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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)

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U.S. Environmental Protection Agency
Washington, DC

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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, Environmental Protection Agency (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 IRIS Hotline at 301-345-2870.

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This document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agency-wide review process whereby the IRIS Program Director has achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Economics, and Innovation; Office of Children's Health Protection; Office of Environmental Information; and the Regional Offices.

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Summaries of the external peer reviewers' comments and the disposition of their recommendations are in Appendix A.

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 $\mu g/m^3$ 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 the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Draft Revised Guidelines for*

Carcinogen Risk Assessment (1999), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998); *Recommend-ations 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. The relevant literature was reviewed through 2002. Pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

Previous IRIS entries for hydrogen sulfide include an RfD listed as 3×10^{-3} mg/kg-day based on an oral pig 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 1,000. 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 $NOAEL_{HEC}$ of 1 mg/m^3 and the $LOAEL_{HEC}$ of 2.6 mg/m^3, as well as an uncertainty factor of 1,000. The cancer assessment for hydrogen sulfide had not yet undergone a complete evaluation and determination under US EPA's IRIS program for evidence of human carcinogenic potential.

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, National Library of Medicine, 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 is a colorless gas and has a strong odor of rotten eggs (HSDB, 1998). Hydrogen sulfide 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 H_2S 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 H_2S 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 decaying organic matter. In residential settings 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, the inhalation of high concentrations of H_2S (greater than 2000 ppm or 2780 mg/m^3) can be fatal within seconds or minutes in both humans and animals, suggesting that it is absorbed rapidly through the lungs. Studies in pigs and rats measuring plasma sulfide levels 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). Although hydrogen is reported elsewhere to be fairly water soluble, Milby and Baselt (1999a) indicated that H_2S is only moderately water soluble and thus not well-absorbed by the moist tissues of the upper respiratory tract. The percutaneous absorption of H_2S has been described as slow (Laug and Draize, 1942).

3.2. METABOLISM

Hydrogen sulfide 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 sulfate (Beauchamp et al., 1984) although thiosulfate may also be isolated from tissues exposed to hydrogen sulfide (Kage et al., 1992). At physiological pH (pH-7.4), H_2S is approximately 69% ionized (HS^-). The metabolic pathway for H_2S is depicted in Figure 1.

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 converted 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 et al. (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 (22 minutes, approx.). 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). They 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 target 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) once for 3 hr and for 70 days (Dorman et al., 2002). The only target organ that demonstrated a clear dose response relationship with increased sulfide levels and concomitant decreases in cytochrome oxidase was the lung examined at the end of the single 3-hour exposure; statistical significance was achieved at 80 ppm (111 mg/m^3) and above for increases in sulfide and at 30 ppm (42 mg/m^3) and above for decreases in cytochrome oxidase. Sulfide concentrations in the lung decreased to preexposure levels within 15 min of the end of exposure. The response was not seen in the lung after the 70-day repeated exposure. Hindbrain sulfide concentrations and cytochrome oxidase activities were unaffected by single or repeated exposures. 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) although sulfide levels did

not appear to be decreased until 400 ppm (556 mg/m^3) H_2S. These results indicate that cytochrome oxidase is a sensitive biomarker of effect for H_2S exposure (more so than sulfide levels) for both lung and nasal epithelial tissues, but only for short term exposures.

Methylation has also been reported as a possible metabolic pathway for 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 dimethylsulfide. 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 et al. (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. When incubated with H_2S, cecal tissues 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. The 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 exogenously-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 heme proteins such as 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 i.p. 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.

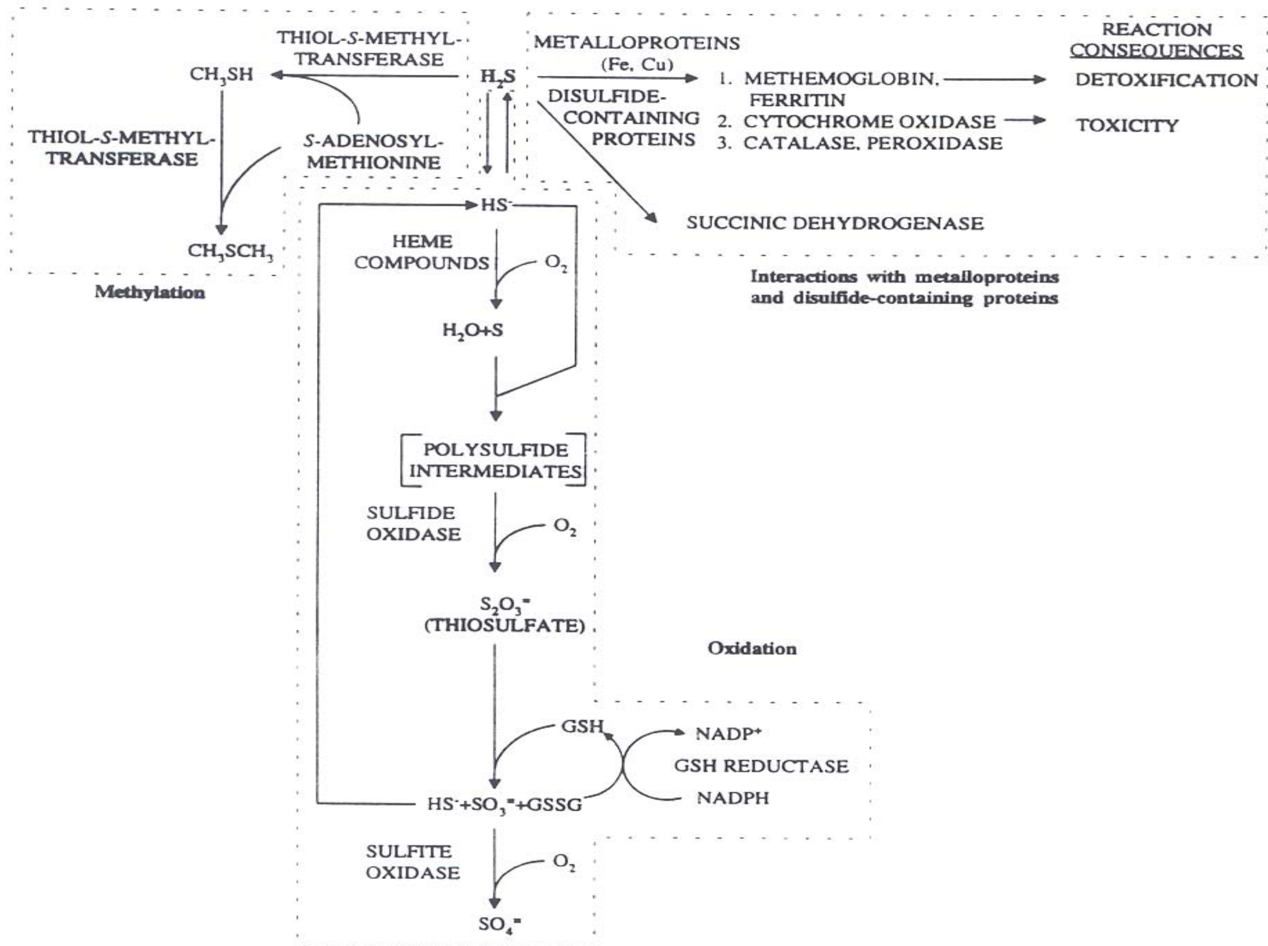

Figure 1. Metabolic pathways of hydrogen sulfide.

Source: adapted from Beauchamp et al., 1984.

3.3. DISTRIBUTION

Forensic autopsies of three men who were overcome by H_2S (estimated concentration 767-906 mg/m^3 or 552-652 ppm) 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. At 24-hours after death, blood sulfide was measured at 0.5, 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 (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 et al. (1996) also reported detectable concentrations of sulfide in the blood, lung, brain, liver, and kidney of a worker overcome by H_2S. 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 et al. (1991) exposed male Wistar rats to 75 ppm (104 mg/m^3) for 20, 40, or 60 min. Following exposure, animals were sacrificed and blood and tissues (brain, lung, heart, liver, spleen, and kidney) were collected within five min 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 exposure duration.

3.4. ELIMINATION

Urine is the primary route of elimination following H_2S 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 postexposure. Kage et al. (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

On December 14, 2001, after internal peer review of this document, the Agency articulated its interim policy on the use of third-party studies submitted by regulated entities (U.S. EPA, 2001). For these purposes, EPA is considering "third party studies" as studies that have not been conducted or funded by a federal agency pursuant to regulations that protect human subjects. Under the interim policy, the Agency will not consider or rely on any such human studies (third-party studies involving deliberate exposure of human subjects when used to identify or quantify toxic endpoints such as those submitted to establish a NOAEL or NOEL for systemic toxicity of pesticides) in its regulatory decision making, whether previously or newly submitted. Some of the supporting studies discussed in this Toxicological Review are third-party studies. The studies cited provide data which suggests and inform a public health concern for hydrogen sulfide, but were not designed or used as principal studies in the derivation of any quantitative value for hydrogen sulfide based on NOAELs or LOAELs. The Agency is requesting that the National Academy of Sciences conduct an expeditious review of the complex scientific and ethical issues posed by EPA's possible use of third-party studies that intentionally dose human subjects with toxicants to identify or quantify their effects.

Recent reviews of the health hazards associated with H_2S exposure and subsequent treatment include Milby and Baselt (1999a) and Guidotti (1996). Earlier reviews of the health effects were provided by Glass (1990), Reiffenstein et al. (1992), and Mehlman (1994). 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). These reviews indicate that the typical "rotten-egg odor" of H_2S is an inadequate warning indicator of exposure since levels in the range of 100-200 ppm (140-280 mg/m^3) can lead to loss of smell followed by olfactory paralysis (Reiffenstein et al., 1992). These authors estimate the odor threshold of H_2S in the range of 3 to 20 ppb (0.004 to 0.03 mg/m^3). The case reports cited below involving overexposure have documented a variety of central nervous system (CNS) transitory symptoms, such as dizziness, nausea, headache and others more long-acting such as abrupt physical collapse or "knockdown," all of which have been attributed to direct effects of H_2S on the brain (Milby and Baselt, 1999b). Prolonged unconsciousness can lead to respiratory failure, hypoxia, and death. In the reviews cited above, levels associated with "knockdown"have been estimated to be in the range of 500-1,000 ppm (695-1,390 mg/m^3). Levels associated with pulmonary edema have been estimated to be in the range of 250-500 ppm (348-695 mg/m^3). A rather extensive list of acute symptoms of intoxication resulting from an accidental release of H_2S in an industrial setting was provided by Kleinfeld et al. (1964).

The following case studies describe exposures to a number of volatile and semivolatile compounds in addition to hydrogen sulfide (e.g., liquid manure) whereby the actual toxic agent is unclear. The point of these descriptions is that exposure to high concentrations of hydrogen sulfide, even for very brief periods, can have life-threatening consequences involving respiratory arrest and secondary CNS effects as well as persistent neurological sequelae.

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, dyspnea, 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 one 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 two weeks in the hospital, his chest radiograph showed improvement and his lung function was normal except for a slightly reduced maximum midexpiratory flow rate. Six months after the accident, he had a normal chest radiograph and was asymptomatic. Blood sulfide levels of the two individuals who had fatally aspirated manure were 5.0 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 while blood samples from random autopsy cases of badly decomposed subjects did not exceed 0.4 mg/L. No blood sulfide measurements were performed on the 41-year-old survivor. Eight days following the accident, air analyzed from the liquid manure storage tank contained 6,360 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 gas in a lavatory facility at his place of work (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 attributed to the inhalation of a "toxic gas," with H_2S suspected as a major component.

Several case studies report on 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 also died during a rescue attempt. Both bodies were deeply cyanosed. A fatality, apparently due to release of H_2S from a load of refinery waste was described by Simson and Simpson (1971). This report also mentioned eyewitness accounts of intense central cyanosis in the victim.

At a poultry feather fertilizer plant, a worker was exposed to H_2S while attempting to repair a leak and was killed (Breysse, 1961). Hydrogen sulfide was created as a byproduct of the putrefaction of the feathers, and was eliminated through a pipe leading to a sawmill 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 4,000 ppm (5,560 mg/m^3) during the cooking and putrefaction of 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. Postmortem examination revealed that he had cerebral edema. A week later, H_2S was measured 30 cm below the manhole of the tank and was found to exceed the upper limit of detection of the monitoring equipment at 150 ppm (208 mg/m^3).

By contrast, Milby (1962) has reported two cases of severe, but non-fatal H_2S poisonings. While disposing of a H_2S-filled gas cylinder and the subsequent release of its contents, two men that were exposed to the gas collapsed immediately and underwent convulsions while unconscious. Upon being rescued from the gas, the men required artificial respiration to begin breathing on their own. Both men were hospitalized in an unconscious state and were later revived. At the conclusion of their hospital stay, both men were without symptoms. 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 of a municipal sewage pumping station, leading to the death of a police officer attempting to rescue an unconscious worker and persistent neurological sequelae in at least one other individual (Snyder et al., 1995). Complications included: decreased ability to communicate, slow speech, marked visual memory impairment with poor acquisition, and difficulty retaining and recalling new information. The findings were reported as essentially unchanged 12-18 months after exposure. Physical findings in affected individuals were limited to pharyngeal and conjunctival erythema and corneal abrasions. Additional reports associated H_2S exposure (via sewer gas) with lung function and neurological decrements and, in certain studies, deaths were reported by Watt et al. (1997) and Hall and Rumack (1997).

Tvedt et al. (1991a) described the follow-up of six H_2S-exposed patients who lost consciousness and experienced signs of long-term neurological sequelae associated with hypoxic brain damage. Immediate symptoms included cyanosis, pulmonary edema, seizures, and coma. Delayed symptoms (5-10 year follow-up) ranged from worker disability due to neurological symptoms to brain damage severe enough to qualify as dementia in one individual. Five patients who had been unconscious in an H_2S atmosphere for 5-20 minutes showed persistent impairment with memory and motor function most affected. The two patients with the most serious symptoms developed pulmonary edema. No measures of H_2S in air, blood or urine were reported. The investigators concluded that the outcome of acute exposures can be variable and that neurological symptoms may not become apparent until a considerable period of time has elapsed.

Tvedt et al. (1991b) also described delayed neuropsychiatric symptoms in an individual who became unconscious for 15-20 minutes during an H_2S exposure. He regained consciousness after two days, but then deteriorated again and remained comatose for a month. Upon waking up, the individual was amnesiac, nearly blind, had reduced hearing and ataxia. At follow-up (five years later) he had not been able to resume work and evidenced slight motor, memory and visual impairments and clinical indications of slight cerebral atrophy. Electroencephalogram and evoked responses were normal at this time.

Symptoms similar to those described by Tvedt et al. (1991b) were also reported by Kilburn (1993) in an oil well tester rendered semiconscious from exposure to high levels of H_2S. Follow-up (39 and 49 months) symptoms suggested damage to the brain stem, basal ganglia, vestibular apparatus, cortex, and other brain structures. There was profound impairment of cognitive function, memory, visual perception and coordination, intelligence and neurophysiologic functions. Malingering was ruled out by the reproducibility of performance during testing sessions.

Persistent neurocognitive impairment from H_2S poisoning was also reported by Wasch et al. (1989). Three cases are described with 2/3 individuals experiencing unconsciousness during exposure. All three individuals had moderate to severe attention and concentration deficits, as well as varying degrees and types of memory, learning, and perceptual deficits and prolonged abnormal event-related potentials (P-300 latency). In one individual, symptoms persisted at follow-up three years after exposure.

In another study, 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 four months, and the maximum recorded level in family's housing scheme was 0.6 ppm (0.8 mg/m^3). 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. This study is discussed at length in Section 4.7.1., Possible Childhood Susceptibility.

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). Results of the survey were compared to health effects in three reference communities not exposed to H_2S. Symptoms were elevated in both exposed communities compared to the referent populations in: CNS effects, respiratory effects, and blood (e.g., anemia, clotting, etc.). Although the odds ratios were elevated over controls, they fell within the confidence intervals. In addition, respondents from the two exposed communities 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 nonexposed 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 nonsmoking subjects, sewer workers were only able to attain 89% of the predicted FEV_1/FVC value compared to 98% in water treatment workers. Although the study author states that chronic low level exposure to H_2S may be associated with decreased lung function, 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 H_2S exposure were assessed in 175 workers who extracted and processed oil and gas (Hessel et al., 1997). The workers received a questionnaire concerning sour gas exposures that caused symptoms or loss of consciousness. Thirty-four percent of the respondents indicated that they had exposures serious enough to cause symptoms, and 8% of the 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. While spirometric values were also not affected in workers that lost consciousness, the workers experienced shortness of breath during physical activity, wheezing with tightness in the chest, and wheezing attacks. The investigators stated that these symptoms are consistent with bronchial hyperresponsiveness. The data also demonstrate the refractiveness of the lung to the effects of H_2S, with lung function parameters being unchanged despite unconsciousness from H_2S exposure.

A cross-sectional pulmonary function study 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. Hydrogen sulfide levels were determined using passive diffusion dosimeters worn by the workers (n = 30 in exposed and matched control workers). The workers had been

exposed to H_2S for an average of 12.3 years. Occupational exposure levels in the 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 the results from the pulmonary function tests were observed in exposed compared to nonexposed workers. Also, no significant differences were noted in the pulmonary function tests taken at the beginning and ending of work shifts.

In addition to the one-workday study, a cross-sectional study was undertaken on 115 exposed workers and 209 nonexposed workers. The subjects underwent forced expiratory flow-volume tests, but were not monitored for exposure. The investigators (Higashi et al., 1983) concluded 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 nonexposed metal, plastic, and starch workers was conducted in which subjects were questioned about eye irritation complaints (Vanhoorne et al., 1995). Exposed and control workers were employed for at least one year by their 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. Workers exposed to H_2S also 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 exposed to CS_2 and H_2S including: pain, tension, burning, hazy sight, photophobia, and irritation. While the percentage of complaints increased with exposure, 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* of evidence suggests that H_2S is responsible. Considering all of the evidence, the investigators further suggested that CS_2 enhances the eye irritation properties of H_2S.

A study by Kilburn and Warshaw (1995) included 13 former workers and 22 near-plant residents of a desulfurization unit within a refinery processing "sour' crude oil. Air monitoring for H_2S and other chemicals at street level near the subjects' homes during one week in July 1990 revealed that the H_2S concentration was 0.01 ppm with periodic peaks of 0.1 ppm along with mercaptans (0.002 ppm), ethane (0.5 ppm), propane (0.5 ppm) and unquantified levels of vanadium and thiodiglycolic acid. In addition, dimethylsulfide, and vanadium (as vanadium pentoxide) 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 H_2S, carbon oxide sulfide (COS: 6 to 52 ppm) and for total reduced sulfur 6 to 71 ppm.

Questionnaires were completed by subjects and controls. Neurobehavioral functions and a profile of mood states were compared to 32 controls, matched by age and educational level. Neuropsychological functions were also evaluated, including: two-choice reaction time, balance,

color discrimination, digit symbol, trail-making A and B, and immediate recall. Abnormal mean values of exposed subjects compared to controls were determined to be statistically significant for these functions. Mood state scores were found to be much higher in the exposed subjects than the controls. However, many of the neurophysiological findings had large standard deviations and consequently were highly-variable. While alcohol use was excluded as a confounder, all of the ex-workers had been exposed to solvents. Although the authors concluded that long-term exposure to "low doses" of H_2S is a plausible cause of the neurological function deficits, they acknowledged that other effects were attributable to other chemicals that were 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, the few numbers of individuals studied and the very limited exposure measurements - it is premature to attribute the deficits cited with H_2S exposure.

Neurobehavioral effects of H_2S were studied in 16 individuals (male and female, ages 21 to 68 years) 2 to 22 years after exposure (Kilburn, 1997). Five individuals had been unconscious after exposure and four had downwind exposures. The exposure level was not reported in any of the cases. Five of the 16 individuals were categorized in the "chronic" exposure group, exposed for 11-22 years. Five were in the "acute" exposure category having been exposed for minutes and the remaining six were in the "hours" category. Twelve of the 16 individuals were plaintiffs in lawsuits. Results from a battery of tests were compared to those from 353 national referents, matched by age, sex, and years of education. Smoking status was not a significant factor. Neurophysiologic and neuropsychologic tests, the latter adjusted for educational level, were employed. Permanent neurobehavioral impairment was apparent in all 16 subjects. Brief high doses were "devastating," whereas protracted low doses of individuals in the chronic category showed effects ($p<0.05$) on the more sensitive neurobehavioral tests, such as balance, visual fields, and choice reaction time. There was no indication of bias from the lawsuit and it was considered unlikely that the symptoms could be attributed to other causes. Kilburn (1997) concluded that moderate occupational exposure and downwind environmental exposure can cause permanent impairment. This finding was assessed in a subsequent study.

Neurobehavioral deficits attributed to low-level environmental exposure of 103 individuals to H_2S were reported by Kilburn (1999). The neurobehavioral battery included 28 tests. One group of 24 homeowners was exposed to H_2S that had collected in crawlspaces and under concrete foundations. Concentrations ranged from 0.1 to 1.0 ppm with several peaks up to 5 ppm during a two-month period in 1996. The homeowners were assessed about one year later. They exhibited abnormal balance, color discrimination, grip strength and delayed verbal recall. It was not stated if the exposure was ongoing at the time of the neurobehavioral assessment. In another group, 48 adults were exposed to measured peak levels ranging from 1 to 20 ppm at street level during a refinery explosion and fire in 1992. They were assessed for

neurobehavioral deficits four years later in 1996. The subjects had 11-12 abnormalities compared to 4 in the first group. A third group consisted of 16 patients with a wide range of exposure who were assessed after latent periods of 1.7 to 22 years [these were the same individuals discussed in the preceding paragraph by Kilburn (1997)]. A fourth group consisted of 13 workers and 22 residents living at or near stack gas emissions from a refinery and desulfurization plant. These individuals were also exposed carbon oxide sulfide (2.6 - 52.1 ppm), mercaptans (1.0 - 21.1 ppm), and 24-hr levels of H_2S at 0 to 8.8 ppm. Referents (357) were selected from four towns (in four states) free of known chemical contamination. Analysis of variance and covariance adjusted for differences in age, education, gender, and height. Kilburn (1997) concluded that peak dose rather than duration of exposure is predictive of effects, and those exposed to nonlethal levels do not recover completely from H_2S. Given the small number (24) of homeowners in the first group exposed to low levels and the type of deficits observed, it is premature to ascribe cause and effect to H_2S.

A five-year retrospective study (Arnold et al., 1985) of 250 workers exposed to H_2S considered compensation claims submitted from 1979-1983. Fifty-four percent of the workers became unconscious after exposure. Of the clinical findings, neurologic, respiratory and opthalmologic ailments predominated. The overall fatality rate was 2.8%.

In conclusion, H_2S exposure levels that result in temporary unconsciousness (e.g., 15-30 minutes) can cause profound neurophysiological, neurobehavioral, neurocognitive, neurophysical, respiratory, and opthalmologic deficits that are persistent. While some of these deficits can occur when the exposed individual remains conscious, it is less clearly established if intermittent or continuous exposure to “low levels” of H_2S cause similar persistent symptoms.

4.2. PRECHRONIC AND CHRONIC STUDIES IN ANIMALS

4.2.1. Oral

Wetterau et al. (1964) examined the effects of H_2S in chickens and pigs that were fed dried green animal fodder. The fodder had been dried by sulfur-containing brown coal or other fuels containing H_2S. The 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 decreased weight gains of 4 and 22%, respectively, compared with the control group. No statistical analysis was presented. Food intake 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 was suddenly changed to diets containing 24% treated dried feed.

In a separate experiment, 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 may have been caused by the sudden change in diet rather than 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. Decreased weight gain observed in the 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 suggested that animals refused to eat food containing H_2S, which may explain the decreased feed intake and the subsequent decreased weight gain observed in the study. Results of the study were likely caused by food avoidance from odor rather than toxicity.

A study by Wetterau et al. (1964) was used previously on IRIS as the basis for deriving an RfD. However, the low concentration of H_2S in the feed, estimated at only about 15 mg/kg-day, coupled with the investigators' conclusions that the diets did not cause adverse or reproducible health effects in pigs precludes the use of this study in the derivation of an RfD in this current assessment.

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. Hydrogen sulfide doses were prepared by purging deoxygenated deionized water with pure H_2S gas. Prepared H_2S solutions 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, kidney, spleen, gonad,

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. Although absolute organ weights were not affected by treatment, relative heart weights increased ($p < 0.05$) in high-dose males, and relative kidney weights 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 an RfD. For example, 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 nongavaged, 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 drawing 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/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 before the first exposure. A final fasted body weight was determined for all animals just before necropsy. Each animal also received an ophthalmological exam before the first day of exposure and within seven 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, 95, and 99. 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 (four levels), trachea, testes, epididymis, esophagus, thymus, prostate, seminal vesicle, and gross lesions.

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 five and six, respectively. Both sacrificed animals exhibited prostration and/or hypoactivity prior to sacrifice. Both animals also had the same black discoloration of the glandular stomach as those in the control group. 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 the use of anterior appendage with the appearance of paralysis while one 30.5 ppm (42 mg/m^3) female and a 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 the nasal tract as the only site where lesions were 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. The lesion was also observed in two 80 ppm (111 mg/m^3) mice 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 (8/9) and female (7/9) animals exposed to 80 ppm (111 mg/m^3) H_2S. The lesions, judged as minimal to mild in severity, were localized primarily in the squamous portion of the nasal mucosa but extended to regions of ciliated respiratory-type epithelium. Since H_2S is 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 (1983a) mouse study were used in a similar study using Fischer 344 rats (CIIT, 1983b). Following a 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. In 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. On the following day, the right and left sciatic nerves and the 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. After staining, 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. Although elevated sulfhemoglobin levels were also observed in females exposed to 30.5 or 80 ppm (42 mg/m^3 or 111 mg/m^3) H_2S, 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. 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 the nasal mucosa and nasolacrimal duct was observed in all rats including the controls. There was also minimal to moderate inflammation of sections I and II of the nasal mucosa, but this effect was not dependent on dose with 45, 40, 75, and 55% 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 depressed body weight gain 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,b), including the special neuropathologic studies in CIIT (1983b) were used in the following 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) H_2S during weeks 1 through 3, and in 80 ppm-treated females exposed throughout the 90-day 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, and there were no H_2S-related lesions in animals selected for the 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 also present in 5 treated rats. However, this effect was not dose-dependent. Organ weights were statistically similar between control and exposure weights, excluding 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; a finding concordant with the results of the CIIT (1983b) study with Fisher 344 rats. The investigators also suggested that the decreased brain weight was due to the chemical nature of H_2S, e.g., 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 in sections III and IV of the nasal mucosa and nasolacrimal duct was observed in all rats, including the controls. Although there was also minimal to moderate inflammation in sections I and II of the nasal mucosa, the 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 (42 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 hr/day, 5 days/week for 7 weeks. At the start of the week 8, dogs were coexposed to 15 ppm (20.8 mg/m^3) H_2S and 10 ppm carbon disulfide. During week 9, dogs were only exposed to 15 ppm (20.8 mg/m^3) H_2S which was increased to 30 ppm (42 mg/m^3) in 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 the investigators determined these parameters was described as "time to time." All measured endpoints were compared to control values that were established during a 4-week preexposure 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 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. Coexposure 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 et al. (2000) exposed 10-week-old male Sprague-Dawley CD rats (12/exposure group) to 0, 10, 30, or 80 ppm (0, 14, 42.7, or 111 mg/m^3) H_2S for 6 hr/day, 7 days/week for 10 weeks. At the end of the 10-week exposure period, animals were euthanized with CO_2 and the noses of the animals were dissected free. The nasal cavities were sectioned at 6 different levels such that sections 3-6 each allowed for histological evaluation of transitional /respiratory and olfactory epithelium. The lesions noted were limited to olfactory tissue, i.e., olfactory neuron loss and basal cell hyperplasia at the olfactory mucosa, and were graded in severity by a subjective scale where 0 = normal, 1 = mild, 2 = moderate, 3 = marked, and 4 = severe.

Table 2 shows that no effects were observed in the control animals or in animals exposed to 10 ppm (14 mg/m^3) H_2S (Brenneman et al., 2000). Lesions, limited to the olfactory mucosa, were observed in animals exposed to either 30 (427 mg/m^3) or 80 ppm (111 mg/m^3) H_2S. These olfactory 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 incidence, mean severity, and distribution of the exposure-related lesions increased in a concentration-dependent manner.

to moderate olfactory neuron loss and mild basal cell hyperplasia mainly affecting the nasal septum, dorsal nasal cavity, and marginal ethmoturbinate were observed in both exposure groups. The nasal septum 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 in the 80 ppm (111 mg/m^3) exposure group.

The critical effects in this study are nasal lesions of the olfactory mucosa, 30 ppm (42 mg/m^3) is the LOAEL and 10 ppm (14 mg/m^3) the NOAEL (Brenneman et al., 2000).

It should be noted that lesions in the nasal tract reported in other studies are not completely in agreement with those reported by Brenneman et al. (2000) where lesions were clearly related to dose and localized in the olfactory epithelium. In mice exposed to similar levels of H_2S (CIIT, 1983a), inflammation of the nasal mucosa was reported and described as occurring primarily in the squamous nasal mucosa and extending into the respiratory epithelium, with no specific mention of olfactory mucosal involvement. Sectioning of the nasal epithelium was through 4- rather than 6-areas examined by Brenneman et al. (2000) explaining, in part, this difference. Effects in the nasal cavity reported in Sprague-Dawley rats in an earlier study (CIIT, 1983c) differ in severity and location from those reported by Brenneman et al. (2000). Reasons for such a discrepancy 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 reproductive performance and 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 continued during a 2-week mating period and 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. Nonpregnant 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 prebreeding females. Feed consumption in presumed pregnant females

was recorded for 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 the 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 (111 mg/m^3) H_2S that was present throughout entire exposure period. 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 ppm (14 mg/m^3) and 80 ppm (111 mg/m^3) but not in the mid-dose of H_2S and a decrease in the relative ovary weight of females in the 10 ppm (14 mg/m^3) exposure group.

There were no statistically significant effects on reproductive performance (mating index, fertility index, postimplantation loss per litter, and number of late resorptions or stillbirths) in F_0 animals. Also, the number of live pups, litter size, average length of gestation, and average number of implants per female were not affected. There were no statistically significant effects on sperm production or morphology noted in F_0 males; 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). In comparing high-exposure and control animals, there were a few histologic diagnoses with a higher incidence in the high-exposure group, including: testicular tubular degeneration, intratubular sperm stasis, tubular mineralization, sperm granulomas, and multinucleated giant cells, degenerate sperm forms in the lumen, aspermia, and oligospermia. While the incidence of testicular tubular degeneration in high-exposures was higher (42%) than the controls (17%), statistical significance was not achieved. 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 females included one each of ovarian cysts and squamous metaplasia of the endometrium in the 10 ppm (14 mg/m^3) and 30 ppm (42 mg/m^3) exposure groups. The olfactory neuron loss and basal cell hyperplasia observed in the male rats in this study are reported elsewhere (Brenneman et al., 2000). The assignment of any effect level for reproductive toxicity is problematic since the study considered only 12 rats/sex/group and the study also lacked any statistical significance in either function or histopathology related to reproduction endpoints. In summary, the reproductive segment of this study did not demonstrate reproductive toxicity in either male or female rats following relatively high H_2S exposure of 80 ppm (111 mg/m^3) under repeated conditions (up to 70 days for males).

With regards to developmental effects from H_2S exposure, 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 was dose-related. There were no significant differences 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 groups were comparable to controls. Although a wide variety of gross observations were noted, they were not considered treatment-related by the investigators. Microscopic examination of six levels of the brain from pups from the control and high-dose group failed to demonstrate any histologic abnormalities.

Saillenfait et al. (1989) reported that the exposure of pregnant Sprague-Dawley rats 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. While there was a slight but significant decrease in fetal weight in all treated groups, 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 (50 ppm) 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 0, 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 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 that prenatal exposure to H_2S may eventually have upon the adult nervous system, early postnatal measurements provide indications of possible neurotoxic effects that may occur in adulthood. Hannah et al. (1989) exposed timed-pregnant Sprague-Dawley rats (number not reported) to 105 mg/m^3 (75 ppm) H_2S, 7 hr/day from day 5 postcoitus until PND day 21. Groups of 8 randomly-selected offspring, exposed *in utero* through PND 21, were sacrificed on PNDs 7, 14, and 21. The levels of putative neurotransmitter aspartate and gamma amino-butyric acid (GABA) were all significantly ($p<0.05$) depressed in

the cerebellum on PND 21. Glycine was unaltered. GABA is an inhibitory neurotransmitter of the central nervous system that appears in Purkinje cells such that decreases in GABA could be due to a decrease in the population of Purkinje cells. Analysis of cerebellum (in the 75 ppm animals only), however, established that this was not the case. There were no significant differences in the brain weights of treated pups compared to controls. The significance of these amino acid changes to neurobehavioral development is uncertain.

In a similarly designed study using the same exposure protocol, Hannah and colleagues (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 randomly-selected pups, exposed *in utero* through PND 21, were sacrificed on PND 7, 14, and 21. Significantly increased ($p<0.05$) serotonin levels were observed in the frontal cortex on postpartum day 21 in pups exposed to 28 mg/m^3 (20 ppm) H_2S, while increased ($p<0.01$) serotonin levels were observed in 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 increased ($p<0.05$) at 105 mg/m^3 (75 ppm) in the cerebellum on PNDs 7, 14, and 21, and in the frontal cortex on PND 21. At 28 mg/m^3 (20 ppm), frontal cortex norepinephrine levels were decreased compared to controls on days 14 and 21.

In a study designed to evaluate the perinatal effect of H_2S on developing cerebellar Purkinje cells, Sprague-Dawley dams were exposed to 28 or 70 mg/m^3 H_2S (20 or 50 ppm) for 7 hr/day from postcoital day 5 until PND 21 (Hannah and Roth, 1991). Control animals were exposed similarly, but without H_2S. Pups born to treated dams were also exposed perinatally up to PND 21 (Roth, 2002), at which time one pup was randomly selected from each litter for analysis, resulting in a total of 10 pups for each concentration and 10 pups from each of the control groups. Cerebella were processed using the Golgi method. One Purkinje cell from the pyramis of each cerebellum was digitized for morphometric analysis. Quantitative analysis of dendritic morphology is generally regarded as a traditional morphometric method appropriate to assess the effect of environmental influences, including chemical insult, on the developing brain. The authors used a vertex analysis (after Berry and Flinn, 1984) that can be used on a small number of nodal vertex types. This method leads to a determination of random versus nonrandom growth. Statistical evaluation was carried out using the Student's *t*-test. Exposure to both 20 and 50 ppm (28 or 70 mg/m^3) produced significantly longer segment length than controls over the low and middle branching orders, but not the higher orders. It was stated that segment lengths are typically increased when afferent fibers are experimentally reduced in number. The mean vertex path length (defined as a measure of the mean distance from any one equivalent order back to the axon hillock) was found to increase ($p<0.001$) in incremental steps in both treated groups compared to controls. When considered over the entire dendritic tree, these changes could alter the capacity of Purkinje cells to integrate afferent impulses and may be associated with an increased likelihood of an abnormal physiological response. Segment

branching was also found to be different in cells from the treated animals. There was a significant increase in branch number of the middle orders and a significant decrease in numbers over the higher orders (considered to represent the 'growth front').

The investigators considered this to be a compensatory response when there is restriction on the 'growth front'. The question of random versus nonrandom growth patterns was evaluated by examining the symmetry of the branching. It was found that vertex analysis indicated that the treated cells were undergoing a significant amount of nonsymmetrical or nonrandom growth, suggesting that H_2S affected the branching pattern. The resulting alterations in dendritic arborization appeared to be in response to H_2S exposure at the 20 and 50 ppm (28 or 70 mg/m^3) levels such that the 20 ppm (28 mg/m^3) level may be considered a low-effect rather than a no-effect level.

The significance of the findings in this and the Skrajny et al. (1992) studies to humans is unclear. Since alterations may constitute a chemically-induced change in the growth or organization of structural (or neurochemical) elements, they are to be regarded as possible indicators of a neurotoxic effect in accordance with guidance in EPA's neurotoxicity risk assessment guidelines (U.S. EPA, 1998). The question as to whether the alterations are adverse, however, remains unclear for several reasons. The effects reported by Hannah and Roth (1991), for example, are highly selective and could be due to environmental factors not directly related to exposure including variability resulting from the restricted sampling technique (i.e., one Purkinje cell/pup). Also, the relationship of these alterations to a functional impairment is unclear and cerebellar neurological sequelae are not the most obvious symptom associated with H_2S exposure. It is acknowledged, however, that predicting functional impairments from specific structural alterations such as reported by Hannah and Roth (1991) is difficult due to the selective nature of the observed alterations and the dynamic self-organizing response of the developing brain to injury.

The effect of H_2S on spermatogenesis in the rat was assessed by Andrew et al. (1980). 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, compared to a control or nonexposed group. Following the last exposure, each male was caged with two unexposed virgin females for 10 consecutive weeks. Four male rats were administered triethylenemelamine and used as positive controls. The females were sacrificed after 18 days cohabitation. Parameters evaluated included fertility, corpora lutea, total implants, and dead implants. There were no adverse effects of exposure on these parameters. Subsequently, the 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 days 1-18 of gestation, or days 7-11 or 12-16 (5 days total). All animals were sacrificed at day 21 and examined for dead implants, fetal malformations, and 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 under acute exposure conditions in a series of studies by Lopez et al. (1987). Fischer 344 rats were exposed to 0, 10, 200, or 400 ppm (0, 14, 278, or 556 mg/m^3) H_2S for 4 hours and then sacrificed at 1, 20, or 44 hours postexposure (Lopez et al., 1987). Bronchoalveolar lavage (BAL) and nasal lavage (NL) were performed on all animals. 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 hour postexposure that returned to normal at 20 hours postexposure. 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 postexposure groups, but these values returned to baseline by 20 hr postexposure. Cellularity was increased by 817, 501, and 73% at 1, 20, and 44 hours postexposure, respectively, for animals exposed to 400 ppm (556 mg/m^3) H_2S. Differential cell counts indicated that the increase in cellularity was initially due to desquamation of nasal epithelial cells (1 hour postexposure) 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 ppm (14 mg/m^3) and 200 ppm (278 mg/m^3) 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 hours postexposure. 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 significantly increased at all postexposure times, but LDH decreased toward baseline at the 20 and 44 hour postexposure times. In the 200 ppm (278 mg/m^3) exposure group, LDH activity in the BAL fluid was significantly decreased in the 44 hr postexposure group. In animals exposed to 400 ppm (556 mg/m^3) H_2S, AP initially significantly increased in the 1 hour postexposure group, but significantly decreased below baseline at 20 and 44 hours postexposure. Animals exposed to 200 ppm (278 mg/m^3) H_2S had AP levels below baseline at 20 and 44 hours postexposure. GGT was elevated (900%) in the 400 ppm (556 mg/m^3) 1 hour postexposure 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 hour postexposure, but which returned to normal at 20 hours and increased to 30% over baseline at 44 hours postexposure.

Pulmonary alveolar macrophages were significantly reduced at 1 hour postexposure in animals exposed to 400 ppm (556 mg/m^3) H_2S, but returned to normal by 20 hours postexposure. PMN increased in all postexposure times (highest at 20 hours) in animals exposed to 400 ppm (556 mg/m^3) H_2S. In the 10 ppm (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 (a cellular degenerative change often from accompanying viral infections), 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 hour postexposure 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 hours 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 postexposure (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 postexposure 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 et al. (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 a lack of structural changes in the

alveolar endothelium, basement membrane, or type I pneumocytes, suggesting 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 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 that fill the alveolar spaces in a retrograde manner.

Lopez et al. (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 hours. Animals were sacrificed at 1, 18, or 44 hours postexposure. 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 et al. (2000). At 44 hours postexposure, 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 et al. (1991) examined the effects of H_2S from the BAL of rats under the hypothesis that the hemorrhagic pulmonary edema observed from exposure to high levels of H_2S is associated with changes in pulmonary surfactant. Male Fischer 344 rats were exposed to 0, 200, or 300 ppm (0, 278, or 417 mg/m^3) H_2S for 4 hours, and at 1 hour postexposure BAL fluid was obtained. Marked abnormalities in surfactant activity were demonstrated in the lavagates from rats exposed to the highest concentration only 300 ppm (417 mg/m^3) and not in the lavagate from the lower dose 200 ppm (278 mg/m^3) or the controls. The lungs of high-dose animals showed areas of red atelectasis, patchy alveolar edema, and perivascular edema. These results are suggestive of a threshold for this surfactant response and subsequent histopathological progression.

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, most of the bacteria was inactivated by the 6-hour postchallenge sacrifice time. Rats exposed to H_2S for 2 hours responded similarly to controls. However, rats exposed to H_2S for 4 and 6 hours 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. The 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 hours.

Although the above studies are acute in nature (i.e., mostly 4 hr) and are not appropriate for the establishment of a chronic guideline value, the results may offer insights into the processes underlying affects observed in the respiratory tract tissues in animals exposed in a more protracted manner to H_2S such as by Brenneman et al. (2002).

Nasal tissues were examined for histology and cytochrome oxidase immunoreactivity in 10-week-old male CD rats 24 hours after exposure to 0, 30, 80, 200, or 400 ppm (0, 42, 112, 280, or 560 mg/m^3) H_2S for 3 hr/day for 1 day or for 5 consecutive days (Brenneman et al., 2002). The nose was histologically examined 24 hours after exposure, and lesion recovery was assessed at 2 and 6 weeks following the 5-day exposure. The single 3-hour exposure to $\geq$ 80 ppm H_2S resulted in regeneration of the respiratory mucosa and full thickness necrosis of the olfactory mucosa localized to the ventral and dorsal meatus, respectively. Repeated exposure to the same concentrations caused necrosis of the olfactory mucosa with early mucosal regeneration that extended from the dorsal medial meatus to the caudal regions of the ethmoid recess. Acute exposure to 400 ppm H_2S induced severe mitochondrial swelling in sustentacular cells and olfactory neurons, which progressed to olfactory epithelial necrosis and sloughing. Cytochrome oxidase immunoreactive cells were more frequently observed in regions of the olfactory mucosa commonly affected by H_2S than in regions that were not. These findings demonstrate that acute exposure to >80 ppm H_2S resulted in reversible lesions in the respiratory and olfactory mucosae of the CD rat and, further, that cytochrome oxidase activity (as judged from cytochrome oxidase immunoreactivity) may be a susceptibility factor for H_2S-induced olfactory toxicity in the CD rat.

Transmission electron microscopy of cultured rabbit alveolar macrophage (AM) cells exposed *in vitro* to 54 ppm (75 mg/m^3) for 24 hours revealed extensive damage to interior organelles and membrane resulting in a decreased percentage of AM capable of phagocytosis (Richardson, 1995). Khan et al. (1991) found evidence that H_2S exposure of F344 rats decreased *in vitro* respiratory rates of collected AM cells (zymosan-stimulated) and at the highest concentration decreased cell viability. Rats had been exposed to 0, 70, 280, or 560 mg/m^3 (0, 50, 200 or 400 ppm) for 4 hours before cell collection.

Moulin et al. (2002) correlated nasal pathology in H_2S-exposed rats with regions of high flux in the rat nasal cavity generated from anatomically accurate computational fluid dynamics (CFD). The specific location of lesions from nasal tract sections approximating sections 4 and 5 of a study of Brenneman et al. (2000) were noted and the predominately olfactory-lined portions of the perimeter of these 2 levels were divided into roughly similar size regions. CFD flux of

H_2S at these regions and the incidence of nasal pathologies within these regions were then calculated and correlated. The analysis showed that H_2S-related lesions were observed only at specific sites within the olfactory epithelium and that areas of respiratory epithelium were not affected. Further, the incidence, severity and distribution of lesions seen within the olfactory epithelium increased with concentration (approximately 50% of the olfactory epithelium affected at 30 ppm [42 mg/m^3] and 70% at 80 ppm [111 mg/m^3]) whereas the olfactory epithelium of rats exposed to 10 ppm (14 mg/m^3) and controls were unaffected. In contrast to the localization of nasal lesions in the olfactory epithelium, CFD simulations predicted higher flux (and therefore a higher dose) to those regions lined by respiratory epithelium where no H_2S-related lesions were observed. Region by region ranking of lesion incidence in the olfactory-lined epithelium with predicted flux revealed a high degree of correlation (Spearman's rank correlation coefficient, $p < 0.005$ for level 5 section) for the 30 and 80 ppm exposure groups.

Results from Moulin et al. (2002) suggest that olfactory epithelium is specifically sensitive to the effects of H_2S while respiratory epithelium is resistant. These results therefore lend credence to considering nasal toxicity from H_2S as relevant to humans having olfactory epithelium that can be impinged upon by inhaled air streams containing H_2S. The high correlation between predicted H_2S flux (a surrogate of dose) in the olfactory epithelium and nasal response (histopathology) indicate dose-response analysis should consider such information. Such procedures could be used, for example, to extrapolate dose-response relationships between animal models and humans to accommodate the differences between these species, i.e., the relatively large area of olfactory epithelium in areas of high flux in rats and mice (Kimbell et al., 1997) compared to the relatively small area of olfactory epithelium in areas of low flux in humans (Subramaniam et al., 1998). In considering this possibility, however, the authors caution that more refined estimates of necessary parameters such as nasal extraction efficiency would be required before such a procedure could be applied in a quantitative manner.

4.4.2. Neurotoxicity

Kombian et al. (1988) examined the acute effects of H_2S on amino acid levels in various brain regions of the rat following i.p. administration of 10 or 30 mg/kg doses. Hydrogen sulfide 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. Hannah et al. (1989; see Section 4.3) also reported depressed levels of these same neurotransmitters (save for glycine) in the cerebella of 21-day-old rats that had been exposed *in utero* and up to postnatal day 21 to airborne levels of H_2S at 105 mg/m^3 for 7 hr/day.

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 hours. The results indicate decreased brain protein synthesis. The authors suggested that the changes may have resulted from the inhibition of cerebral CytOx activity. The authors also noted that 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 the addition of 0.13μM Na_2S can inhibit CytOx and carbonic anhydrase. 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 CytOx 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 H_2S levels 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., approximately 500 ppm or 695 mg/m^3) has been associated with memory loss and varying degrees of learning and perceptual deficits (Kilburn, 1993; Tvedt et al., 1991a,b; 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 hr/day for 5 days. Results, recorded during the last 10 minutes of the exposures, indicated that repeated exposure to H_2S produced a dose-related increase in the amplitude of hippocampal theta activity the total of which was cumulative with exposure duration. The effect of H_2S on hippocampal EEG was dose-dependent and highly significant at all concentrations. The authors postulated that elevated catecholamine and/or serotonin levels 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 LOAEL for this study is 35 mg/m^3 (25 ppm). 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.

In an earlier study, Skrajny et al. (1992) examined the effect of H_2S exposure on serotonin and norepinephrine levels in the developing cerebellum and frontal cortex of Sprague-Dawley rats. Timed-pregnant rats were exposed to 20 or 75 ppm (28 or 104 mg/m^3) for 7 hr/day from day 5 postcoitus until PND 21. At parturition, treated and control animals were culled to 6 dams, with a maximum of 12 pups per litter with 10 pups in each group randomly selected for euthanization at PND 7, 14, and 21. The number of litters in each group of dams was not stated nor was there an indication if pups were selected from randomly selected litters. Exposure to 75

ppm (104 mg/m^3) resulted in a statistically significant ($p<0.01$) increase in serotonin in the cerebellum from PND 7 to 14, compared to controls. At PND 21, the value remained unchanged from PND 14. The increase was even more pronounced in the frontal cortex from PND 7 through 21 ($p<0.01$). The increase at 20 ppm (28 mg/m^3) in the frontal cortex was less pronounced than at 75 ppm (104 mg/m^3) and reached statistical significance only at PND 21 ($p<0.05$). There was no significant increase in the level in the cerebellum. Changes in norepinephrine were more difficult to interpret as there was no clear pattern of change. The investigators concluded that the effects of exposure on serotonin and norepinephrine, coupled with the laboratory's prior evidence demonstrating possible morphological changes in Purkinje cell development and the known influence of monoamines on neural development, suggest a mode of action for H_2S on the CNS.

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. Hydrogen sulfide was generated from the reaction of iron sulfide and hydrochloric acid. The exposure duration was 2 hours. 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 demonstrate that the neurological sequelae following exposure to high concentrations of H_2S is due to neuronal necrosis, Baldelli et al. (1993) administered sodium sulfide (Na_2S) to unventilated and anesthetized-ventilated (to compensate for the loss of central respiratory drive) male Wistar rats by i.p. 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 that were 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 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. None of the 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 one 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. Mean arterial blood pressure decreased 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 minutes 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 $\geq$ 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.

Three reports describe effects of H_2S on neurological function in rats (Partlo et al., 2001; Struve et al., 2001). Partlo et al. (2001) exposed adult rats to125 ppm (174 mg/m^3) H_2S for 4 hr/day for 5 days/week for 5 weeks. Controls were exposed to a nitrogen/air mixture. Overt signs of eye irritation, respiratory distress, behavioral dysfunction or impaired consciousness were not observed at any time during the experiment. Testing of the animals revealed that this level of exposure did not affect memory retention or acquisition, but did impair one measure of performance during the reacquisition of a reversed contingency (radial arm maze task). These data provide evidence of mild brain dysfunction following a prolonged period of repeated hydrogen sulfide at this exposure level. The authors discuss the prefrontal cortex as a potential target site. Struve et al. (2001) exposed adult 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/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, body temperature, and water maze performance (spatial learning); it is to be noted, however, that the latter result may have been confounded by the former. Pregnant Sprague-Dawley rats (12/group) were exposed to either 0, 10, 30, or 80 ppm (0, 14, 42, or 111mg/m^3) H_2S throughout gestation and postnatally to both dams and pups (8 rats/litter after culling) PND 5 - 18. No decrement in behavioral performance, as judged by testing for motor activity (conducted on PNDs 13, 17, 21, and 60), passive avoidance (conducted on PND 22 and 62), functional observational battery (conducted blind on PND 60), and acoustic startle response (conducted on PND 21 and 62), was noted in the offspring at any exposure concentration (Dorman et al., 2000).

4.4.3. Cardiovascular Toxicity

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 et al. (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 postexposure. 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, during the postexposure monitoring period. Electrocardiograms revealed that the P-Q intervals were longer during the exposure and postexposure periods, which suggests some disorder in stimulus transmission. No changes were noted in blood pressure either during or after exposure.

4.4.4. Ocular Toxicity

Hydrogen sulfide has previously been reported to affect the eyes in a condition called "sore eye" or "gas eye". Lefebvre et al. (1991) examined this effect by exposing male Fischer 344 rats to 560 mg/m^3 (403 ppm) H_2S for 4 hours or 2100 mg/m^3 (1511 ppm) H_2S for 4 min. 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 at very high concentrations.

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. Hydrogen sulfide 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 TA 102. The investigators

believed that the increased mutagenicity was due to H_2S-mediated formation of iron sulfide, which converts hydrogen peroxide to hydroxyl radicals more efficiently than ferrous iron.

4.5. EVALUATION OF MODE OF ACTION FOR NONCANCER EFFECTS

4.5.1. Mode of Action of Noncancer Effects

At low levels of exposure H_2S is an irritant such that effects on tissues-of-contact would be anticipated, either in laboratory animals or humans (Shusterman, 1992).

At high levels, neurotoxic effects have been reported. Neurotoxicity is thought to occur via the ventilatory centers of the brain that lead to secondary effects including respiratory paralysis, pulmonary edema and death. Some investigators interpret the potential mode(s) of action for H2S very differently, whether it be via a neurotoxic pathway or effects on the respiratory system.

The potential mode-of-action of the neurotoxicity of H_2S has been investigated. Studies 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 that, in part, may 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, while 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 (Warenycia et al., 1989b). It was concluded that the inhibition of MAO activities and increased neurotransmitter levels in the brainstem may be important in the loss of central respiratory drive after H_2S exposure.

On the other hand, 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), possibly by alterations in pulmonary surfactants (Green et al., 1991). Other investigators, using dogs, have suggested that sulfide was an irritant to the afferent endings of the pulmonary vagi, and that high amounts of sulfide tend to paralyze the ventilatory center (Haggard and Henderson, 1922).

Almeida and Guidotti (1999), on the basis of rat studies, suggest that the lung may be the primary locus of HS ion action that can lead to vagal paralysis. Khan et al. (1990) investigated the action of H_2S on a molecular level in the lung, in which lung mitochondria incubated with H_2S exhibited a dose-dependent inhibition of cytochrome oxidase (CytOx) activity. Exposure to