indicate that it would be identified as a Category 2 gas, following guidance for derivation of inhalation RfC values (U.S. EPA, 1994). The human equivalent concentration

(HEC) for H_2S was derived by multiplying the animal NOAEL by an interspecies dosimetric adjustment for gas:respiratory effects in the region of critical effect. Since the critical effect is in the nose (point of contact), the dosimetric adjustment was calculated for the extrathoracic (ET) region.

For H_2S, this regional gas dose ratio (RGDR) was calculated as the ratio of rat to human ventilation rate over the ET region surface area. The ventilation rate (V_E) was calculated for male Sprague-Dawley rats using the default body weight of 0.267 kilograms shown in Table 4-5 of U.S. EPA (1994). The ventilation rate for male rats was calculated as follows:

$$\ln V_E = b_0 + b_1 \ln(0.267\text{kg})$$

The parameters, b_0 and b_1, are species specific parameters listed in Table 4-6 of U.S. EPA (1994). This gave a V_E of 0.19 liters/minute for male rats. The default ventilation rate for humans is 13.8 liters/minutes given on page 4-27 of U.S. EPA (1994). The default ET surface areas for the rat and human are 15 and 200 cm^2, respectively. These values are given on page 4-26 of U.S. EPA (1994). The RGDR was calculated as follows:

$$RGDR_{ET} = (V_E/SA_{ET})_{animal}/(V_E/SA_{ET})_{human} = (0.19/15)/(13.8/200) = 0.184$$

The animal NOAEL is then multiplied by the $RGDR_{ET}$ to yield the $NOAEL_{HEC}$.

$$NOAEL_{HEC} = NOAEL_{animal} \times RGDR_{ET} = 3.48\ mg/m^3 \times 0.184 = 0.64\ mg/m^3$$

5.2.3. RfC DERIVATION—INCLUDING APPLICATION OF UNCERTAINTY FACTORS (UFS) AND MODIFYING FACTORS (MFS)

The RfC for H_2S is derived by dividing the $NOAEL_{HEC}$ for nasal effects by the UF of 300. Uncertainty factors were applied for interspecies extrapolation from animal to human (3) and intraspecies variability (10). The interspecies uncertainty factor of 3 ($10^{1/2}$) was used rather than 10 because of a dosimetric adjustment from rat to human was used. The use of an additional uncertainty factor of 10 was employed for the use of a subchronic, rather than chronic, study.

Therefore, a total uncertainty factor 300 (10 subchronic to chronic, 10 for intraspecies variability, and 3 for interspecies variability) is applied to the $NOAEL_{HEC}$ of 0.64 mg/m^3, yielding an inhalation RfC of 0.0021 mg/m^3.

5.3. CANCER ASSESSMENT

No data pertaining to the potential carcinogenicity of H_2S were identified.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HAZARD IDENTIFICATION

H_2S is a colorless gas and has a strong odor of rotten eggs. Its primary uses include the production of elemental sulfur and sulfuric acid, the manufacture of heavy water and other chemicals, in metallurgy, and as an analytical reagent.

Although quantitative data are lacking, toxicity studies suggest that H_2S gas is absorbed rapidly through the lungs. Oral exposure is not likely to occur. In animals and humans, it distributes to the blood, brain, lung, heart, liver, spleen, and kidney. Oxidation is the primary metabolic pathway for H_2S, with thiosulfate and sulfate as metabolites. H_2S is excreted in the urine.

Human data pertaining to inhalation exposure consist of case reports and occupational studies; these studies have limitations that preclude their use for quantitative risk assessment. Studies in rodents demonstrate that nasal lesions of the olfactory mucosa are the critical effects. Relevant quantitative human oral toxicity data are not available. One oral rat study demonstrated neuromuscular and behavioral signs and mortality as the critical effects (Anderson, 1987).

There is no evidence to indicate that exposure to H_2S is associated with carcinogenesis.

6.2. DOSE RESPONSE

The effects of H_2S in humans can be acute and/or chronic. The exposure-response relationship for acute effects, particularly CNS and respiratory, can be very steep. Initial effects are those related to nausea and airway irritation followed by respiratory complications and unconsciousness that can occur around 200 ppm (278 mg/m^3). Higher levels such as around

1,000 ppm (1,390 mg/m^3) and above can lead to respiratory paralysis and death. Precise determination of acute effect levels is precluded by the lack of accurate monitoring data since nearly all cases were those involving acute accidental overexposure. Levels dangerous to human health may not be detected since high levels of H_2S can paralyze the olfactory nerves making detection impossible.

Little is known of the low level chronic effects of H_2S exposure. Occupational studies have been confounded by exposure to other substances and inadequate monitoring to establish cause-effect levels. For this reason, controlled studies in laboratory animals were used to develop an inhalation reference concentration (RfC) for humans. The daily inhalation exposure to the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime (RfC) has been determined to be 0.002 mg/m^3 or 1 part-per-billion. This value was derived from a subchronic inhalation study by Brenneman et al. (2000). This study reported nasal lesions of the olfactory mucosa in male rats exposed to 30 or 80 ppm (42 or 111 mg/m^3). An overall uncertainty factor of 300 was applied to the NOAEL of 10 ppm (14 mg/m^3) to account for interspecies extrapolation from animal to human, intrapecies variability, and subchronic exposures. The use of nasal lesions permits the derivation of an RfC that is considered protective against any long-term CNS and respiratory effects in ambient exposure environments.

The overall confidence in the RfC assessment is low; confidence in the principal study is medium because it was well-designed and conducted, and examined sensitive endpoints. However, it was subchronic in duration, and examined only male rats. The overall confidence in the database is medium because the endpoints are supported by other subchronic studies. A chronic inhalation study in rats and mice, with additional dose groups, would increase the confidence in the database.

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