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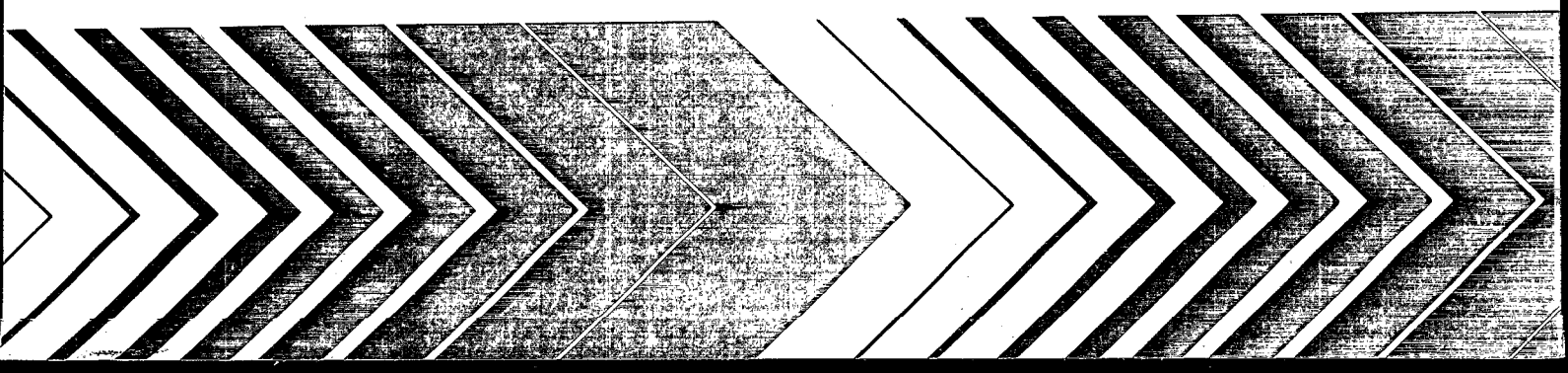
Health Assessment Document for Hydrogen Sulfide



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Health Assessment Document for Hydrogen Sulfide

Environmental Criteria and Assessment Office
Office of Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

DISCLAIMER

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

PREFACE

The Office of Health and Environmental Assessment has prepared this health assessment to serve as a source document for U.S. Environmental Protection Agency use.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been evaluated, and summary/conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified. The relevant literature for this document has been reviewed through July 1992. Observed effect levels and other measures of exposure-response relationships are discussed, where appropriate, so that the nature of the adverse health responses is placed in perspective with observed environmental levels.

Any information regarding sources, emissions, ambient air concentrations, and public exposure has been included only to give the reader a preliminary indication of the potential presence of this substance in the ambient air. While the available information is presented as accurately as possible, it is acknowledged to be limited and dependent in many instances on assumption rather than specific data. This information is not intended, nor should it be used, to support any conclusions regarding risk to public health.

ABSTRACT

Hydrogen sulfide (H_2S) is a highly toxic gas that is immediately lethal in concentrations greater than 2,000 ppm. Lethality appears to be due to anoxia in brain and heart tissues resulting from the interaction of H_2S with the cellular enzyme cytochrome *c* oxidase. Inhibition of this enzyme halts oxidative metabolism, which is the primary energy source for cells. Another toxic endpoint is the irritation of the mucous membranes, particularly those of the respiratory tract and the eyes. Pulmonary edema occurs at sublethal concentrations (250 to 500 ppm) in which sufficient exposure occurs before consciousness is lost. Pulmonary edema also has been reported after long-term exposure to levels as low as 50 ppm. Concentrations above 50 ppm can cause initial loss of coronary reflex, changes in visual acuity, and perception of blue or rainbow colors around lights, followed by very painful inflammation, with ulceration in severe cases. Subchronic studies with mice have shown that exposure to 80 ppm causes nasal lesions.

Olfactory sensation is lost at 150 to 200 ppm; hence, the characteristic odor of rotten eggs is not sufficient to warn of lethal exposure. At concentrations equal to or less than 150 ppm, symptoms such as the inability to think logically and incoherence have been reported. Recovered victims of exposure report neurologic symptoms such as headache, fatigue, irritability, vertigo, and loss of libido. Long-term effects are similar to those caused by anoxia due to exposure to other toxic agents such as carbon monoxide. Hydrogen sulfide is not a cumulative poison. No mutagenic, carcinogenic, reproductive, or teratogenic effects have been reported in the literature.

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1. SUMMARY AND CONCLUSIONS

1.1 BACKGROUND INFORMATION

Hydrogen sulfide (H_2S) is a colorless gas. At low concentrations it has a characteristic obnoxious odor similar to that of rotten eggs. Its molecular weight is 34.08, and it is heavier than air. It is flammable in air, can explode, and can be ignited by static discharge. It burns with a pale blue flame, and its combustion products are sulfur dioxide and water. Hydrogen sulfide is the only thermodynamically stable, binary, sulfur-hydrogen compound that occurs frequently in nature; because of its relative lack of hydrogen bonding, it is a gas under normal conditions. It is soluble in water and in a number of organic compounds.

Produced in nature primarily through the decomposition of organic material by bacteria, H_2S is also a constituent of natural gas, petroleum, sulfur deposits, volcanic gases, and sulfur springs. Such natural sources constitute approximately 90% of the air emissions of H_2S, which have been estimated to be 90 to 100 million tons annually.

Industrial sources and other anthropogenic activities contribute about 10% to the total air burden of H_2S. In the United States, 125,000 employees in 73 industries are potentially exposed to H_2S, according to the National Institute of Occupational Safety and Health. The gas is used mainly as an intermediate and reagent in the preparation of other compounds of reduced sulfur. It is also a by-product of many industrial processes that release it into the atmosphere. Generally, it is not found in high concentrations in the ambient air. Occasional catastrophic releases in processing and transport have exposed the general public to concentrations high enough to elicit toxic symptoms and death.

Hydrogen sulfide is oxidized by photochemically-generated free radicals, especially by hydroxyl radicals. It has a half-life in air typically ranging from 12 to 37 h, but this varies depending on the presence of photoactive pollutants and temperature, so that seasonal and geographic differences in concentrations are found. The half-life in air can exceed 37 h during very cold and dry winter conditions.

Ambient levels of H_2S measured over short time intervals (e.g., 1 to 8 h) tend to be low, in the range of 0.001 mg/m^3 (0.00072 ppm). Pollution episodes have reached levels of nearly 0.5 mg/m^3 (0.358 ppm) in severe cases, and accidental releases such as well blowouts

have produced levels as high as 14.3 mg/m^3 (10.26 ppm). At least one release in Poza Rica, Mexico, emitted lethal levels of gas.

Ecologic effects have been studied primarily with bacteria and naturally generated H_2S (i.e., geothermally produced). Ambient levels generated by anthropogenic sources are well below those known to cause symptoms of injury to higher plants. Hydrogen sulfide can act as a nutrient sulfur source in sulfur-deficient plants. Hydrogen sulfide in water, generated through decay, can be damaging to plants such as rice. Fish can be injured by high sulfide levels; the toxicity is similar to that shown in mammals, including humans. However, several marine macroinvertebrates are capable of tolerating long exposures to high concentrations of H_2S. This is explained by two protective mechanisms, one described in the tubeworm, *Riftia pachyptila*, in which a sulfide-binding protein acts as a sulfide trap and prevents it from spontaneous oxidation and subsequent inhibition of the respiratory chain enzymes, and the second described in the clam, *Solemya reidi*, in which the mitochondria possess a capability to oxidize sulfide to thiosulfate. The latter can function as an energy source for the bacterial symbionts in the host clam gill tissue.

Effects on wildlife have not been demonstrated from ambient H_2S levels, although high levels from accidental releases can be lethal.

1.2 METABOLISM AND TOXICITY

Hydrogen sulfide is an extremely toxic gas and is a leading cause of sudden death in the workplace. The cellular mechanism of toxicity is like that of cyanide, reversibly inhibiting the respiratory enzyme, cytochrome *c* oxidase.

Absorption of H_2S through the skin is limited, but absorption through the nasal and lung mucosa occurs readily. Hydrogen sulfide is not considered to be a cumulative poison, since it is fairly rapidly oxidized to sulfates and excreted by the kidneys. Thiosulfate is also a product of H_2S oxidation. Hydrogen sulfide is distributed to various organs such as the lung and the brain.

The immediate effect of inhaling H_2S at concentrations of 1,000 to 2,000 ppm (1,390 to 2,780 mg/m^3) for a few minutes are unconsciousness and respiratory paralysis, which may lead to death due to inhibition of the respiratory center of the brain. Inhalation of only 1 or

2 breaths of air containing 5,000 ppm (7,000 mg/m^3) H_2S causes unconsciousness. At concentrations of 500 to 1,000 ppm (695 to 1,390 mg/m^3), respiratory paralysis is preceded by a period of rapid breathing or hyperpnea, and death will result unless the victim is removed from the contaminated area and given artificial ventilation.

At concentrations between 250 and 500 ppm (347 to 695 mg/m^3), the gas is extremely irritating to the mucous membranes of the respiratory tract and the eyes. Pulmonary edema, which can be life-threatening, almost always occurs. Prolonged exposure to the gas at concentrations above 50 ppm (70 mg/m^3) can result in pulmonary edema, although dryness and inflammation of the epithelia of the entire respiratory tract are more common. The epithelia of the eye, especially of the conjunctiva and the cornea, are similarly affected, resulting in "sore eye" or "gas eye" characterized by inflammation, lacrimation, and mucopurulent exudate; in some cases, permanent scarring of the cornea occurs after ulceration.

It is a fallacy to assume that the obnoxious odor of H_2S (like that of rotten eggs) will give warning of the presence of the gas; this occurs only at low concentrations. The odor threshold in humans is low (0.1 to 0.2 ppm; 0.14 to 0.28 mg/m^3), but at levels of 150 to 250 ppm (208 to 347 mg/m^3), the olfactory sense is lost. Those recovering from potentially lethal exposures recall either no smell at all or a "sweetish" smell before losing consciousness. Similarly, it should not be assumed that pain from the irritant effect, especially in the eyes, will warn of dangerous exposure, since the gas anesthetizes the nerve endings in these mucous membranes.

The levels of gas that produce these severe effects generally have not been encountered in the ambient air or even in the workplace. Limited ambient air-monitoring data for various U.S. locations, obtained prior to 1965, indicated maximum concentrations of less than 1 ppm (1.4 mg/m^3) when measured over short time intervals (e.g., <8 h) (see Table 6-1). Routine measurements of the concentrations of H_2S in ambient air were not made by the National Air Sampling Network, and more recent monitoring information, which could aid in establishing current ambient exposure levels, does not exist in the published literature.

It is during only catastrophic releases or failures of containment processes that the public is exposed to the high concentrations of H_2S gas (>50 ppm; 70 mg/m^3) that have been associated with chronic or acute pathological changes. During such accidents, there is

often loss of life. Such an accident occurred in 1950 in Poza Rica, Mexico, when a flare burning off H_2S at a natural gas desulfurization plant failed. The nearby community was inundated with gas for 20 min. As a result, 320 people were hospitalized; of these, 22 died. After the Lodgepole, Canada, gas well blowout, ambient exposure levels of gas reached 15 ppm (21 mg/m^3), and the exposed population complained of eye and respiratory irritation. No long-term effects were recorded, and affected people and animals recovered completely.

Physicians reporting on recovered victims indicate that neurological and cardiological lesions persist after high-level exposure, but no clear-cut sulfide toxicity has been implicated. The damage has not been differentiated from that which occurs as a result of anoxia or ischemia of the brain or heart. While there are also clear indications of damage to the eighth cranial nerve and its associated central nervous system (CNS) connections, manifested as disturbances in balance and gait, these too may be the result of anoxia rather than direct sulfide toxicity.

The available literature contains insufficient human data on chronic exposure to low-level concentrations of H_2S. However, a health survey conducted by Dales et al. (1989) on Canadian residents living downwind from two natural gas refineries at Pincher Creek, Alberta, Canada, clearly indicated the health hazards associated with chronic low-level exposure to sour gas, which may contain very high H_2S levels. Children showed respiratory symptoms, whereas no physiological changes were noted among adults.

The effects attributed to chronic low-level exposure (<10 ppm; 14 mg/m^3), such as headache, fatigue, dizziness, irritability, and loss of libido, may also result from single or recurring high-level exposures. Other workplace factors such as high humidity, temperatures, noise levels, and work-shift effects have not been ruled out.

Animal toxicity studies showed that acute and repeated exposures to H_2S can affect various tissues, such as brain, lungs, nose, and heart. In a 90-day inhalation study B6C3F1 mice were exposed to levels of 10, 30, and 80 ppm (14, 42, and 111 mg/m^3) H_2S. The only exposure-related histopathological lesion was inflammation of the nasal mucosa in animals from the high exposure group. A similar study with Sprague-Dawley and Fischer-344 rats at levels of 10, 30, and 80 ppm (14, 42, and 111 mg/m^3) revealed no histopathological abnormalities of the nasal tract. In female Sprague-Dawley rats of the high-exposure group,

mean body weights were <90% of controls and brain weight was significantly reduced in males of this group. These observations suggest that 80 ppm (111 mg/m^3) is a LOAEL.

Farm animals exposed to 10 to 15 ppm (14 to 21 mg/m^3) H_2S during the Lodgepole gas well blowout in 1982 experienced nasal and eye irritation, coughing, decreased food intake, diarrhea, and bloody stool and urine. Similar effects were seen in cattle and horses during the Drummond gas well blowout near Claresholm, Alberta, Canada, in 1984. No data were available on chronic exposure of farm animals to H_2S.

It is not possible to unequivocally state that mutagenic, carcinogenic, teratogenic, or reproductive effects do not occur because data are insufficient.

1.3 RECOMMENDATIONS

The acute toxicity associated with exposure to H_2S is clearly established, but false assumptions about the recognition of danger by odor need to be dispelled, and adequate information for dealing with catastrophic accidents needs to be promulgated.

There is a clear need for epidemiologic studies of long-term, low-level exposures of populations near or involved in industries producing H_2S. Neurological examination followups of H_2S accident victims are also imperative. Studies that resolve questions of genotoxicity and carcinogenicity also need to be performed, and reproductive effects in animals need to be evaluated.

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2. PHYSICAL AND CHEMICAL PROPERTIES

Hydrogen sulfide (H_2S) is a colorless gas, heavier than air under conditions of standard temperature and pressure (specific gravity = 1.192), with a characteristic offensive odor, like that of rotten eggs, at low concentrations. Its molecular weight is 34.08 (Weast, 1982). It is flammable in air, burns with a pale blue flame, and is oxidized to sulfur dioxide (SO_2). Its autoignition temperature is 260 °C, with explosive limits of 4.3 and 46% by volume. The gas has flammability limits from 44% to 4.0% (National Fire Protection Association, 1978). It may be ignited by static discharge (Manufacturing Chemists Association, 1968). Its combustion products are water and SO_2 (Compressed Gas Association, 1981). Hydrogen sulfide is soluble in water (437 mL/100 mL at 0 °C, and 186 mL/100 mL at 40 °C) (Weast, 1982), which may be important from a health viewpoint. It is also soluble in ethanol, carbon disulfide (Weast, 1982), and a number of other organic solvents including ether, glycerol, and solutions of amines, alkali carbonates, bicarbonates, and hydrosulfides (National Research Council, 1977). The vapor pressure of H_2S is 18.75×10^5 Pa at 20 °C and 23.9×10^5 Pa at 30 °C. Its melting point is −85.5 °C, and its boiling point is −60.3 °C (Macaluso, 1969). In air, 1 ppm (w/v) of H_2S is equivalent to 1.4 mg/m^3.

Hydrogen sulfide can be oxidized by a number of oxidizing agents. The type of reaction and its rate are dependent on the nature and type of the oxidizing agent involved. Principal products of these reactions are SO_2, sulfuric acid (H_2SO_4), and elemental sulfur. Reaction with oxides of nitrogen in the atmosphere can result in the formation of SO_2 and/or H_2SO_4; in water the primary product is elemental sulfur. Interaction with photochemically produced oxidants and hydroxyl radicals (•OH) and ozone produces SO_2, with further oxidation eventually producing H_2SO_4 and/or sulfate ion (SO_4^{2-}).

Hydrogen sulfide is the only thermodynamically stable binary sulfur-hydrogen compound that occurs frequently in nature. It is the sulfur analogue to water. Because of the relative lack of hydrogen bonding, it exists as a gas under normal conditions. However, it is easily liquefied by reduced temperature or increased pressure. The liquid is colorless, with a viscosity one-hundredth that of water (Bailar et al., 1973).

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3. MEASUREMENT AND ANALYSIS

A number of sampling and analytical techniques used for the measurement of hydrogen sulfide (H_2S) in ambient air and occupational settings are available. Low concentrations of H_2S in ambient air are measured in field samples using paper or tiles impregnated with lead acetate, which darkens with exposure. The detectable range of concentrations is 0.11 to 1.1 ppm (0.15 to 1.5 mg/m^3). The color of the exposed samplers fades with exposure to turbulent air and light. However, the use of lead acetate filter tape in continuous volume air samplers is questionable because of fading that is due not only to interaction with light but also to exposure to any oxidant (Sanderson et al., 1966). Tapes impregnated with mercuric chloride do not fade (Paré, 1966), but sulfur dioxide in the air may change its sensitivity to H_2S (Dubois and Monkman, 1966). A standard reference method for the testing of H_2S in ambient air utilizes gas chromatography with a photoionization detector. This method can detect concentrations below 0.7 ppb (0.001 mg/m^3) without preconcentration (Environment Canada, 1984).

A combination of gas chromatographic analysis and flame photometer detection is a dynamic system for sampling sulfur-containing gases, including H_2S, in ambient air. The system's sensitivity depends on a number of variables, including the material on which the sample is collected and the handling of the sample as it goes through the gas chromatograph. The detection limits for this method range from 3.5 to 93 ppb (0.005 to 0.13 mg/m^3) (Pecsar and Hartmann, 1971).

Adams and Koppe (1967) developed a technique using a gas chromatograph coupled with a microcoulometric bromine filtration cell to determine H_2S emitted into the air from kraft paper mills. Concentrations down to a lower limit of 11 ppb (0.015 mg/m^3) can be measured on electronic titration equipment developed by Thoen et al. (1968).

Concentrations of 0.05 to 1 ppm (0.07 to 1.4 mg/m^3) H_2S in the air can be determined by trapping the gas in an aqueous sodium hydroxide solution using an ascorbic acid adsorber, and titrating the resulting sulfide ion with a standard cadmium sulfide solution using a sulfide ion-selective electrode as an indicator (Ehman, 1976).

However, the most sensitive method for the determination of H_2S in ambient air was reported by Natusch et al. (1972). It involves the use of fluorescence and has a sensitivity of 0.1 ppt (0.0002 $\mu g/m^3$).

Occupational exposure can be sampled via the use of personal samplers or monitors, and samples can be taken intermittently or continuously. A number of commercially available H_2S monitors were evaluated for their suitability in continual monitoring situations and their ability to measure concentrations (Smith and Shulman, 1988). The monitors used metal oxide semiconductor sensors and employed a one- or two-point calibration with linearization to obtain concentration data from the sensors. Performance information evaluated included long-term zero and span stability, response time, and the effects of various temperatures, humidities, and interferences on response. Because of good zero stability, all monitors could be used to warn industrial employees of dangerous levels of H_2S. However, to be useful for concentration measurement, a two-point calibration monitor with electronic linearization would be required. It was noted that the faster the response time, the greater the dependence on humidity control. Detection limits for these monitors correspond to the exposure standards and recommended occupational guidelines for H_2S.

The National Research Council of Canada (1981) cited two analytical techniques used in industry to determine worker exposure to H_2S. One method employed a chemical reaction with *N, N*-dimethyl-p-phenylenediamine and ferric chloride to form methylene blue, which is spectrophotometrically measured for H_2S. The limits of detection are 0.7 to 72 ppb (0.001 to 0.1 mg/m^3) air (more concentrated samples must be diluted). This method is considered to be the most accurate means of determining H_2S in air and water. The other technique uses iodometric titration, which has a detection limit of 0.5 ppm (0.7 mg/m^3)/30 L of air sampled.

A micromethod for the determination of H_2S was described by Delwiche (1960). It is a refinement of a method developed by Winkler (1913) and involves the precipitation of colloidal lead sulfide, stabilization and particle size control with gum arabic, and quantification in a photoelectric colorimeter. The sulfide is calculated by direct reference to a standard curve prepared by the use of crystalline lead acetate or lead nitrite as a secondary standard. This method is simple, convenient, and rapid, and it is accurate in the range of 0.01 to 0.1 μg H_2S. An improvement on this method was made by Kruszyna et al. (1975).

Standard methods are available for the determination of H_2S and mercaptal sulfur in natural gas and H_2S and SO_2 in industrial aromatic hydrocarbons; however, no details are available (American Society for Testing and Materials, 1981). Procedures for the analysis of H_2S in biosamples have been described by Goodwin et al. (1989).

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4. SOURCES

4.1 NATURAL OCCURRENCE

Hydrogen sulfide (H_2S) is produced in nature primarily through the decomposition of organic material by bacteria. It develops in stagnant water that is low in oxygen content, such as bogs, swamps, and polluted water (Denmead, 1962; Dixon and Lodge, 1965; Alexander, 1974; Barrett and Clark, 1987). The gas also occurs as a natural constituent of natural gas, petroleum, sulfur deposits, volcanic gases, and sulfur springs. Natural sources constitute approximately 90% of the atmospheric burden of H_2S. This has been estimated to be 90 to 100 million tons, of which 60 to 80 million are produced annually from land sources and approximately 30 million tons from aquatic areas (Urone, 1976). Ambient air concentrations of H_2S due to natural sources are estimated to be between 0.11 and 0.33 ppb (0.15 and 0.46 $\mu g/m^3$) (Miner, 1969).

4.2 PRODUCTION SOURCES

Industrial processes and other anthropogenic sources contribute approximately 10% of the air burden of H_2S. The National Institute for Occupational Safety and Health (1977) lists 73 industries that emit H_2S (Table 4-1). The gas is used mainly as an intermediate and reagent in the preparation of other compounds of reduced sulfur. Processing operations in kraft paper mills and manufacturers of viscose rayon and polyethylene and polyester resins release H_2S to the air. Petroleum refineries, natural gas plants, petrochemical plants, coke oven plants, iron smelters, food processing plants, tanneries, heavy water processing plants, and a variety of metal alloy manufacturers release H_2S as a by-product.

Hydrogen sulfide found in natural gas may be present in concentrations ranging from 1.5 to 90%. It must be removed prior to use of the natural gas for heating or power production. It is an important source of elemental sulfur. Natural gas is usually sold only when the H_2S content is less than <16.4 ppm (23 mg/m^3), but some H_2S can escape during the transport and processing of natural gas (Miner, 1969).

TABLE 4-1. OCCUPATIONS WITH POTENTIAL EXPOSURE TO HYDROGEN SULFIDE

Animal fat and oil processors	Gold-ore workers
Animal manure removers	Heavy-metal precipitators
Artificial-flavor makers	Heavy-water manufacturers
Asphalt storage workers	Hydrochloric acid purifiers
Barium carbonate makers	Hydrogen sulfide production and sales workers
Barium salt makers	Landfill workers
Blast furnace workers	Lead ore sulfidizers
Brewery workers	Lead removers
Bromide-brine workers	Lithographers
Cable splicers	Lithopone makers
Caisson workers	Livestock farmers
Carbon disulfide makers	Manhole and trench workers
Cellophane makers	Metallurgists
Chemical laboratory workers, teachers, students	Miners
Cistern cleaners	Natural gas production and processing workers
Citrus root fumigators	Painters using polysulfide caulking compounds
Coal gasification workers	Papermakers
Coke oven workers	Petroleum production and refinery workers
Copper-ore sulfidizers	Phosphate purifiers
Depilatory makers	Photoengravers
Dyemakers	Pipeline maintenance workers
Excavators	Pyrite burners
Felt makers	Rayon makers
Fermentation process workers	Refrigerant makers
Fertilizer makers	Rubber and plastics processors
Fishing and fish-processing workers	Septic tank cleaners
Fur dressers	Sewage treatment plant workers
Geothermal-power drilling and production workers	Sewer workers
Gluemakers	Sheepdippers
Silk makers	Tank gagers
Slaughterhouse workers	Tannery workers
Smelting workers	Textiles printers
Soapmakers	Thiophene makers
Sugarbeet and sugarcane processors	Tunnel workers
Sulfur spa workers	Well diggers and cleaners
Sulfur products processors	Wool pullers
Synthetic-fiber makers	

Source: National Institute for Occupational Safety and Health (1977).

Processing of high-sulfur coal and oil can also result in the release of H_2S. Crude oil stock of 20,000 barrels may form up to 50 tons of H_2S (Miner, 1969). Gebhart and Andersen (1988) measured and evaluated ambient concentrations of H_2S in and around two oil wells in western North Dakota. The effectiveness of flaring exhaust gases to reduce ground-level H_2S concentrations was also studied. Measured concentrations ranged from 0.002 to 0.671 ppm (0.08 to 0.9 mg/m^3). It was found that flaring exhaust gases reduced the range of H_2S concentrations to 0.002 to 0.097 ppm (0.08 to 0.13 mg/m^3).

Combustion of sulfur-contaminated fuels releases some H_2S to the atmosphere, a problem that industries have generally mitigated by both decreasing the sulfur content of fuels and by catalytically oxidizing the H_2S. In automobiles, the latter method is used, but is circumvented when carburetors and/or catalytic converters are not functioning properly.

Agriculture, too, is a source of H_2S, particularly in large feedlot or barn operations, where bacteria produce the gas in manure piles and tanks, and in settling ponds. Some fatal cases of H_2S poisoning have occurred in connection with the processing of manure and with work associated with human sewage treatment and latrines. Deaths have been reported in pigs and cattle following the emptying of slurry (manure) tanks, when agitation releases toxic gases (McAllister and McQuitty, 1965; Lawson and McAllister, 1966; Clarke and Clarke, 1975).

Most cases of acute toxicity occur in accidental or episodic releases associated with leaks from storage tanks or processing equipment, or in transfer or transport of the gas or mixtures containing the gas. (See Chapter 8: Toxicity).

4.3 ATMOSPHERIC TRANSPORT AND ENVIRONMENTAL FATE

The lifetime of H_2S is affected by ambient temperature and other atmospheric variables, including humidity, sunshine, and presence of other pollutants. The decreased temperatures and decreased levels of $\bullet OH$ in northern regions in winter increase the residence time of H_2S in air (Bottenheim and Strausz, 1980).

Studies of photo-oxidation by Cox and Sandalls (1974) and Stuhl (1974) concluded that free radicals such as $\bullet O$ and $\bullet OH$ generated photochemically were of importance in oxidizing H_2S. Rate constants for the reaction of H_2S with $\bullet OH$, ranging from $< 10^{-13}$ to

10^{-10} cm^3 $mole^{-1}s^{-1}$, were used to derive a lifetime for H_2S in the troposphere ranging from 12 to 27 h (Sprung, 1977; Eggleton and Cox, 1978; Wine et al., 1981; Servant and Delaport, 1982).

Robinson and Robbins (1970), using data from other researchers, estimated that the surface-catalyzed reactions of H_2S with ozone (O_3) are sufficiently rapid to cause H_2S to have a mean residence time in the troposphere from 2 h in urban areas to about 2 days in more remote, unpolluted areas. However, Hales et al. (1974) suggest such catalysis is negligible.

Spedding and Cope (1984) carried out a limited number of experiments at ground level in a geothermal plume, in both summer and winter, and concluded that atmospheric lifetimes of H_2S oxidation to sulfur dioxide (SO_2) were less than those deduced in the laboratory reactions of H_2S with •OH. They proposed that at least one other mechanism that occurs in the dark when •OH is not present is responsible for H_2S oxidation. Their calculated lifetime for H_2S in air was about 10 h.

Studies by Becker et al. (1975) and Hales et al. (1974) show that homogeneous reactions of H_2S with O_3 are very slow, and can be considered negligible when compared to reaction with •OH (Sprung, 1977). Becker et al. (1975) calculated the rate constants for the hypothetical bimolecular reaction at $k_1 = <2 \times 10^{-20}$ cm^3/molecule/s. The authors state: "This number reflects the technically limited accuracy in measuring slow reaction rates at sufficiently low reactant concentrations to exclude chain processes rather than a true bimolecular rate constant, k, which may still be substantially lower."

Microorganisms in soil and water are involved in oxidation-reduction reactions, which oxidize H_2S to elemental sulfur (see Chapter 5). Members of the genera *Beggiatoa*, *Thioploca*, and *Thiotrix* function in transition zones between aerobic and anaerobic conditions where both molecular oxygen and H_2S are found (National Research Council, 1977). Joshi and Hollis (1977) described how *Beggiatoa* protects rice plants from the inhibitory effects of H_2S that accumulates in the soil (see Chapter 5). Other genera such as *Thiobacterium*, *Macromonas*, *Thiovulum*, and *Thiospira* also interact at interfaces of water containing oxygen and water containing H_2S, but since these organisms have not been isolated in pure culture, their specific role is less well understood. Some photosynthetic bacteria oxidize H_2S to elemental sulfur. Members of the families Chlorobiaceae and Chromatiaceae (purple sulfur

bacteria) are obligate aerobes, phototropic, and are found in waters with high H_2S concentrations (National Research Council, 1977). The interactions of these organisms form part of the global sulfur cycle, which is diagrammed in Figure 4-1.

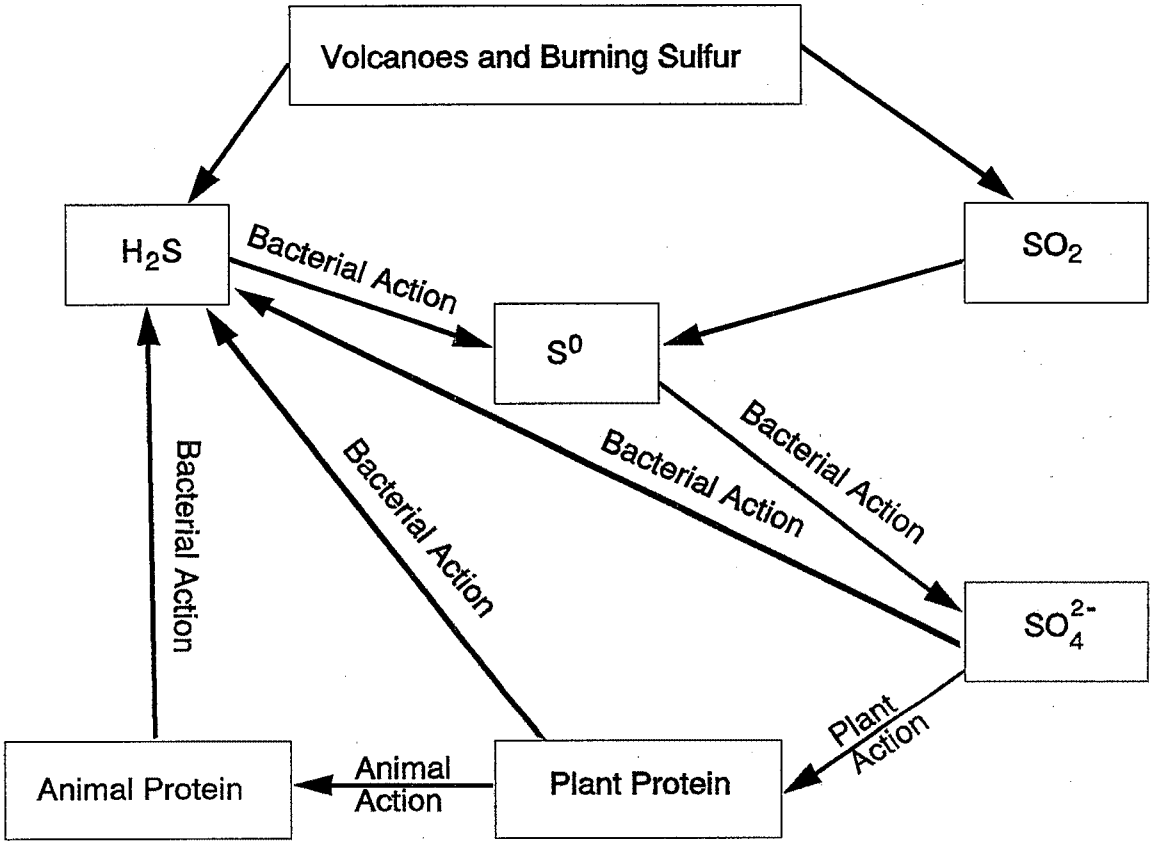

Figure 4-1. The sulfur cycle.

Source: National Research Council (1977).

Hydrogen sulfide is oxidized by microbes to elemental sulfur, and finally to sulfate, which is chemically relatively stable. Sulfate can be taken up by plants and incorporated into plant protein, which in turn is incorporated into animal protein by herbivorous animals, and on through the food web by carnivores. Decay of plant and animal material releases H_2S again through the action of decay microorganisms; some strictly anaerobic sulfate-reducing bacteria can also reduce sulfate directly to H_2S.

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5. ECOLOGICAL EFFECTS

5.1 INTRODUCTION

Available data on the ecological effects of hydrogen sulfide (H_2S) relate more directly to bacteriological or geothermal sources than to anthropogenic sources. Hence, more information is available about the effects on plants and animals coming into contact with H_2S through soil and water than through air.

5.2 EFFECTS ON HIGHER PLANTS

Ambient levels of H_2S are well below those known to cause signs of injury to higher plants (National Research Council of Canada, 1977). Field injury of plants generally has not been reported from ambient exposures. A report from a gas well blowout in Alberta, Canada, in which monitored H_2S concentrations ranged from 5 to 10 ppm (7 to 14 mg/m^3) for several hours, with higher peak exposures, indicated the possibility for effects on vegetation. Alfalfa and hay crops in the exposure area were reported to have as low as one-half to one-third of their normal yield. No comparisons with unexposed croplands were made, and the effects of seasonal parameters such as moisture and temperature were not ruled out. It must be noted that the blowout occurred in winter; therefore, growing field crops were not affected (Lodgepole Blowout Inquiry Panel, 1984).

Relatively few air exposure or fumigation experiments have been done with higher plants. McCallan et al. (1936) and Benedict and Breen (1955) conducted short-term, high-exposure fumigation studies on 29 vegetation and 10 weed species, respectively.
In McCallan's study, plants were exposed for 5 h in the middle of the day to concentrations ranging from 20 to 400 ppm (28 to 560 mg/m^3) H_2S. A wide range of injury was seen; eight species showed no injury at 400 ppm (560 mg/m^3), and other species displayed visible injury at less than 40 ppm (56 mg/m^3). Young, growing tissues were the most susceptible to injury. Benedict and Breen (1955) fumigated 10 species of weeds, 3 to 6 weeks of age, with 100 to 500 ppm (140 to 700 mg/m^3) H_2S for 4 h. They also observed species differences in susceptibility to injury and noted that younger plants were more sensitive to damage than

older ones. Both studies indicated that increases in temperature and dry soil exacerbated the damage.

The damage to young shoots and leaves consisted of scorching; basal and marginal scorching of the next oldest leaves were also observed (Heck et al., 1970). Mature leaves were unaffected. Heck et al. (1970) provided a table that divides 38 selected plants into sensitive, intermediate, and resistant groupings. Included among plants sensitive to H_2S are kidney bean (*Phaseolus vulgaris* L.), buckwheat (*Fagopyrum esculentum* Moench), clover (*Trifolium* sp.), cucumber (*Cucumis sativus* L.), soybean, (*Glycine max.* Merr.), tobacco (*Nicotiana glauca* Grah. and *Nicotiana tabacum* L.), and tomato (*Lycopersicon esculentum* Mill.). Among intermediately sensitive plants are Kentucky blue grass (*Poa pratensis* L.), pepper (*Cupisium futescens* L.), and rose (*Rosa* sp.). Some plants resistant to the effects of H_2S are apple (*Malus pumila* Mill.), cherry (*Prunus serotina* Ehrhe.), mustard (*Brassica campestris* L.), and strawberry (*Fragaria* sp.).

Thompson and Kats (1978) fumigated various crop and forest plants in continuous, long-term exposure experiments. Two procedures using concentrations of 0, 0.03, 3.0, and 30 ppm (0, 0.04, 4 and 40 mg/m^3) or 0, 0.03, 1.0, and 3.0 ppm (0, 0.04, 1.4, and 4 mg/m^3) were employed. Alfalfa was exposed for 28 to 35 days, grapes for 117 or 145 days, and ponderosa pine for 76 days. In contrast to the low sensitivity to H_2S shown by plants in the high-concentration, short-term exposures conducted by McCallan et al. (1936) and Benedict and Breen (1955), plants exposed to very low concentrations of H_2S over long periods of time showed considerably more damage (Thompson and Kats, 1978). For instance, alfalfa (*Medicago sativa* L.) suffered visible leaf lesions after 5 days of exposure to 3 ppm (4 mg/m^3) H_2S, but no damage was seen at 0.03 ppm (0.04 mg/m^3). The alfalfa yield, which is normally cut and regrown in farming practice, was reduced at 3 ppm and 0.3 ppm (4 and 0.4 mg/m^3), but not at 0.01 ppm (0.014 mg/m^3); exposure to 0.03 ppm (0.04 mg/m^3) significantly increased yields. Seedless grapes (*Vitis vinifera* L.) suffered severe damage at 3 ppm (4 mg/m^3) and easily detectable damage at 0.3 ppm (0.4 mg/m^3). Ponderosa pine (*Pinus ponderosa*) showed no visible effect until 4 to 6 weeks of exposure at 3 ppm (4 mg/m^3); at 8 weeks, defoliation occurred. At 0.3 ppm (0.4 mg/m^3), tip burn occurred after 8 weeks. No effect was seen at 0.03 ppm

(0.04 mg/m^3). The exposed plants accumulated sulfur in leaves, although pine did less than alfalfa or grape, perhaps because of lower normal growth rates.

California buckeye (*Aesculus californica*), sugarbeet (*Beta vulgaris*), and lettuce (*Lactuca sativa*) were resistant to damage, and actually the latter two species exhibited considerable growth stimulation at 0.3 ppm (0.4 mg/m^3). As indicated in repeat experiments, temperature variation might play a role in differential growth rates. Buckeye was exposed for 117 days, sugar beets for 123 or 134 days, and lettuce for 59, 88, or 96 days.

Airborne SO_2 has been shown to contribute to the nutrition of plants, especially those grown in sulfur-deficient soils. Faller and Linser (1972), using H_2S in addition to SO_2, confirmed the findings of earlier researchers regarding this phenomenon. In the H_2S experiments, Faller and Linser exposed mature, flowering, and viable seed-bearing sunflowers growing in a sulfur-free nutrient solution to 3 weeks of H_2S fumigation ranging from "a few" ppm to 200 ppm (280 mg/m^3). Growth of all parts of the plants was stimulated very significantly over that of the sulfur-deficient controls; the stem alone approximately doubled in height. The sulfur content in all plants was elevated above that of controls; this result has not been previously observed in nutrient experiments with SO_2.

Gas uptake in plants occurs primarily through the stomata, which can be opened or closed in response to changes in environmental conditions (e.g., illumination, humidity, and perhaps pollutant concentrations). The cell surface available for gas exchange within leaves can be considerably larger than the external leaf surface, which is covered with cuticle and, therefore, not permeable to gas. For example, the lilac leaf has 6 to 8 times the external surface internally, while the bluegreen eucalyptus has 31.3 times the surface area internally (Turrell, 1936). Closure of stomata can, therefore, reduce gaseous uptake dramatically and perhaps protect against short-term, high-level exposure (Hosker and Lindberg, 1982). Conversely, stomatal opening can increase gas uptake which may constitute a nutrient effect.

Closure of stomata in response to air pollution ("smog") was observed by Mansfield and Heath (1963). Sulfur dioxide, in concentrations as low as 0.05 ppm (0.07 mg/m^3), decreases stomatal resistance (indicating opening of stomata), but higher concentrations do not cause a corresponding decrease in resistance (Biscoe et al., 1973). The possible effects of H_2S on stomatal opening or closing have not been investigated. Taylor et al. (1983) measured the flux of sulfur-containing gases to vegetation, however. Using bush bean (*Phaseolus vulgaris*)

and soybean (*Glycine max*), they showed that internal flux, through stomata, was less for H_2S than SO_2 but greater for H_2S than carbonyl sulfide, methyl mercaptan, or carbon disulfide. No direct effect on stomatal function could be deduced from these experiments.

Uptake of sulfide from soil and water has been studied far more extensively than air uptake, since this can represent plant toxicity in soils that are waterlogged or raised in water (e.g., rice). The sulfide found in soils and water results more from bacterial action during the decay of plant and animal protein than from any anthropogenic source of air pollution. Ford (1973) reported that citrus trees in poorly drained areas of Florida suffered root injury at a threshold concentration of 2.8 mg/L aqueous sulfide after 5 days of exposure. Several investigators have examined the effect of disulfide on rice (*Oryza sativa* L.). Hollis and his co-workers (Pitts et al., 1972; Allam and Hollis, 1972; Joshi et al., 1975; Joshi and Hollis, 1977) found that 1 mg/L of sulfide inhibited nutrient uptake, oxygen release, and phosphate uptake by rice seedlings. Some varieties, however, showed enhanced nutrient uptake with exposure to 0.05 mg/L of sulfide. It was learned that the presence of the bacterium *Beggiatoa* in the soil prevented the toxic effect of H_2S, while the rice seedlings' presence symbiotically enhanced the survival of the bacterium. *Beggiatoa* oxidizes H_2S (Joshi and Hollis, 1977). Respiration in rice roots was investigated by Allam and Hollis (1972). Increasing H_2S concentrations were found to increasingly inhibit respiration, so that 0.1 mg/L inhibited respiration 14%, while 3.2 mg/L inhibited this function 25.6%. Assays of root homogenates were made after 3 to 6 h of exposure to 0.1 to 3.2 mg/L sulfide. Assayed enzymes that showed inhibition of respiration included ascorbic acid oxidase, polyphenol oxidase, catalase, peroxidase, and cytochrome *c* oxidase. Of these, cytochrome *c* oxidase was most dramatically inhibited. Forty percent inhibition was measured after a 6-h root exposure to 0.1 mg/L sulfide. This evidence is consistent with the known mode of toxicity of H_2S, which is inhibition of metal-containing enzymes, most specifically cytochrome *c* oxidase, the final electron acceptor of the respiratory chain. When it is incapable of accepting electrons, electron transport along the entire cytochrome chain stops, thereby halting oxidative respiration.

5.3 EFFECTS ON ALGAE AND BACTERIA

Other plant communities in the ecosystem are also affected by H_2S in natural waters. Czurda (1941) found that some species and strains of algae were inhibited by 1 to 2 mg/L sulfide, while others seemed unaffected at concentrations of 8 to 16 mg/L. He found that effects on various physiologic functions such as cell division, respiration, uptake of nutrients and anaerobic respiration were variably affected in different species of algae. Nakamura (1938) delineated enzyme inhibition in two species of algae, *Pinnularia* sp. and *Oscillatoria* sp. Concentrations of sulfide of 0.1 mM (3.2 mg/L) completely inhibited catalase in both species and stimulated oxygen uptake in darkness. Photosynthetic oxygen production was strongly inhibited even at 0.01 mM (0.32 mg/L), while CO_2 fixation was unaffected. Cell division was slightly inhibited by 1.0 mM (32 mg/L) in *Oscillatoria*, and was stimulated twofold in *Pinnularia*.

The role of bacteria in the sulfur cycle, both in the evolution of H_2S during decay processes and in the oxidation of sulfide to sulfate, is discussed in Section 4.3, Atmospheric Transport and Fate.

5.4 EFFECTS ON AQUATIC ANIMALS

The effect of dissolved H_2S gas and dissociated hydrosulfide ion (HS^-) has been examined in a number of studies of aquatic organisms. In typical seawater with a pH of about 8, less than 4% of the sulfide pool exists as H_2S; in sediments of pH 7, about 50% (Vetter and Bagarinao, 1989). A variety of studies have shown that HS^- does enter aquatic species and is rapidly oxidized to thiosulfate and other products (Vetter et al., 1987; Vetter and Bagarinao, 1989; Bagarinao and Vetter, 1989).

Hydrogen sulfide is highly toxic to several fish species. Broderius and Smith (1976) reported the effect of H_2S, HS^- ion, and pH variation on the lethal concentration for 50% of the test organisms (LC_{50}) in the fathead minnow (*Pinephales promelas*). The 96-h LC_{50} values for dissolved H_2S gas decreased linearly from 57.3 μg/L to 14.9 μg/L, with pH increases ranging from 7.1 to 8.7. The more alkaline the pH, the more H_2S, which is a weak acid, dissociates. Undissociated H_2S is thought to be the primary toxic sulfur species

that interacts with respiratory enzymes, so the increase in toxicity indicated by the decreased LC_{50} seems paradoxical. Ions are transported across membranes such as lung epithelia less readily than neutral chemical species. However, transport across the gill surface of fish involves a complex ion exchange mechanism for ridding fish blood of CO_2 in the form of bicarbonate ion (HCO_3^-), formed through the action of the enzyme carbonic anhydrase, which is found in gill tissue. The authors (Broderius and Smith, 1976) suggest that acidic microenvironments at the gill surface may re-form the undissociated H_2S, which is easily transported. It is equally plausible to assume that HS^- exchanges for HCO_3^- through the ion exchange transport system, which normally involves a chloride ion, and that the hydrogen ion (H^+) released from the cleavage of carbonic acid (H_2CO_3) by carbonic anhydrase associates with HS^- within the cell to re-form undissociated H_2S. The 96-h LC_{50} values of the dissolved HS^- ion increased linearly from 64.0 to 780.1 μg/L with increasing pH ranging from 6.5 to 8.7. The data for the HS^- ion are straightforward: the more alkaline the pH, the more HS^- ion forms; therefore the transport rate and the resulting toxicity are lower.

Cleland and Kingsbury (1977) reported that the bluegill *Lepomis macrochirus* was adversely affected at 1 μg/L dissolved H_2S. A 96-h exposure study of northern pike, *Esox lucius*, by the same authors, reported an LC_{50} ranging between 17 and 32 μg/L H_2S. Walleye eggs (*Stizostedion vitreum vitreum*) would not hatch at concentrations of 0.02 to 0.7 μg/L. Smith (1978) exposed several species of freshwater fish to low concentrations of H_2S and determined no-effect levels of $\approx$5 μg/L for all the exposed fish. The 96-h LC_{50} values for the various fish species ranged from 25 to 145 μg/L. The author recommended a 2-μg/L H_2S concentration as a safe limit for freshwater fish. Smith and Oseid (1972) also investigated H_2S effects on walleye eggs and fry in 96-h exposure studies. The LC_{50} values they report are 74 to 87 μg/L for eggs and 7 μg/L for fry. Reynolds and Haines (1980) exposed newly hatched brown trout to H_2S in concentrations ranging from 2 to 13 μg/L for periods of 8 to 22 days. In contrast to the damaging effect mentioned in other studies, these authors reported that the survival rate increased in fry exposed to concentrations of 2 to 5 μg/L H_2S, and that the exposed group's growth was enhanced by 50 to 200%.

Colby and Smith (1967) investigated the effect of H_2S generated by paper fiber sludge deposits ("mats") on the survival of walleye (*Stizostedion vitreum vitreum* Mitchill) eggs and fry, and on the amphipod crustacean *Gammarus pseudolimnaeus* in field and laboratory

investigations. In the field studies, green eggs (36- and 48-h postfertilization) and eyed eggs (2-weeks postfertilization) were placed on paper fiber sludge mats (five stations) and normal river bottom (three stations) in which pH, dissolved oxygen, and dissolved sulfide varied. Exposure times for two separate experiments were 6 and 13 days, to 5,800 eggs and 3,300 eggs, respectively. The latter study was followed by a survival-through-hatching study on 14-day-old eggs. The lowest survival for green eggs occurred when the dissolved oxygen concentration dropped below 3.0 ppm and dissolved sulfide reached a concentration of 0.58 mg/L. The mortality rate for eyed eggs and sac-fry was 100% after a 6-day exposure to the highest dissolved sulfide concentration of 0.14 mg/L. At 0.28 mg/L, all eyed eggs and sac fry died within 2 days. Green eggs (3 and 4 days old) showed greater tolerance to dissolved sulfide when oxygen concentrations in the water were higher. At 5.6 ppm dissolved oxygen, little mortality was noted at 0.08 and 0.20 mg/L dissolved sulfide; at 0.34 mg/L, 98% died after 6 days; and at 0.52 mg/L, 100% died within 72 h. In contrast, at 8.3 ppm dissolved oxygen, up to 96% of eggs exposed to 0.09, 0.21, and 0.27 mg/L survived the experiment. At 0.47 mg/L dissolved sulfide, mortality was 97% within 5 days. In laboratory investigations, gammarids (*Gammarus pseudolimnaeus*) were intolerant to dissolved sulfide concentrations of 0.16 to 0.36 mg/L, especially at low dissolved oxygen concentrations (1.2 to 1.3 ppm). They were far more tolerant to similar sulfide concentrations when dissolved oxygen was 5.0 to 5.1 ppm.

Torrans and Clemens (1982) noted in their work with channel catfish (*Ictalurus punctatus*) that not only oxygen, but also temperature had an effect on H_2S toxicity. They investigated possible reasons for mortality of catfish during harvesting, when the black, malodorous sediment of pond bottoms is disturbed (and H_2S is released into the water). Harvesting usually occurs in the summer, when water temperatures are higher and dissolved oxygen is lower, and when transport over distances exposes fish to heat. The catfish were implanted with electrodes in the opercular muscle and near the heart so that ventilation and heart rates could be monitored. Acute exposure (0.5 mg/L H_2S for 1 min at 20 °C) resulted in an initial stimulation of heart rate and amplitude of ventilatory movement. Heart rate increased from a resting rate of 88 to 128 beats/min (b.p.m.), while ventilation rate decreased from 140 to 128 cycles/min (c.p.m), but with greater amplitude of opercular movement. After 5 min of exposure, the heart rate decreased to 60 b.p.m.; ventilation rate decreased to

88 c.p.m., and both became shallow and irregular. After 6 min and 40 s of exposure, the opercular muscle went into a state of tetany and ventilation ceased. When the fish were returned to freshwater after 8 min of exposure, the opercular muscle showed occasional spasms, and ventilation was not restored, although the heart continued to beat with a steadily decreasing rate for 1 h. The effect of H_2S in vivo and in vitro on cytochrome *c* oxidase and on blood lactate levels was determined and is discussed in detail in Chapter 7, Section 7.2, Metabolism and Pharmacokinetics. Fish exposed so that brain cytochrome *c* oxidase was inhibited 50% recovered full enzyme activity 6 h after they were returned to freshwater, showing that inhibition is reversible and noncumulative.

Channel catfish and fathead minnows (*Pimephales promelas*) exposed to 20 mg/L total dissolved sulfide at 20 °C, pH 8.0 (1.0 mg/L H_2S) were removed from the solution when respiration ceased, and their tissues were assayed for cytochrome *c* oxidase activity. For the fathead minnow, enzyme activity varied from control levels in the testes to 55% inhibition in the kidney. In the channel catfish, the inhibition ranged from 28% for brain to 66% for heart. The enzyme in the gill was affected before the brain and was inhibited to a greater extent. Blood lactic acid levels rose, indicating active anaerobic metabolism. The time course for recovery from H_2S poisoning was determined; the enzyme returned to normal levels 6 h after fish were returned to freshwater.

In subchronic toxicity studies with *Gammarus pseudolimnaeus* (gammarids), the maximum safe level determined for 65-, 95-, and 105-day exposures was 2 μg total sulfide/L, while the 96-h LC_{50} was determined to be 20 μg total sulfide/L (Oseid and Smith, 1974). Chronic studies on juvenile and adult bluegills (*Lepomis macrochirus*) demonstrated a no-effect level of 2 μg/L H_2S, but minnows, suckers, amphipods, and some aquatic insects did show toxic effects at levels slightly higher than this limit (Smith et al., 1976; Smith, 1978).

In 1972, it was proposed that a water quality criterion for undissociated H_2S should be set at 2 μg/L for fish and other aquatic life in both fresh and marine waters (Cleland and Kingsbury, 1977). The National Academy of Sciences-National Academy of Engineering, Environmental Studies Board had earlier recommended such a standard for freshwater organisms, but proposed 10 μg H_2S/L as a standard for marine life.

Bagarinao and Vetter (1989) evaluated sulfide toxicity in ten species of shallow-water marine fish and found a wide range of tolerance. Tidal-marsh fishes (e.g., killifish and mudsucker) show high tolerance, while those from open coastal areas (e.g., northern anchovy) have low tolerance. Sulfide concentrations in various marine habitats were reviewed by Vetter and Bagarinao, 1989.

While the majority of aquatic organisms tested have exhibited very low tolerance for H_2S, it must be noted that several marine macroinvertebrates are capable of withstanding long-term exposure to the compound at concentrations above expected lethal limits. Examples of these organisms include the gutless protobranch clam (*Solemya reidi*), which is found almost exclusively in habitats such as sewage outfalls and pulp-mill effluent zones (Reid, 1980); the acoel turbellarians (*Solenofinomorpha funilis* and *Pseudohaplogonaria* sp.) and the gastrotrich (*Dolichodasys carolinensis*) inhabiting the reduced zone of sediments (Powell et al., 1980); and inhabitants of oceanic hydrothermal vent areas such as the brachyuran crab (*Bythograea thermydron*), the vestimentiferan tubeworm (*Riftia pachyptila*), the vesicomyid clam (*Calyptogena magnifica*), and the mussel (*Bathymodiolus thermophilus*) (Williams, 1980; Cavanaugh et al., 1981; Felbeck et al., 1981; Cavanaugh, 1983). Research on the organisms inhabiting deep ocean volcanic fumaroles has shown symbiotic relationships with chemolithoautotrophic bacteria (Cavanaugh, 1983), which exploit the H_2S of the vent effluent to synthesize reduced carbon and nitrogen compounds used for the nutrition of the symbionts and the host animal (Felbeck, 1981; Felbeck et al., 1981).

A focus of further research into hydrothermal vent symbiotic organisms has been the ability of the host organism to withstand the comparatively high levels of H_2S. Two protective mechanisms have been described. The first mechanism was noted in the blood of the vestimentiferan tubeworm *R. pachyptila*, which contains a sulfide-binding protein that functions as a sulfide carrier (Arp and Childress, 1983). When bound to this protein, the sulfide is stabilized against spontaneous oxidation and, consequently, does not disrupt aerobic respiration (Powell et al., 1987). This transport system enables the tubeworm to transport sulfide from ambient seawater to organs containing symbiotic bacteria without endangering electron transport in the host organism's mitochondria. The second mechanism has been proposed based on work with the gutless protobranch clam, *S. reidi*. The mitochondria of *Solemya* possess a sulfide-oxidizing capability that is linked to oxidative phosphorylation

(ATP synthesis) (Powell and Somero, 1986; Powell et al., 1987). The major product of *Solemya* sulfide oxidation is thiosulfate, which is proposed to function as an energy source for bacterial symbionts in the host clam gill tissue.

Marine macroinvertebrates lacking chemolithoautotrophic symbionts also exhibit a similar sulfide detoxification mechanism. The foraging predatory brachyuran crab (*B. thermydron*) achieves protection from sulfide toxicity through a detoxification system located in the hepatopancreas (Vetter et al., 1987). The initial step in this process is the oxidation of sulfide by a sulfide oxidase enzyme to produce thiosulfate. However, it is not known whether this sulfide oxidation process is ATP-generating. The investigators concluded that crabs are sensitive to sulfide, but tolerances vary between habitats.

The presence of a variety of avoidance mechanisms for H_2S toxicity combined with their expression in different phyla suggests that H_2S tolerance may be more widespread among marine organisms than presently thought.

Some research suggests that a defense mechanism of some marine animals against sulfide toxicity may be resistance of hemoglobin to reaction with sulfide. In marine animals, mitochondrial sulfide oxidation appears to be the primary defense mechanism for protecting cytochrome *c* oxidase against sulfide. This area has been reviewed by Vetter and Bagarinao (1989).

5.5 EFFECT ON WILDLIFE

Very few studies exist that attempt to measure natural or accidental exposure of wildlife to H_2S, or to determine its effects. One investigation by Siegel et al. (1986) examined the ambient levels of H_2S at Sulphur Bay Wildlife area on Lake Rotorua, New Zealand, where shore and water birds are exposed to H_2S of geothermal origin in concentrations of 0.125 to 3.90 ppm (0.17 to 5.4 mg/m^3). The authors state that exposure of these birds is higher than would be expected for humans at these concentrations because small birds have a higher oxygen utilization rate and, therefore, a higher ventilation rate than mammals of human size. Populations in this wildlife area have nevertheless thrived, as indicated by the increasing number of nests found for several species in the preserve. No other parameters of exposure were measured on either a population level or an individual level.

An attempt to determine the effect of exposure to fumes from a gas well blowout in Alberta, Canada, on wildlife was made by the Canadian Wildlife Service (New Norway Scientific Committee, 1974). A flight over the well and surrounding area the day of the mishap to examine the lakes and larger sloughs for any evidence of dead or distressed waterfowl, and over the areas between lakes, draws, and valleys to search for dead deer, did not reveal any ill or dead wildlife. At the time of the blowout, all young fowl had reached flying size, so both young and adults tending them could fly from the contaminated area. A next-day overflight in the downwind area showed no dead or distressed birds, and the distribution and activity of all birds seen appeared normal. Monitoring at various sites ranged from 0 ppm (8 h) to as high as 0.02 ppm (1 h), although higher concentrations were probable at time of release.

The gas well blowout that occurred at Lodgepole, Alberta, Canada, was investigated by a board of inquiry. During the blowout, three moose and a raven were found dead near the well site. Cause of death was not established. Animal track surveys indicated that large ungulates such as elk were avoiding the immediate well-site area during the winter of the blowout, but that they moved in normal patterns throughout the nearby forested areas, conforming to those seen in surveys conducted in 1981. A small mammal survey conducted by Alberta Fish and Wildlife in the cleared and perimeter areas of the well-site determined a shift in species composition but no significant changes in numbers. Local residents said that birds and small wild mammals disappeared from the area following the blowout. At times, concentrations between 5 and 10 ppm (7 to 14 mg/m^3) H_2S were measured at various sites in the area (Lodgepole Blowout Inquiry Panel, 1984). Averaging times are unknown.

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6. EXPOSURE TO HYDROGEN SULFIDE

6.1 INTRODUCTION

Hydrogen sulfide (H_2S) is a leading cause of sudden death in the workplace (Ellenhorn and Barceloux, 1988). The National Institute for Occupational Safety and Health (NIOSH) (1977) lists 73 categories of workers with potential for exposure to H_2S (see Table 4-1). Among those with the greatest likelihood of hazard are natural gas drillers, processors, and producers; petroleum production and refinery workers; kraft pulp industry, coke oven, blast furnace, and smelter workers; coal gasification workers; heavy water manufacturers; synthetic fiber and rayon makers; pipeline maintenance workers; miners; livestock farmers and manure processors; sewage treatment plant workers; sugarbeet processing workers; and tannery workers (National Institute for Occupational Safety and Health, 1977).

Ambient concentrations of H_2S tend to be low, constituting an odor nuisance. Populations around sulfide-producing industries have been exposed to accidental releases of widely varying concentrations, ranging from levels that caused malaise to higher levels that were lethal.

6.2 AMBIENT CONCENTRATIONS

Ambient levels of H_2S are not routinely measured. Examples of average and maximum atmospheric concentrations of H_2S found in various U.S. geographical locations before 1965 are listed in Table 6-1. More recent data on ambient levels of H_2S in the United States were not found in the published literature. Motor vehicles, especially those whose carburetors and/or catalytic converters are functioning improperly, are one source of concern for contribution to the H_2S air burden (Harvey, 1983).

Elevated ambient concentrations in two recorded episodes, one in the Great Kanawha River Valley, WV, in 1950, and one in Terre Haute, IN, in 1964, were reported as 0.3 and $\approx$0.33 ppm (0.41 mg/m^3 and $\approx$0.46 mg/m^3) (averaging times unknown), respectively (West Virginia Department of Health, 1952; U.S. Department of Health, Education, and Welfare,

TABLE 6-1. ATMOSPHERIC HYDROGEN SULFIDE CONCENTRATIONS (mg/m^3)[a]

Location	Average[b]	Maximum
New York City, NY		
1956-1961	0.001	0.013
1962	0.001	0.006
Elizabeth, NJ		
August-October 1963	0.001	0.247
Hamilton Township, NJ		
May-October 1962	0.001	0.049
Woodbridge Township, NJ		
April-May 1961	0.001	0.305
Greater Johnstown Area, PA		
1963	0.003	0.210
Winston-Salem, NC		
November-December 1962	0.003	0.011
Lewiston-Clarkston Area, North Lewiston, ID		
Near pulp mill, 1962		0.037
Great Kanawha River Valley, WV		
Industrial area		
February 1950-August 1951	0.003-0.092	0.410
Camas, WA		
1962	0.001	0.006
Santa Barbara, CA		
1949-1954		1.4
St. Louis, MO		
1964	0.002-0.006	0.094
Terre Haute, IN		
May-June 1964		>0.460

[a]1.4 $mg/m^3 \simeq 1$ ppm.
[b]Averaging times not stated.

Source: Miner (1969).

1964). In the Terre Haute incident, levels of H_2S measured at a nearby lagoon ranged from 2 to 8 ppm (2.8 to 11.2 mg/m^3) (1-h averaging time).

During the Lodgepole oil well blowout in the foothills of Alberta, Canada, in 1982, transient levels of up to 14.5 ppm H_2S (averaging times unknown) were detected in communities located 20 km from the site. The maximum concentration detected in the city of Edmonton, 130 km away, where the odor level was substantial even at concentrations well under the peak, was 0.52 ppm (0.73 mg/m^3) (Lodgepole Blowout Inquiry Panel, 1984).

Rotorua, New Zealand, is a major recreational and sports center for travelers from all over the world. The proximity of the city to an active geothermal system is evident from the widespread use of this energy source and the prevailing odor of H_2S. Ambient concentrations ranging from 0.005 to 1.9 ppm (0.007 to 2.6 mg/m^3) (averaging times ranged from 10 to 60 min) have been measured. A preliminary study revealed no evidence of health impairment (Siegel et al., 1986).

The states reported to have ambient air quality standards for H_2S are identified in Table 6-2.

6.3 OCCUPATIONAL CONCENTRATIONS

In the United States alone, H_2S has been cited as a potential hazard in 73 occupations in which approximately 125,000 employees are subject to exposure (National Institute for Occupational Safety and Health, 1977) (see Table 4-1). Low-level concentrations routinely occur in certain industries such as viscose rayon production, pulp processing, oil refining, and gas and oil well operation. In all such occupations, potentially hazardous gases such as carbon disulfide, mercaptans, SO_2, and diverse hydrocarbons form a mixture with H_2S, and individual effects of these pollutants have been difficult to delineate. Information regarding effects from exposure to low concentrations is scant and is often confounded by the presence of other gases in the work environment.

In 1977, NIOSH recommended a ceiling limit of 15 mg/m^3 or approximately 10 ppm H_2S for 10 min, for up to a 10-h work shift in a 40-h work week (NIOSH, 1977). The present Threshold Limit Value (TLV) for H_2S, expressed as a Time-Weighted Average

TABLE 6-2. AMBIENT AIR QUALITY STANDARDS FOR HYDROGEN SULFIDE

State	Concentration (ppm)	Average Time (hours)
California	0.03	1
Connecticut	0.2	8
Kentucky	0.01	1
Massachusetts	0.014	24
Minnesota	0.05[a]	0.5
	0.03[b]	0.5
Missouri	0.5[a]	0.5
	0.03[b]	0.5
Montana	0.05[c]	1
Nevada	0.24	8
New York	0.01	1
North Dakota	0.20[d]	1
	0.10[c]	24
Pennsylvania	0.10	1
Rhode Island	0.01	1
Texas	0.08	0.5
Virginia	0.16	24
Hawaii	0.04	1
Delaware	0.03	1
Indiana	0.05	1

[a]Not to be exceeded more than two times/year.
[b]Not to be exceeded more than two times/five consecutive days.
[c]Not to be exceeded more than one time/year.
[d]Not to be exceeded more than one time/month.

Source: Environmental Reporter (1991).

(TWA), is 10 ppm (≈14 mg/m^3) (ACGIH, 1989). (Threshold Limit Value is set by the American Conference of Governmental Industrial Hygienists for exposure of healthy workers 8 h/day, 40 h/week. The TLV for short-term exposure limit (STEL), which represents the maximum concentration to which workers may be exposed for up to 15 min, is 15 ppm

(≈21 mg/m^3). Accidental exposures of workers and the general population have occurred in which the levels were much higher, sometimes by several orders of magnitude. For example, in Poza Rica, Mexico, in 1950, an accidental release of H_2S from an absorption unit in a natural gas refining plant killed 22 people and hospitalized 320 more in the nearby community, even though the release lasted only 20 to 25 min (McCabe and Clayton, 1952).

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7. METABOLIC FATE AND DISPOSITION

7.1 ABSORPTION

The most common route of entry for hydrogen sulfide (H_2S) is the lung. Experimentally, sodium sulfide (Na_2S) has been injected intravascularly or intraperitoneally, or instilled orally by gavage, to determine its distribution and fate in tissues as well as its metabolism. Absorption of H_2S through the skin is limited. Exposure of large areas of skin to pure H_2S was lethal in guinea pigs after 45 min but did not affect dogs (Walton and Witherspoon, 1925). In rabbits, exposure of the entire body, except the head, allowed a qualitative detection of H_2S in expired air (Laug and Draize, 1942). Absorption through the tympanic membrane of workers wearing respirators was not a significant route of toxicity (Ronk and White, 1985).

In aqueous solution, for instance in body fluids, H_2S has two acid dissociation constants and can thus exist as the hydrosulfide anion (HS^-) and as the sulfide anion (S^{2-}). The pKa for Step 1 is 7.04; and the pKa for Step 2 is 11.96 (in solutions 0.01N to 0.1N at 18 °C).

$$H_2S \longleftrightarrow H^+ + HS^- \longleftrightarrow H^- + S^{2-}$$

(1) (2)

At human physiologic pH and temperature of 7.4 and 37 °C, respectively, about one-third of the total sulfide exists as undissociated H_2S, about two-thirds as HS^-, and minuscule amounts as S^{2-}. Since unionized small molecules tend to diffuse across membranes more readily than ionized molecules do, it is likely that H_2S is absorbed more rapidly than the negatively charged ions. Absorption of H_2S in protozoans occurred more rapidly than the ionic species (Beerman, 1924). Absorption of H_2S from the peritoneal cavity of mice occurred more rapidly with an acidic carrier, which prevented sulfide ion formation, than in an alkaline carrier, which enhanced ion formation (Smith and Abbanat, 1966).

7.2 METABOLISM AND PHARMACOKINETICS

Hydrogen sulfide can be metabolized via three pathways: (1) oxidation to sulfate, (2) methylation, and (3) reaction with metallic ion or disulfide-containing proteins (Figure 7-1) (Beauchamp et al., 1984). Oxidation and methylation represent means of detoxification, while the interaction with essential proteins, particularly the iron-containing proteins of the respiratory chain, is largely responsible for the toxic actions of the gas.

The oxidation of sulfide to sulfate has been studied for nearly 40 years and is not as yet precisely defined. While early in vitro studies with liver and kidney preparations postulated intermediates such as free sulfur, polythionates, and thiosulfate, Garabedian (1945a,b) proposed that sulfide oxidase enzymatically catalyzed the oxidation of sulfide. Baxter et al. (1958) and Baxter and Van Reen (1958) confirmed the existence of a liver sulfide oxidase.

The observation was made by Sorbo (1958) that heme catalyzed sulfide oxidation to thiosulfate. Several studies were initiated to determine the precise site of sulfide oxidation. ^{35}S-Sodium sulfide incubated in vitro with blood rapidly bound to blood proteins (Curtis et al., 1972). It was demonstrated too that this route of oxidation worked very slowly and was insufficient to account for high levels of sulfate formation in living systems. Other in vitro experiments (Bartholomew et al., 1980) showed that thiosulfate was the major oxidation product of sulfide in liver mitochondria, and that this could then be converted to sulfate by sulfide oxidase, which has been purified from rat and dog liver and kidney (MacLeod et al., 1961a,b). The precise location for major oxidation of sulfide in vivo has not been unequivocally established, but the liver is the most probable site.

The lung participates little in metabolism of sulfide to sulfate. Using whole-body autoradiography after intraperitoneal injection or gavage instillation of ^{35}S-sulfide, Curtis et al. (1972) showed that while the lung accumulated ^{35}S-sulfide, very little was converted to radioactively labeled sulfate. This confirms the work of MacLeod et al. (1961a) that sulfide oxidase is absent in lung tissue.

Whole-body autoradiography of young male M.R.C. hooded rats, following intraperitoneal injection of ^{35}S-sulfide and ^{35}S-sulfate and sacrifice of animals at time intervals ranging from 3 min to 6 h after injection, showed that the label widely distributed and accumulated in tissues, including the gastrointestinal tract and cartilage. The uptake into bones indicated that oxidation to sulfate occurred prior to incorporation into

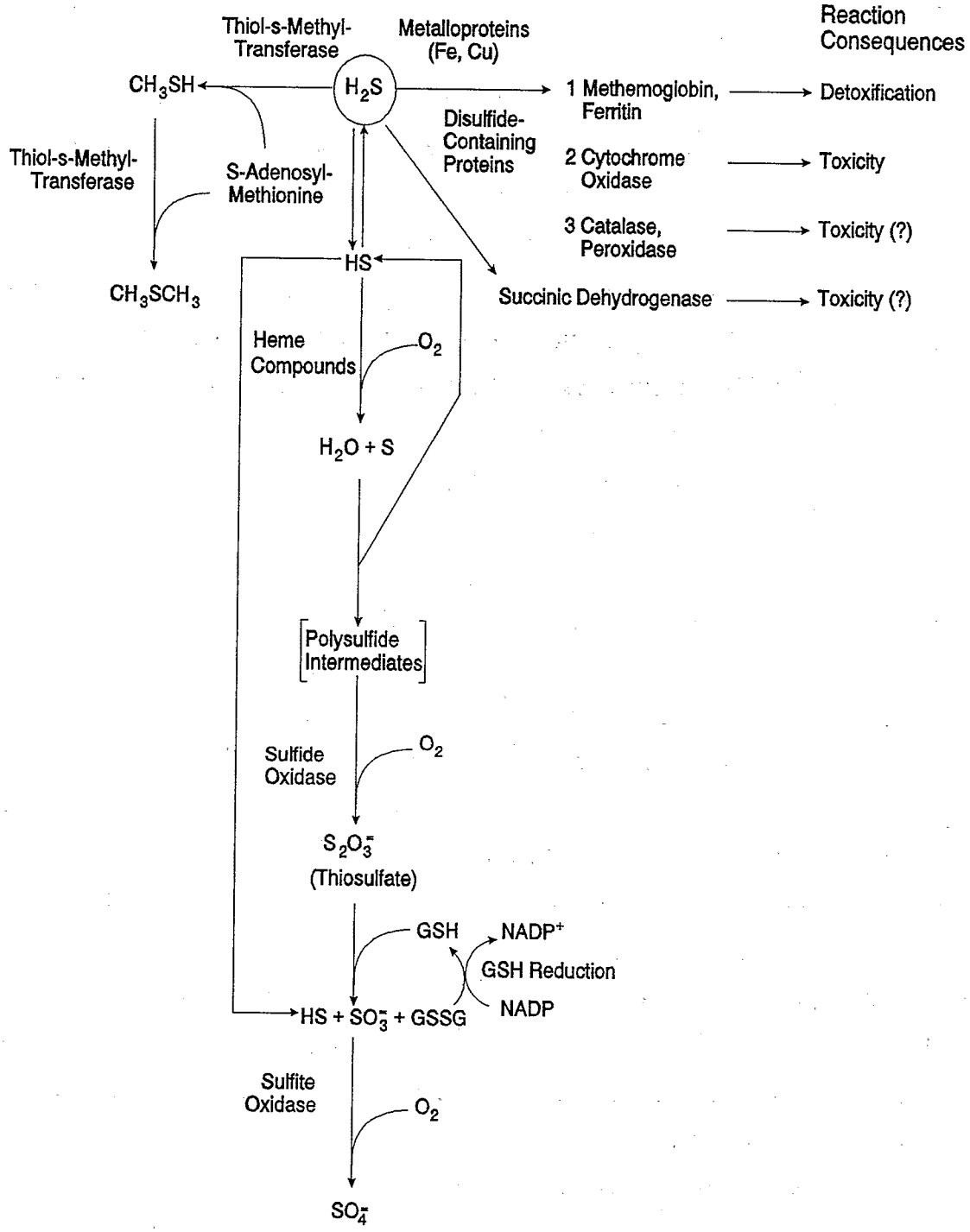

Figure 7-1. Metabolism of hydrogen sulfide.

Source: Beauchamp et al. (1984).

mucopolysaccharides. In addition to these tissues and lung, radioactive label also accumulated in brain tissue and persisted there up to 20 min after sulfide injection (Curtis et al., 1972).

Further attempts to identify the locale of sulfide oxidation were made by Bartholomew et al. (1980) using ^{35}S-sulfide and isolated, living, perfused rat livers, lungs, and kidneys. These experiments confirmed the plasma binding of sulfide (up to 90% bound) and the lack of sulfate formation in the lung. Release from carrier proteins in plasma and volatilization of sulfide to H_2S occurred, and 32% of the administered dose was lost from the blood through the lung. Sulfide remaining in the blood was oxidized slowly, possibly within red cells.

The same experiments with kidney confirmed the findings of Curtis et al. (1972) that sulfate was the major radioactive component in renal vein blood and urine, and that the kidney can oxidize sulfide. Bartholomew et al. (1980) found a mechanism for rapid oxidation of sulfide in liver mitochondria, which worked rapidly but only at low sulfide concentrations that did not inhibit cytochrome *c* oxidase activity. Studies with isolated rat liver perfused with heparinized homologous blood to which (a) $Na_2{}^{35}S$ in phosphate buffer and (b) $Na_2{}^{35}S$ and unlabeled thiosulfate in buffer were added showed significant metabolism of the sulfide to sulfate. After perfusion for 15 min in experiment (a) above, 70% of the radioactively labeled sulfur was associated with sulfate, and the percentage increased to 82% after 2 h of perfusion. In experiment (b) above, 54% of the radioactive sulfur was found in thiosulfate after 15 min of perfusion, with 22% ^{35}S in sulfate. After 30 min, the amount of label present in thiosulfate had decreased to about 30%, while that in sulfate had increased to about 46%. At the end of 2 h perfusion time, only 13% of the label remained in the unreacted sulfide, and no radioactivity could be detected in thiosulfate. The work of these researchers confirmed the earlier work by MacLeod et al. (1961a,b) and Koj et al. (1967), which found that thiosulfate is a major oxidation product of sulfide and that thiosulfate was oxidized to sulfate in mitochondria. They proposed that glutathione (GSH) mediated thiosulfate oxidation according to the following equations:

(1) $(S \bullet SO_3)^{2-} + 2GSH \longrightarrow HS^- + HSO_4^- + GSSG$ (oxidized glutathione)

(2) $SO_3^{2-} + \frac{1}{2}O_2 \longrightarrow SO_4^{2-}$

(3) $2HS^- + 2O_2 \longrightarrow (S \bullet SO_3)^{2-} + H_2O$

MacLeod et al. (1961 a,b) suggested that sulfide oxidase converted the sulfide intermediate to sulfate.

Weisiger and Jakoby (1979) have identified an enzyme, thiol-S-methyltransferase, which catalyzes the methylation of H_2S to methanethiol (CH_3SH), then dimethylsulfide (CH_3SCH_3). The authors regarded this methylation as a means of detoxification because both products are less toxic than H_2S. The enzyme is found primarily in gut mucosa and liver, and may thus serve to detoxify absorbed H_2S that was produced by anaerobic bacteria in the intestinal tract. The role of this enzyme in the detoxification of inhaled H_2S has not been determined.

Reaction of H_2S with metallic ion-containing protein is considered its major mechanism of toxicity (Smith and Gosselin, 1979). Chance and Schoener (1966) had found sulfide to be a stable inhibitor of mitochondrial heme-containing cytochrome enzymes, which are involved in oxidative metabolism. Cytochrome *c* oxidase is the last enzyme in this complex of the cytochrome chain that transfers electrons to oxygen as the final electron acceptor, combining them with hydrogen ions to form water. In the presence of H_2S, transfer of electrons to oxygen cannot occur, all electron transport down the chain is stopped, and oxidative metabolism, which is the primary energy source for mammalian cells, stops. Work by Wever et al. (1975), Nicholls (1975), Nicholls et al. (1976), Smith et al. (1977), and Smith and Gosselin (1979) showed that H_2S causes chemical reduction of one of the hemes of this enzyme, preventing electron transfer to oxygen. Chance and Schoener (1966) found that H_2S inhibits cytochrome *c* oxidase slightly more potently than does hydrogen cyanide (HCN), but the mechanism of action appears to be similar. Smith et al. (1977) also conducted in vitro experiments using sub-mitochondrial particles prepared from beef heart. They confirmed that sulfide is a more potent inhibitor of cytochrome *c* oxidase than is cyanide. Nicholls (1975) showed similar results and determined the k_i for H_2S to be $\approx 0.02\ \mu M$.

Inhibition of cytochrome *c* oxidase through in vivo and in vitro experiments, and recovery from inhibition, was shown by Torrans and Clemens (1982) in channel catfish (*Ictalurus punctatus*), in addition to measurement of some physiologic parameters (see Chapter 5). Both fathead minnows (*Pimephales promelas*) and channel catfish were exposed to 1.0 mg/L H_2S (20 mg/L total sulfide) at 20 °C, water pH 8.0. Individual fish were removed from the sulfide solution when ventilation ceased (13 to 23 min for the channel catfish and 9 to 15 min for the fathead minnows), and tissues were removed for

homogenization and assay of enzyme activity. Cytochrome *c* oxidase activities in the fathead minnows ranged from control levels in testes to 55% inhibition in kidney. In the channel catfish, the brain enzyme was inhibited 28% and heart enzyme 66%. Hydrogen sulfide (unionized) affected the catfish brain and gill cytochrome *c* oxidase more than dissolved sulfide ion. When fish were exposed to 0.1 mg/L H_2S at 10 °C, brain enzyme was not affected, even at 30 min exposure, but gill enzyme was inhibited 15% after 5 min and 39% after 30 min exposure. At 0.3 mg/L H_2S, brain enzyme activity was reduced by 25%, and at 0.5 mg/L brain enzyme activity was inhibited 56%, while gill enzyme activity was reduced by 48% after 5 min exposure. The latter was the maximum effect at that concentration and coincided with ventilatory arrest. Temperature had a great effect on enzyme activity of fish exposed in vivo. Channel catfish exposed at 20 °C to 0.1 mg/L H_2S showed enzyme inhibition similar to those exposed to 0.5 mg/L at 10 °C. Thus, after 10 min of exposure to 0.1 mg/L H_2S, brain cytochrome *c* oxidase activity was 58% reduced, while gill enzyme was 41% decreased; after 20 min, brain enzyme was 40% reduced, while gill enzyme was reduced 33%; after 30 min, brain enzyme was 40% reduced, and gill enzyme was 26% reduced. Blood lactate levels increased as cytochrome *c* oxidase levels decreased, indicating high levels of anaerobic metabolism, and the fish became rapidly fatigued. High levels of methemoglobin induced by pre-exposing fish to nitrite sulutions reduced the degree of cytochrome *c* oxidase inhibition produced upon exposure to H_2S.

Torrans and Clemens (1982) also measured in vitro cytochrome *c* oxidase inhibition by sulfide. Even very low concentrations inhibited the enzyme in tissue homogenates. Catfish brain-homogenate cytochrome *c* oxidase activity was decreased 18% at 10^{-7}M H_2S, 64% at 10^{-6}M H_2S, and 100% at 10^{-4}M H_2S. Effects were similar for fathead minnow brain-homogenate. The pH of the solution influenced dissociation of H_2S and consequently its toxicity. At pH 5, and 10^{-6}M, 98% of the H_2S is unionized, and greatest inhibition (65.4%) occurs. As the pH of 7.04 was approached, inhibition decreased and more sulfide ion formed, and at pH 7.5 only 14% H_2S remained unionized, and enzyme inhibition decreased to 45.7%. The reaction was reversible, as was also shown in vivo, and showed competitive kinetics.

Since the effect of H_2S poisoning is to deprive the cellular cytochrome chain of oxygen, those cells having the highest oxygen requirement are most rapidly and severely affected.

Nerve tissue and cardiac tissue have large oxygen demands and show the first effects of H_2S toxicity. Warenycia et al. (1989) reported on several case studies in which brain stem levels of sulfide were measured shortly after the fatalities. Analysis indicated sulfide levels of about 1 μg/g tissue compared to a normal human brain stem concentration of about 0.7 μg/g. Such elevated levels were used to establish cause of death.

Besides cytochrome *c* oxidase, other metallo-proteins also react with H_2S. When these are enzymes, perturbations of other pathways may occur, although this effect would be nearly overshadowed by the cessation of oxidative metabolism. Interactions of H_2S with horseradish peroxidase (Wieland and Sutter, 1928), potato polyphenol oxidase (Keilin, 1928), and catalase (Stern, 1932) produced inhibition of these enzymes, but the importance of these reactions to detoxification has not been further explored. Tenhunen et al. (1983) assayed in vitro enzyme activity for heme synthetase, and δ-amino-levulinic acid synthetase (ALA-S) from human venous blood. These enzymes are part of the pathway in the synthesis of protoporphyrin, which is a precursor of heme. In 17 workers exposed to H_2S and methylmercaptan, these enzymes showed decreased activity when assayed. Erythrocyte and protoporphyrin concentration in seven of these cases were below the control range. In the in vitro experiments, both H_2S and sulfide anion inhibited heme synthetase and ALA-S synthetase. These results may be of importance for their indication of a possible additional pathologic mechanism for H_2S poisoning, as well as a means of assessing worker exposure and/or health. However, it must be noted that the in vitro concentrations used to produce inhibition were considerably higher (3.4 to 10 mmol/L) than the concentrations that workers exposed to low levels would experience.

Jappinen (1989) evaluated 21 cases of acute H_2S poisoning (<10 min) that occurred in sulfate pulp mills. In 6/21 cases, blood samples were collected in less than 2 h and changes in heme metabolism were assessed. A decrease in ALA-S activity was most prominent when blood sulfide concentrations were more than 100 μg/L. Initial mean levels of heme synthase and protoporphyrin in blood from these six individuals were also lower than mean control values. Levels continued to be lower than controls 1 mo after the acute poisoning episode.

Hydrogen sulfide can act as a reducing agent for disulfide bridges in proteins. Such change in protein structure has been proposed as an explanation for H_2S inhibition of succinic

dehydrogenase (Hayden, 1989). Whether inhibition of this enzyme has a role in the toxicity of H_2S has not been elucidated.

Reaction of H_2S with methemoglobin constitutes a pathway for detoxification, resulting in the formation of sulfmethemoglobin. Smith et al. (1977) using submitochondrial particles from beef heart in vitro, showed that methemoglobin relieved the inhibition of cytochrome *c* oxidase by H_2S by re-initiating the oxidation of ferricytochrome c. They also indicated that the undissociated H_2S is a more potent inhibitor of the enzyme than the hydrosulfide anion. This is in agreement with findings related to HCN and hydrogen azide molecules. Similar to the work by Scheler and Kabisch (1963), Smith and Gosselin (1966) pretreated mice with sodium nitrite. Nitrite causes the formation of methemoglobin. Smith and Gosselin (1966) also preinjected mice intraperitoneally with human methemoglobin prior to injection of sodium sulfide. Both injected nitrite and methemoglobin protected the mice from death from subsequent injections of sodium sulfide.

Detoxification may also take place via interaction of pyruvate with H_2S (Dulaney and Hume, 1988). These investigators found that administration of pyruvic acid to mice prior to ip sodium sulfide injection reduced sulfide-induced mortality.

Beck et al. (1982, 1983) demonstrated an anesthetic-like effect of both H_2S and HCN at concentrations ranging from 5,300 ppm (7,420 mg/m^3) to 99% pure H_2S on isolated nerve preparations from the frog *Rana pipiens*. Changes in membrane function led them to suggest not only an inhibition of cytochrome *c* oxidase, but also a conformational H_2S or HS^- induced change in membrane proteins, which they suggest might account for some of the evidence of permanent nerve damage seen in some recovered victims of H_2S poisoning. The exposure concentrations used far exceed those from which victims usually recover, however. Such possible change in membrane protein conformation has not been further investigated. Other explanations for permanent nerve damage are equally plausible or more so. Examples of phenomena that have been explored include nerve cell damage as a result of anoxia (Yap and Spector, 1965; Yanagihara, 1976; Elovaara et al., 1978; Savolainen et al., 1980; Metter and Yanagihara, 1979) and damage done by ischemia following anoxia.

7.3 EXCRETION

While H_2S usually enters via the lung, this organ can also serve in an excretory capacity. Evans (1967), working with cats, showed that some of the sulfide from injected sodium sulfide was exhaled. The percentage eliminated depended on the site of injection, and the variation in injection site was related to a variation in the length of time that sulfide was free in the blood. Zero to 37% of H_2S and NaHS injected into the abdominal aorta was eliminated through the lung, while 26.5% was exhaled when sulfide was injected into the external jugular vein. The external jugular joins the vena cava, and blood flowing through it enters the pulmonary circulation almost immediately. There is little time for interaction of sulfide with blood components, or with organs whose tissues can metabolize H_2S, before it is exchanged in the lung. The abdominal aorta, in contrast, is near the beginning of the systemic circulation, and sulfide injected here has to make a full circuit of the vascular system before reaching the lung. Curtis et al. (1972) demonstrated clearly that sulfide binds to plasma proteins, primarily the albumin fraction, until it is oxidized to sulfate and excreted in the urine. The bound sulfide would not be exhaled.

Sulfate is the end-product of oxidation and is excreted in the urine (Curtis et al., 1972). A small amount of sulfide is oxidized to sulfate by sulfide oxidase, and is eliminated in the bile, appearing in the feces for excretion. The sulfate that is not excreted is widely distributed in tissues and incorporated into tissue proteins, as shown through autoradiography and other radioactive tracer methodology by Curtis et al. (1972).

The principal fate of injected sulfide is oxidation to sulfate and excretion in urine (Curtis et al., 1972). Sodium ^{35}S-sulfide administered intravenously to rats resulted in 45% of the radioactively labeled sulfur appearing in the urine as sulfate within the first 6 h after injection. Only small amounts (4.7 to 5.0%) appeared in the bile, indicating that the liver is not a major site of excretion.

Similarly, intragastric administration of ^{35}S (1.66 mg of sulfide sulfur) into rats showed that sulfate, both as inorganic and ethereal sulfur, was mainly excreted in urine. Radioactivity was also high in bone marrow. The significance of the latter finding was unclear (Dziewiatkowski, 1945).

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8. TOXICITY

8.1 ANIMAL EFFECTS

8.1.1 Introduction

Studies involving laboratory animal species have used inhalation exposures to hydrogen sulfide (H_2S), administration of sodium bisulfide (NaHS) and sodium sulfide (Na_2S) by other routes of exposure, and oral gavage in which test solutions were preparing by bubbling H_2S through water. It should be noted that administration by gavage and other noninhalation routes of exposure does not permit determination of direct effects on the respiratory tract and does not allow estimation of inhaled concentration responses on the brain, principal target organs for H_2S. Thus, the focus of this section is upon studies involving inhalation of H_2S.

While most of the toxicological observations described in the following studies are specifically attributable to H_2S poisoning, some effects are also characteristic of brain anoxia. The number and kind of cellular changes, as well as the enzymatic changes, delineated in tissues of animals exposed to low levels of H_2S correlate very closely with those observed in animals recovering from anoxia episodes (Yap and Spector, 1965; Yanagihara, 1976; Elovaara et al., 1978; Savolainen et al., 1980). While there is some evidence of other enzymes that play a role in cellular dysfunction, inhibition of cytochrome *c* oxidase is a singularly important event since tissues with the highest oxygen demand, such as neural and cardiac tissues, sustain the most rapid, consequential, and permanent damage.

8.1.2 Effects Associated with Acute Exposure

Some LC_{50} (concentration that is lethal to 50% of test animals) values for the exposure of laboratory animals to H_2S are presented in Table 8-1. Acute toxicity values for Na_2S and HS^- are intended for comparison.

Lopez (1989) carried out a study to determine if an acute lethal exposure of H_2S for less than 5 min is capable of producing life-threatening pulmonary edema similar to that observed in persons killed by accidental exposure to H_2S. Male Fischer 344 (F344) rats (10 per group) exposed to ≥1,438 ppm (≥2,000 mg/m^3) developed severe dyspnea and died

TABLE 8-1. ACUTE TOXICITY VALUES IN LABORATORY ANIMALS

Route of Administration	Species	Sex	Strain	LD_{50}/LC_{50}	Reference
H_2S					
Inhalation	Rat	M/F	Sprague-Dawley; Long Evans; Fischer 344[a]	587 ppm/2 h 501 ppm/4 h 335 ppm/6 h	Prior et al. (1988)
	Rat	--[b]	Sprague-Dawley	444 ppm/24 h	Tansy et al. (1981)
	Mouse	--	--	100 ppm/7.5 h 50 ppm/15 h 30 ppm/18.5 h	Hays et al. (1972)
Intraperitoneal	Cat	--	--	0.025 mM/kg	Evans (1967)
Na_2S					
Oral	Rat	--	Charles River	55 mg/kg[c]	Bitterman et al. (1986)
Intraperitoneal	Mouse	--	--	0.55 mM/kg[d]	Smith and Gosselin (1966)
	Mouse	M	--	0.25 mM/kg	Smith et al. (1976)
	Mouse	--	--	0.32 mM/kg	Smith and Gosselin (1979)
HS^-					
Intraperitoneal	Mouse	--	--	0.50 mM/kg	Elovaara et al. (1978)

[a]LC_{50} values are pooled for the three strains.
[b]Not reported.
[c]5 min; LD_{75}.
[d]LD_{67}.

within 5 min. Postmortem examination revealed severe edema characterized by a massive accumulation of fluid in the interstitium and bronchoalveolar spaces. Prior et al. (1989) also reported pulmonary edema in groups of 10 male F344 rats exposed to 1,000, 1,142, 1,428, or 2,000 ppm (1,400, 1,588, 1,986, or 2,781 mg/m^3) H_2S for 7 min. Responses were categorized into three stages. The first stage included avoidance of irritant gas, hyperactivity, and lethargy; the second included unconsciousness and a change in the breathing patterns; and the third was characterized by shallow breathing and gasping. Pulmonary edema developed in all animals.

To compare pulmonary injury induced by inhalation of H_2S to that caused by NaHS injection, Lopez et al. (1989) exposed Sprague-Dawley (SD) rats to 1,655 ppm (2,301 mg/m^3) H_2S, or injected them intraperitoneally with 30 mg/kg NaHS. All rats in both treatments died within 3 min, but only those exposed to H_2S showed severe respiratory distress, severe dyspnea, and presence of frothy fluids coming out of the nose and mouth. These signs were indicative of pulmonary edema and were confirmed by postmortem examination of the trachea and lungs of the rats exposed to H_2S.

Lopez et al. (1987) studied both the biochemical and cytological changes induced by H_2S inhalation in rats. Male F344 rats were exposed to 0, 10, 200, or 400 ppm (0, 14, 278, or 556 mg/m^3) H_2S for 4 h and killed at 1-, 20-, or 44-h postexposure. Both nasal and bronchoalveolar lavage (BAL) fluids were obtained to determine enzyme activities and epithelial cell morphology as markers of cellular injury. At 400 ppm (556 mg/m^3), H_2S caused a marked but transient 320% increase in lactate dehydrogenase found in the nasal lavage, and a significant increase (up to 90%) in alkaline phosphatase and aldehyde dehydrogenase in BAL. Such elevations are markers of cell death. In addition, BAL protein concentrations used as markers for changes in membrane permeability were elevated by more than 3,000% at 44-h postexposure in rats exposed to 400 ppm (556 mg/m^3) H_2S. This increase in BAL protein concentrations together with a 933% increase in gamma glutamyl transpeptidase (GGT) enzyme activity were indicators of edema. Exposure of rats to 10 or 200 ppm (14 or 278 mg/m^3) for 4 h did not cause these changes in BAL. The only significant changes caused by lower concentrations (10 and 200 ppm; 14 and 278 mg/m^3) were a 139 and 483% increase, respectively, in the cellularity of the nasal lavage fluid. Gamma GT has been found in the sputum of individuals with cariogenic pulmonary edema,

chronic bronchitis, or pulmonary embolism (Rosalki, 1975). The origin of H_2S-induced pulmonary edema was suggested to result from permeability as opposed to hydrostatic or neurogenic factors (Lopez et al., 1988b).

Male F344 rats were exposed to 0, 9.6, 198, or 387 ppm (0, 13, 275, or 538 mg/m^3) H_2S for 4 h, and four levels of the nasal cavity were examined histologically at 1, 18, and 44 h after exposure (Lopez et al., 1988b). Necrosis and exfoliation of respiratory and olfactory mucosal cells, but not squamous epithelial cells was observed in rats exposed to 387 ppm (542 mg/m^3). No nasal lesions were seen in the controls or the two lower exposure levels. Male F344 rats (4 rats/exposure level) were exposed to 0, 83, or 439 ppm (0, 115, or 610 mg/m^3) for 4 h (Lopez et al., 1988b). In rats exposed to 83 ppm (115 mg/m^3), only mild perivascular edema was observed. Rats exposed to 439 ppm (610 mg/m^3) had marked perivascular and alveolar edema, and bronchioles contained polymorphonuclear leukocytes, proteinaceous fluid, fibrin, and exfoliated cells. Necrosis of bronchiolar ciliated cells and hyperplasia of alveolar Type II cells was also observed in this group. Nasal structures were not examined. These changes, as well as pulmonary edema and fibrinocellular alveolitis, were reversible.

The nasal lesions were confined to specific areas of the nasal cavity (Lopez et al., 1986, 1987, 1988a,b). The most damage was observed in the lateral wall of the nasal turbinates in both F344 and Long Evans rats. Moreover, pulmonary lymphatics were distended and lympharrhexia was observed in the thymus and lymph nodes (Lopez et al., 1986, 1988b).

Khan et al. (1990) reported no mortalities in F344 male rats exposed to 10 to 400 ppm (14 to 556 mg/m^3) H_2S for 4 h. Mortality only occurred at levels above 500 ppm (695 mg/m^3). There were no adverse clinical signs in the 10, 50, and 200 groups (14, 70, and 278 mg/m^3); at 400 ppm (556 mg/m^3), lethargy was observed immediately following exposure. Exposure to sublethal concentrations (50 to 400 ppm; 70 to 556 mg/m^3) produced marked and highly significant depressions in the activities of cytochrome *c* oxidase and succinate oxidase complexes of the respiratory chain. The inhibition of cytochrome *c* oxidase activity in lungs was most severe (>90%) in rats that died from acute exposure to >500 ppm (>695 mg/m^3) H_2S. In rats exposed to 200 and 400 ppm (278 to 556 mg/m^3), a marked recovery in cytochrome *c* oxidase activity of lungs was observed at 24- and 48-h postexposure. In vitro studies with rat lung mitochondria showed that low concentrations of

sulfide also caused a similar and selective inhibition of cytochrome *c* oxidase activity. This effect was reversed upon removal of sulfide either by washing or by oxidation with methaemoglobin.

Khan et al. (1987a) reported a 65% decrease in cytochrome *c* oxidase and 50% decrease in succinate oxidase activities in lung mitochondria from F344 rats exposed to 500 ppm (695 mg/m^3) H_2S for 2 h. Exposure to the same level for 4 h resulted in 50% mortality and a 90 to 95% decline in cytochrome *c* oxidase activity in the lung mitochondria of dead animals. In surviving rats, cytochrome *c* oxidase activity remained 60% depressed 48 h postexposure; however, full recovery was evident within 2 weeks. Khan et al. (1987b) showed that various sulfur-containing compounds such as S^{2-} inhibited enzyme activities in bovine erythrocytes in a dose-dependent manner. At a concentration of 10 mM, superoxide dismutase activity was inhibited by 48%; at 0.2 mM, the activity of catalase was inhibited by 25.7%.

Khan et al. (1991) exposed male F344 rats (6/group) to 0, 50, 200, and 400 ppm (0, 70, 278, and 556 mg/m^3) H_2S for 4 h. After anesthesia and exsanguination, lungs were lavaged and alveolar macrophages (AM) were collected. Although treatment had no effect on basal respiratory rates of AM, a significant decrease in cell viability was observed in the high exposure group.

Green et al. (1991) investigated the effect of acute exposure of H_2S on lung surfactant in F344 rats. Decreases in surfactant could increase fluid transport to lungs resulting in reduction in gas exchange and could result in increased surface tension within the lungs. Groups of 6 male rats were exposed for 4 h to actual concentrations of 194 and 290 ppm (270 and 403 mg/m^3). Controls were exposed to filtered air. At 1-h postexposure, animals were sacrificed and the right lung was lavaged. Samples of the left lung were assessed by light microscopy.

Exposure to 194 ppm (270 mg/m^3) produced no adverse clinical signs or visible gross changes in the lungs. There was, however, a statistically significant increase in protein and lactic dehydrogenase in BAL compared to controls. Microscopic evaluation revealed focal areas of perivascular edema. Animals exposed to 290 ppm (403 mg/m^3) were visibly stressed during and immediately after exposure. At necropsy, lungs exhibited focal areas of red

atelectasis, and alveolar edema with substantial perivascular and peribronchial interstitial edema.

The minimum surface tension values for BAL from controls and animals exposed to 194 ppm (270 mg/m^3) H_2S were nearly identical. By contrast, exposure to 290 ppm (403 mg/m^3) H_2S resulted in a substantial increase in minimum surface tension and lowered stability. The effects of H_2S on surfactant activity were considered to be due to the leakage of serum proteins into the alveoli.

Prior et al. (1988) examined the exposure-time relationships in male and female SD, Long Evans, and F344 rats. All three strains were exposed to H_2S for 2, 4, or 6 h. There was a significant, sex-related difference in mortality and body weight loss; the males were more sensitive (30%) than females (20%) in all three strains. Body weight loss was also increased proportionately to H_2S concentration. For every 10-ppm increase in exposure concentration, the body weight loss increased by 0.21 g greater in males than in females and was different between strains (F344 < SD < Long Evans). Marked differences were seen in LC_{50}/LC_{10} ratios for the length of exposure: 587/549 ppm for 2 h, 501/422 ppm for 4 h, and 335/299 ppm for 6 h. These results are in agreement with those reported by Lopez et al. (1987), which indicate that once H_2S reaches its threshold, pulmonary edema and death ensue.

Substance P (SP), an endogenous neurotransmitter, has a protective role in H_2S poisoning (Prior et al., 1990). Rats were injected with capsaicin, the irritant found in red pepper that depletes the body of SP and other tachykinins, and subsequently exposed to 400 ppm H_2S for 4 h. There was 100% mortality, but no mortalities in control rats injected with saline. Animals depleted of SP exhibited a significantly greater degree of bronchial epithelial cell exfoliation and ulceration following H_2S exposure. These results with capsaicin suggest a role for afferent C-fibers in pulmonary defense against H_2S exposure.

Husain (1976) and Husain and Zaidi (1977) investigated various enzyme activities in H_2S-treated lung homogenates from albino rats. Homogenates were exposed to H_2S for 1 h prior to measurement of enzyme activities. At 18 ppm (25 mg/m^3), H_2S inhibited acid phosphatase, alkaline phosphatase, glutamic-pyruvic transaminase, glutamic-oxaloacetic transaminase, and ATPase by 16.8, 11.0, 25.9, 15.9, and 13.3%, respectively. As the H_2S concentration increased, the inhibition of these enzymes also increased. Fructose

1,6-diphosphate aldolase activity was unaffected by H_2S, while arginase activity was stimulated with increasing gas concentrations. The authors postulated that metallo-sulfate complexes are formed from the interaction with H_2S, and that H_2S also combines with the enzyme cofactor pyridoxal phosphate in the case of the transaminases. Such interactions with enzymes other than cytochrome *c* oxidase could contribute to possible cumulative cellular damage from either long-term, low-level, or repeated exposure to H_2S gas. However, direct evidence for the formation of such complexes is lacking. Exposure of rabbits to 72 ppm (100 mg/m^3) H_2S for 30 min/day, for 7, 10, and 14 days resulted in relative increases in activities of acid and alkaline phosphatase, ATPase, and deoxyribonuclease-II in lungs (Jonek and Konecki, 1966).

Yang and Hulbert (1990) observed that exposure of guinea pigs for up to 1 h to H_2S concentrations up to 500 ppm (695 mg/m^3) did not alter airway resistance or dynamic compliance. However, there was an increase in airway responsiveness to methacholine in animals exposed to 300 and 500 ppm (417 and 695 mg/m^3). Airway responsiveness to methacholine was also increased when Cam-Hartley guinea pigs were exposed to 100 ppm (139 mg/m^3) H_2S for 1 h (Hulbert et al., 1989).

Cralley (1942) found a correlation between the irritation of rabbit throats by H_2S and other irritant gases, and the suppression of mucociliary activity of the trachea. Exposure of rabbits to 400 and 600 ppm (556 and 834 mg/m^3) for 10 and 5 min, respectively, resulted in the cessation of ciliary motility without recovery in air.

Haggard et al. (1922) demonstrated the dramatic lethal effect of H_2S, as well as striking differences in the dose response, when dogs were exposed to concentrations of 0.05, 0.1, and 0.3% H_2S by volume (500, 1,000, and 3,000 ppm, respectively). At 500 ppm (695 mg/m^3) (considered to be the minimal lethal concentration), the respiratory rate of the animals showed a slight, progressive decrease. The depth of respiratory rate was also progressively depressed. Death resulted from pulmonary edema after many hours (not reported) of exposure. At 1,000 ppm (1,400 mg/m^3), death ensued within 15 to 20 min of exposure. Respiration was immediately stimulated as the dogs inhaled the gas, which led to strong hyperpnea; this was followed by cessation of breathing and death. When the concentration of H_2S was increased to 3,000 ppm (4,200 mg/m^3), respiratory arrest occurred after a few gasps.

When dogs were injected iv with Na_2S, pulmonary edema was not observed (Haggard et al., 1922), but dogs exhibited immediate hyperpneic breathing. This was followed by variable periods of apnea, which was relieved by artificial ventilation. Haggard et al. (1925) indicated that vagotomy eliminated the stimulatory effects of H_2S on respiration.

Inhalation of H_2S, leading to elevated sulfide levels in brain tissue, has been reported to result in histopathological damage and changes in neurotransmitter levels which alone or together may be responsible for the CNS effects observed in humans.

Citing a Norwegian report (Savolainen, 1982), the World Health Organization (1987) reported that acute H_2S intoxication caused brain edema, as well as degeneration and necrosis of the cerebral cortex and the basal ganglia in rhesus monkeys. Exposure parameters were not specified. Effects of H_2S on brain tissue of monkeys were also reported by Lund and Wieland (1966).

Lund and Wieland (1966) found that inhalation of H_2S resulted in pronounced histopathological changes in brain tissues of rhesus monkeys. A lethal exposure of one monkey to 500 ppm (695 mg/m^3) H_2S for 35 min caused no pathologic changes in fixed and stained tissue sections of brain, kidneys, adrenal glands, or heart. However, necropsy revealed a severely hyperemic liver and dilation of the blood vessels. The second monkey was exposed to 500 ppm (695 mg/m^3) H_2S for 35 min until breathing ceased; it was revived, exposed again until it lost consciousness, and then revived. At 5 days postexposure, it was sacrificed and the tissues were examined. Histologic examination of the brain revealed spotty regions of altered cells and a noticeable vascularization in the region of the basal ganglia, in the upper parts of the putamen, and on the caudate nucleus. The lesions characteristically had newly formed capillaries and increased glial formation. The cortex of the occipital lobe was altered, with lamellar separations between the lower layers of cortex. The smaller blood vessels of the cortex were hyperemic. Necrosis of the parenchymal cells of the cortex was evident. No pathologic lesions were seen in tissues other than the brain. The liver, however, was severely hyperemic. The third monkey was exposed in similar fashion; however, exposure was interrupted after 22 min. Spontaneous respiration never ceased, but the monkey was somnolent, ataxic, anorexic, and relatively immobile, and exhibited uncoordinated movements. The animal showed only slight improvement and was sacrificed after 10 days. Examination of the brain showed damage in the basal ganglia, an increase in

glia, and spotty lesions of the cortex in the parietal and occipital lobes. There was a decrease in Purkinje cells in the cerebellum. No pathologic lesions of the kidneys, adrenals, heart, or liver were seen.

Administration of NaHS by ip injection of SD rats (10 or 30 mg/kg) resulted in increases in amino acid levels in brainstem tissue, while other brain regions were unaffected. Since some of these amino acids (e.g., taurine) may be involved in neuronal control of breathing, it was speculated that alteration of amino acid neurotransmitter levels may result in H_2S-related arrest of the respiratory drive (Kombian et al., 1988). Taurine has been shown to depress respiration upon intraventricular administration (Mueller et al., 1982; Wessberg et al., 1983). However, decreases in amino acid (including taurine) levels in brainstems of SD rats, but not ICR mice, following repeated ip dosing with NaHS, was reported by Reiffenstein and Warenycia (1987). Warenycia et al. (1989c), using patch clamp studies of neuroblastoma cells, found that addition of taurine or cysteic acid in the presence of NaHS resulted in reversible abolition of inward sodium currents. Neither compound alone had any effect. The sulfhydryl agents, β-mercaptoethanol and dithiothreitol also reversibly abolished sodium currents.

Warenycia et al. (1989a) measured the brain sulfide levels in rats following inhalation of H_2S or ip injection of NaHS. Male SD rats either were exposed to 1,650 ppm (2,294 mg/m^3) H_2S until death (time to death, 4.9 min $\pm$ 1.4 min) or injected ip with various concentrations of NaHS with corresponding sulfide levels ranging from 7.5 to 50 mg/kg and sacrificed 2 min later. The brainstem and cortex of control rats were found to contain endogenous levels of sulfide; corresponding values were 1.26 and 1.66 μg/g. Injection of NaHS resulted in sulfide levels of 4.42 and 4.76 μg/g in the brainstem and cortex, respectively. Analysis of the brainstem, cerebellum, hippocampus, striatum, and cortex revealed a high correlation between brain sulfide levels and corresponding NaHS dose levels. Brain sulfide levels in rats inhaling H_2S were approximately 10% of those expected from the dose administered. These low amounts reflect either the extreme lethality of H_2S or the probable metabolism and possible formation of nonlabile species of sulfide. Subcellular fractionation clearly demonstrated that H_2S exposure resulted in sulfide uptake into nerve cells, as evidenced by an increase of sulfide content in the synaptosomes, mitochondria, and myelin. It was concluded that the brainstem selectively accumulates H_2S and that this

preferential accumulation may, in part, account for the lethal action of H_2S on respiratory centers (Warenycia et al., 1987).

Warenycia et al. (1989b) found that administration of NaHS to rats at 30 mg/kg resulted in significant increases in regional catecholamine levels in brain. This dose was described as $2 \times LD_{50}$. The hippocampus, striatum, and brainstem all showed increases in nor-adrenaline and adrenaline. In vitro studies showed that NaHS inhibited monoamine oxidase.

Elovaara et al. (1978) demonstrated a marked decrease in mouse brain protein synthesis after a 2-h exposure to 100 ppm (139 mg/m^3) H_2S, as evidenced by a decrease in ^{14}C-leucine incorporation. In subsequent experiments, Savolainen et al. (1980) found that this decrease in protein synthesis correlated with an increasing inhibition of cerebral cytochrome *c* oxidase when mice were repeatedly exposed to 100 ppm (139 mg/m^3) H_2S for 2 h at 4-day intervals. Nicholls (1975) showed that H_2S forms a heme-sulfide complex, which is very slow to dissociate ($K_i \approx 0.02$ μM for H_2S). Repeated exposure to the gas would cause increasing numbers of complexes to form, resulting in less oxidative metabolism in the affected cells. The limiting factor in recovery would be the rate of synthesis of new heme (Shanley et al., 1977). While these studies indicate a cumulative effect on the brain resulting from H_2S exposure, similar damage is seen as a result of anoxic episodes (Yap and Spector, 1965; Yanagihara, 1976). In anoxia, there is a decrease in protein synthesis as well as RNA synthesis, and a decrease in the formation of polyribosomal complexes (Yanagihara, 1976).

Higuchi (1977) studied the effects of exposure to H_2S on rat behavior. Rats were exposed to concentrations of 100 to 500 ppm (139 to 695 mg/m^3) H_2S. At 200 ppm (278 mg/m^3), there was an immediate inhibition of discriminated avoidance response; at 300 to 500 ppm (417 to 695 mg/m^3), the Sidman-type avoidance response was also inhibited.

Exposure of guinea pigs to 20 ppm (28 mg/m^3) H_2S, 1 h/day, for 11 days was shown to cause significant reduction in total lipids (14 to 34%) and phospholipids (11 to 21%) of the cerebral hemisphere and brainstem tissues (Haider et al., 1979, 1980). Levels of amino acids were not determined. The associated increase (18%) in malonaldehyde in the cerebral hemisphere suggest peroxidation of polyunsaturated lipids. Exposure of rabbits to 72 ppm (100 mg/m^3) H_2S for 1 h/day, for 2 days resulted in a reduction in adenosine triphosphatase (ATPase) and alkaline phosphatase in brain tissue (Kosmider and Zajusz, 1966).

Dahne and co-workers (1983) examined the brains of eight cattle whose survival time after H_2S poisoning ranged from 18 h to 10 days. Histological examination of the brain disclosed spotty regions of neuronal necrosis with vascular proliferation and gliosis in the basal ganglia. Laminar necrosis of the cerebral cortex was also noted, particularly in the occipital cortex. Up to 60 h after intoxication, bilaterally symmetrical lesions were seen in the dorsal neocortex and, to a somewhat lesser degree, in the cornu Ammonis of the hippocampus, the lateral geniculate nucleus, the globus pallidus, the caudate nucleus, and the cerebellar Purkinje cell layer. These lesions were characterized by eosinophilic neuronal necrosis and astrocytic edema, and were accompanied by low-grade edema of the white matter. After 10-days postexposure, the lesions had progressed to laminar necrosis with resorption of necrotic tissue by macrophages. The lesions described in this study are similar to those seen in systemic hypoxia and in intoxications that impair tissue utilization of oxygen, such as carbon monoxide poisoning.

Doses in the range of 20 μmol/kg Na_2S injected intravenously into cats caused immediate hyperpnea, which was often followed by permanent respiratory arrest (Evans, 1967). If the carotid sinus region was locally anesthetized, the hyperpnea did not occur; however, in a single trial where the sulfide was injected into the ascending aorta allowing interaction with the aortic chemoreceptors, hyperpnea still occurred.

Hays et al. (1972) exposed cows, goats, and mice to various concentrations of H_2S. Each animal served as its own control. The LC_{50} for mice is reported in Table 8-1. Body weight and food and water consumption were measured in all animals. Rectal temperature was measured in mice and goats, heart rate in goats and cows, and milk production in cows. Plasma cortisol concentration in goats and carbonic anhydrase activity and phenobarbital sleeping time in mice were also recorded. Goats were individually exposed, but data were pooled in experimental or control groups of three to five animals.

All goats exhibited an initial decrease in food and water consumption; one goat/group developed a fever and one goat exposed to 100 ppm (139 mg/m^3) died after 19 h of exposure. All goats exhibited eye irritation, coughing, shivering, and a reduction in urinary output. Some goats exhibited an increased heart and/or respiratory rate; a 50% mean increase in plasma cortisol levels was observed in animals exposed to 100 ppm (139 mg/m^3) H_2S. Cows exposed to 20 to 50 ppm (28 to 69 mg/m^3) exhibited lacrimation and discomfort,

and an alteration in normal body function. No change in milk production was observed. It was suggested that exposure to low levels of H_2S for short periods of time results in irritation and facilitates the establishment of bacterial infection.

Limited information suggests that H_2S decreases the ability of animals or humans to withstand infection. Rogers and Ferin (1981) exposed male Long Evans rats in nose-only exposure chambers to 45 ppm (63 mg/m^3) H_2S for 2, 4, or 6 h. Immediately following exposure, rats were anesthetized and challenged with a 30-min staphylococcal (coagulase negative *Staphylococcus epidermidis*) aerosol through a nose-only exposure chamber. Rats were sacrificed at 30 min (time 0), 3-h, and 6-h postbacterial challenge. Exsanguinated lungs were homogenized, plated, and grown on a selective growth medium for staphylococci, and colonies were counted. Rats exposed for 4 h to H_2S had 6.5-fold greater percent colony-forming units (CFU) than controls, while the 6-h H_2S-exposed group had a 52-fold greater percent CFU than controls. Since there was no evidence of pulmonary edema to promote bacterial growth, it was concluded that H_2S significantly affected the antibacterial system of the rats by impairing AMs.

8.1.3 Effects Associated with Repeated Exposure

Hulbert et al. (1989) exposed F344 rats (9/sex/group) to 1, 10, or 100 ppm (1.4, 14, or 139 mg/m^3) H_2S, 8 h/day, 5 days/week for 5 weeks. The only significant histopathological difference was an increase in tracheal ciliated cells in animals from the 100 ppm (139 mg/m^3) group. Measurement of airway resistance and dynamic compliance after animals were anesthetized indicated there was no effect of exposure on these parameters. However, some exposed rats in each group responded to a 10-fold lower dose of methacholine.

In 90-day inhalation toxicity studies conducted for the Chemical Industry Institute of Toxicology on SD rats (Toxigenics, 1983c), F344 rats (Toxigenics, 1983b) and B6C3F1 mice (Toxigenics, 1983a), animals (15/sex/exposure group) were exposed to 0, 10.1, 30.5, or 80 ppm (0, 14, 42, or 111 mg/m^3) H_2S for 6 h/day, 5 days/week.

This highly detailed study included neurologic function tests assessing posture, gait, and tone of facial muscles, and examined pupillary, palpebral, extensor thrust, and crossed-extensor thrust reflexes, before and after exposure. Eyes were examined with both a monocular ophthalmoscope and a slit-lamp biomicroscope at the end of the exposure period.

Extensive clinical pathology included blood volume, appearance, urine specific gravity, protein, pH, ketone, and glucose. Hematologic parameters and serum chemistry parameters were determined. Detailed necropsy examination was performed, individual major organs were excised, and tissues were collected and examined microscopically. These included the brain (cerebellum and two levels of cerebrum, medulla, optic nerve), spinal cord (cervical, thoracic, and lumbar—two sections each), peripheral nerves (sciatic and anterior tibial, with remaining sciatic nerve removed and stored in buffered formalin), eyes, pituitary, thyroid, parathyroid, salivary glands (submaxillary), heart, lungs (four levels), spleen, liver, pancreas, adrenals, lymph nodes (mesenteric and mandibular), kidneys, bladder (inflated with formalin), lacrimal glands, ovaries, uterus, oviducts, vagina, cervix, stomach, small intestine (duodenum, jejunum, ileum), large intestine (large and small colon and caecum), skeletal muscle (thigh), skin, mammary glands (males and females), bone (femur), bone marrow (smear and section), aorta, ear canal with zymbal gland, nasal turbinates (four levels), trachea, testes, epididymis, esophagus, thymus, prostate, seminal vesicle, and any gross lesion(s).

In addition, a neurological study was performed on the two strains of rats. Male and female rats (5/sex/strain) from each exposure and control group were used. Following anesthesia with sodium pentobarbital, rats were perfused with glutaraldehyde and the intact animal was then refrigerated at approximately 4 °C overnight. The right and left sciatic nerve and their branches were dissected together with specimens of the cervical and lumbar spinal cord and placed in a 4% glutaraldehyde solution. Specimens were examined by routine light microscopy for evidence of pathologic change. The control and highest exposure groups were examined initially. If changes were detected, lower exposure groups were examined.

In mice, the only exposure-related histopathological lesion was inflammation of the nasal mucosa in the anterior segments of the nose which was observed in 8/9 male mice and in 7/9 female mice in the group exposed to 80 ppm (111 mg/m^3). This lesion was also present in two high dose mice that died during the course of the study. The lesion was generally minimal to mild in severity and was located in the anterior portion of the nasal structures, primarily in the squamous portion of the nasal mucosa, but extending to areas covered by respiratory epithelium. This lesion was not observed in any animals in the other

exposure groups. Thus, 80 ppm (111 mg/m^3) is considered a LOAEL for nasal inflammation in mice, while 30.5 ppm (42 mg/m^3) is the corresponding NOAEL.

Significant reductions in body weight gain were noted in all exposure groups at various times during the study. Decreased weight gain in animals exposed to 80 ppm (111 mg/m^3) occurred consistently in both male (approximately 90% of control during last 7 weeks of study) and female (<90% of control during last 3 weeks of study) mice.

A significant reduction in body weight gain was noted in all rats exposed to 80 ppm (111 mg/m^3). In F344 and male SD rats, mean body weights were never <93% of control. In female SD rats, the effect on body weight was statistically significant at various time points in all exposed groups, but mean body weight in the 80 ppm (111 mg/m^3) group was <90% of the control groups during most of the study. Statistically significant changes in absolute kidney, liver, and spleen weight were also observed in the male rats exposed to 80 ppm (111 mg/m^3), but no differences were apparent when organ weights were normalized to body weight. Brain weight was significantly reduced in the male SD rats in the high-exposure group and slightly, but not significantly reduced in females (Toxigenics, 1983c). There were no exposure-related clinical signs in rats. Neurologic function examinations yielded negative results. Blood volume, appearance, occult blood, urine specific gravity, protein, pH, ketone, and glucose values were all normal. Ophthalmoscopic examination, hematology, serum chemistry parameters and urinalysis were also normal. Histopathological examination, which included four sections of the nasal turbinates, revealed no abnormalities in comparison with controls.

The effects of a 3-mo exposure of rats to H_2S were reported by Fyn-Djui (1959). Groups of 10 male white rats were exposed to 0, 0.14, or 7.14 ppm (0, 0.2, or 10 mg/m^3) H_2S, 12 h/day. Body weights were measured, and motor function was observed. At study termination, gross necropsy was performed on two rats/group.

Exposure to 0.14 ppm (0.19 mg/m^3) produced no changes in body weight; however, motor chronaxy changes were observed. At 7.14 ppm (9.9 mg/m^3), a decrease in body weight was seen and similar changes in motor chronaxy were noted. It was suggested that these fluctuations in the extensor and flexor chronaxy were the result of a cerebral cortex effect and were indicative of changes in the functional state of the brain. Necropsy of rats exposed to 7.14 ppm (9.9 mg/m^3) revealed irritation of the tracheal and bronchial mucosa;

less pronounced irritation was observed in animals exposed to 0.14 ppm (0.19 mg/m^3). Brain cortex changes in the animals at 7.14 ppm (9.9 mg/m^3) consisted of prickled, thickened, and swollen dendrites.

Significant weight loss was observed in monkeys, rats, and mice exposed to 20 ppm (28 mg/m^3) for 3 mo; however, CNS disorders were not evident (Sandage, 1961). When exposed to concentrations of 20-25 ppm (28 to 35 mg/m^3) H_2S for 150 days, one rabbit lost weight and four others exhibited variable weight gain. Gamma albumin was also increased (Kuwai, 1960).

Wakatsuki (1959) exposed groups of rabbits (number, sex, and strain not reported) to 100 ppm (139 mg/m^3) H_2S, 300 ppm (417 mg/m^3) carbon disulfide (CS_2), or a combination of the two gases, 30 min/day for 4 mo. Clinical observations made during the study and continuing for 4 mo postexposure included general conditions, body weight, peripheral blood picture, serum calcium, blood specific gravity, total serum protein, and serum protein fraction.

Rabbits exposed only to H_2S exhibited comparatively slight changes and no measureable abnormal findings in general condition, body weight, number of erythrocytes, serum calcium, total serum protein, and serum protein fraction. Slight changes such as oligochromemia, reticulocytosis, leucopenia, decrease of pseudoacidophilic cells, relative lymphocytosis, and an increase in toxic granules were observed; however, recovery was complete within 4 mo. Rabbits exposed to a combination of the two gases exhibited more pronounced effects, and complete recovery was not observed.

Kosmider et al. (1967) exposed rabbits to 71 ppm (100 mg/m^3) for 1 to 5 h (until they lost consciousness) or for 0.5 h/day for 5 days. Electrocardiograms revealed disorders of repolarization in acutely exposed animals. Repeated exposure resulted in arrhythmias in the form of ventricular extrasystoles, bigeminal rhythms, and disorders of ventricular repolarization manifested as flattened T-waves. When animals with H_2S-induced arrhythmias were treated with calcium-binding compounds, such as sodium citrate, normal rhythms were restored. Arrhythmias returned in several instances, and repeated doses of sodium citrate had to be used after several hours to restore physiologic rhythms.

Kosmider et al. (1967) followed these experiments with histochemical studies. Fragments from the apical region of the heart and heart vasculature were examined for the

activity of two enzymes. They found that ATP phosphohydrolase activity in blood vessels and the sarcolemma of the heart muscle cells was decreased in exposed animals as compared with controls. Nicotinamide adenine dinucleotide phosphate, reduced (NADPH) oxidoreductase activity in heart muscle cells and vascular endothelium was likewise reduced. It is not possible to distinguish whether these effects result directly from H_2S toxicity on the cells examined or whether they are secondary effects of H_2S poisoning of the whole animal. The authors state that these effects are the result of H_2S action directly on the heart.

Changes in activity of these enzymes affected the active transport of sodium and potassium ions in the heart muscle cells and the walls of blood vessels. These changes led to changes in concentrations of these ions across heart cell membranes, which in turn caused changes in electrical activity. These changes can account for the observed differences in rhythm and repolarization in the experimental animals. The significance of these observations is that changes in heart function may be the direct response of the heart cells to H_2S exposure, rather than a secondary response elicited by the action of the nervous system on the heart. Since other enzyme activities were not measured and in vitro enzyme assays were not done, it is unclear whether the decrease in activities is directly attributable to action of H_2S on the enzymes, or to interference with oxidative metabolism by the gas.

Lowering of alkaline phosphatase and succinate dehydrogenase in heart tissue was reported in rabbits exposed to 72 ppm (100 mg/m^3), 1 h/day, for 7 or 14 days (Dwornicki, 1979).

A series of studies conducted by Renne et al. (1980) and reported in preliminary form investigated the potential toxic and synergistic effects of exposure to geothermal effluents (H_2S and ammonia). In the first study, groups of 10 rats and 10 guinea pigs (sex, strain, and number not reported) were exposed for 7 days to 100 ppm (139 mg/m^3) H_2S, 250 ppm (174 mg/m^3) NH_3, or a combination of the two gases. Complete necropsies were performed on all animals. No significant histopathological lesions or clinical pathological alterations were observed.

A subsequent 7-day exposure to 220 ppm (306 mg/m^3) H_2S, 250 ppm (174 mg/m^3) NH_3, or a combination of the two gases resulted in a significant increase in the incidence of respiratory tract lesions in guinea pigs. Mild interstitial pneumonitis was observed in 70% of guinea pigs exposed to a combination of H_2S and NH_3 compared to 30% of controls and a

40% incidence in groups exposed to either gas alone. An increased incidence of mild acute suppurative tracheitis and laryngitis and mild chronic nephritis was observed in all groups. The increased incidence of respiratory tract lesions appeared to be treatment-related; however, the significance of the increased incidence of chronic nephritis was not known.

Curtis et al. (1975) exposed groups of three pigs (sex and strain not reported) to 8.5 ppm (12 mg/m^3) H_2S, 24 h/day for 17 days, or 2 ppm (2.8 mg/m^3) H_2S in combination with 50 ppm (35 mg/m^3) NH_3, 24 h/day for 19 days. No statistically significant changes in body weight gain or respiratory tract structure were observed.

The 1982 Lodgepole, Alberta, Canada, gas well blowout exposed farm animals to levels of 10 to 15 ppm (14 to 21 mg/m^3) H_2S as well as to other gaseous constituents of the well effluent. Members of the community described problems in cattle, pigs, horses, and household pets. They noted that the animals exhibited runny noses and eyes, coughing, and decreased food intake. Most cattle in the exposed area were affected, young animals showing more severe signs of irritation of mucous membranes than old. Residents also indicated that some animals exhibited diarrhea, red stools, red urine, and decreased weight gain. A local veterinarian and members of five families described an almost total disappearance of small wild animals and birds; these did not reappear for a "long time" after the blowout had been controlled (Lodgepole Blowout Inquiry Panel, 1984; Herbert, 1985).

The Alberta Environmental Centre staff reported some "significant" changes in the activity of certain enzymes in the blood of cattle exposed to emissions from the Lodgepole blowout. The changes were not characterized further. The enzymes superoxide dismutase, glutathione peroxidase, glucose-6-phosphate-dehydrogenase, acetylcholine esterase, and aspartase aminotransferase were found to be involved in the detoxification of H_2S or otherwise affected by it (Beck, 1985). The changes appeared to be transient and reversible, and their importance and possible relationship to clinical disease in the exposed animals are not known (Harris, 1986).

Calves continually exposed to 20 or 150 ppm (28 or 208 mg/m^3) H_2S for 7 days exhibited a number of clinical signs (Nordstrum, 1975; Nordstrum and McQuitty, 1976). At 20 ppm, toxic effects included distress, lethargy, restlessness, occasional diarrhea and vomiting, coughing, irregular respiration and dyspnea, photophobia, keratitis, corneal opacity, nasal irritation, and epistaxis. These signs were also observed at 150 ppm

(208 mg/m^3); in addition, calves exhibited severe keratoconjunctivitis, clinical blindness, reduced food and water consumption, and elevated temperature.

Similar findings of eye and respiratory irritation in cattle and horses were reported by a veterinarian following a well blowout in 1984 (Drummond 6-30 Sour Gas Well Blowout). The Alberta Environment Centre and Alberta Agriculture staff conducted followup research of the livestock on 16 farms beginning the day following the blowout and continuing over the next 3 mo. Owners of livestock were contacted a year later to determine if any unusual health problems had occurred. Immediate complaints following the blowout generally consisted of irritation of ocular and respiratory membranes, respiratory disease (pneumonia), reduced exercise tolerance, and reproductive failure. The investigation team concluded that eye and respiratory irritation could be attributed to exposure to the wellhead emissions and may have made animals more susceptible to the effects of infective keratoid conjunctivitis (pinkeye) and infective respiratory disease (pneumonia). Decreased exercise tolerance of horses, and loss of weight, condition, and appetite may have been caused by exposure to gases. No consistent patterns of animal disease could be identified. Hydrogen sulfide concentrations ranged from 0.01 to 3.50 ppm, (0.014 mg/m^3 to 4.90 mg/m^3), with a mean concentration over the 4 days of the episode of 0.36 $\pm$ 0.57 ppm (0.51 $\pm$ 0.80 mg/m^3) (Alberta Agriculture, 1986).

The effects of H_2S have also been examined in a study involving oral gavage with solutions prepared by bubbling H_2S through water (Arthur D. Little, Inc., 1987). Sprague-Dawley rats (20/sex/dose) were administered solutions containing 1.0, 3.5, or 7.0 mg/kg/day H_2S, once daily, 7 days/week, for 89 days. The principal findings were dose-related clinical signs of restlessness (males) and salivation (females). These signs occurred also in the low exposure group. Significant increases in mortality occurred in the high dose group (males only). There were no adverse, dose-related histopathological findings in the respiratory tract or other organs.

8.1.4 Chronic Toxicity

No chronic toxicity studies were found in the available literature.

8.1.5 Effects on Respiration Control Receptors

Carotid sinus chemoreceptors (carotid bodies) may play a role in stimulating the ventilatory reflex upon interaction with blood sulfide at sublethal levels. Both the rate and depth of ventilation increase to the point of hypernea. Heymans et al. (1931, 1932) showed that injecting a small amount of Na_2S into the common carotid artery of dogs resulted in hyperpnea. After denervation of the sinus by transection of the sinus nerve, larger doses of sulfide had no immediate effect on respiration, and the late effect was respiratory depression. Injection of Na_2S into the internal carotid or vertebral arteries had the same effect as denervation. The sulfide would be diluted by the general circulation and metabolized before it reached the chemoreceptors.

These results were confirmed by the use of cross-perfusion techniques, in which isolated carotid sinuses of a recipient dog received the entire blood supply from a donor dog (Heymans et al., 1931, 1932). Sodium sulfide injected into the recipient dog's general circulation elicited no stimulatory effect on respiration, since the carotid chemoreceptors were not part of its circulation. However, the donor dog, whose blood perfused the recipient's chemoreceptors, elicited the response when injected systematically with Na_2S. A similar, although secondary, response was observed with the aortic chemoreceptors (Heymans and Neil, 1958).

However, the effect on carotid and aortic bodies seems inconsistent with the depressant effect on the central nervous system (CNS). Early researchers of this phenomenon did not offer an explanation for this seeming contradiction, yet clearly ascertained that it existed (Haggard et al., 1922; Heymans et al., 1931, 1932; Evans, 1967). It is possible to resolve this paradox if the normal function of the carotid and aortic bodies is examined together with the cellular effect of H_2S.

The physiological function of the reflexes associated with the chemosensors of the carotid and aortic bodies is to maintain a ventilation rate and depth that is adequate for supplying tissue cells with oxygen. The chemosensors are primarily sensitive to the partial pressure of oxygen (pO_2), or oxygen tension, in blood flowing through the carotid sinuses and the aortic arch. Under normal conditions, no oxygen is removed from the blood before it reaches these vessels; therefore, the pO_2 is between 100 and 104 mmHg and the hemoglobin is saturated with oxygen. Oxygen tension must decrease considerably for the

reflexive increase in ventilation to be activated. The carotid and aortic chemosensors do not respond with rapid impulse firing until the pO_2 falls into the range between 30 and 60 mmHg (Biscoe, 1971). Such a decrease normally occurs only with hypotension if the systolic arterial blood pressure falls below 80 mmHg. When the oxygen tension falls together with blood pressure, the chemosensors, in concert with the baro- or pressure sensors in the same blood vessels, initiate reflexes to increase the rate and depth of ventilation and blood pressure; this can lead to restoration of normal pO_2 under normal circumstances.

This same response is seen in sublethal H_2S poisoning; however, it also inhibits neural function. Hydrogen sulfide most rapidly affects the intracellular mitochondrial enzyme cytochrome *c* oxidase, interfering with the transfer of electrons and hydrogen ions to oxygen, thus blocking oxidative metabolism. Cells that are dependent on oxidative metabolism, and/or those having a high oxygen demand such as those of the nervous system or the heart, would be most rapidly and severely affected. In the case of the carotid and aortic chemoreceptors, halting of oxidative metabolism has the same effect as a decrease in oxygen supply. As oxidative metabolism in these highly sensitive nerve endings ceases, they respond with rapid-fire impulses to the respiratory centers, initiating the reflexive increase in rate and depth of ventilation. Reflexive hyperpnea is therefore a logical consequence of the inhibition of cytochrome *c* oxidase in the chemosensors of the carotid and aortic bodies by H_2S (Ammann, 1986).

8.1.6 Cellular Mechanism(s) of Toxicity

Reiffenstein (1989) investigated the cellular mechanism(s) of sulfide intoxication in rat brains using neurochemical and neurophysiological approaches. Several "model" systems were utilized, including the in vitro hippocampal slice (considered to be the best model for studying human "knockdown" seen in H_2S poisoning) and the in vivo iontophoresis of HS^- onto single hippocampal pyramidal cells to test the effects of H_2S on the rates of spontaneously firing neurons. Within 2 min of an intraperitoneal injection of 10 or 30 mg/kg NaHS (corresponding to LD_{30} and LD_{99} doses), there were significant increases in brainstem aspartate, glutamate, taurine, gamma aminobenzoic acid (GABA), and alanine neurotransmitter concentrations. However, no changes were found in the cerebral cortex, striatum, and hippocampus. At the low dose, aspartate (an excitatory neurotransmitter) and

glycine (an inhibitory neurotransmitter) levels were decreased in the cerebellum, and the glutamine (precursor of glutamate) level was elevated in the brainstem. In contrast to these findings, Kombian et al. (1989) using the push-pull perfusion technique, observed no changes in brainstem glutamate, aspartate, glycine, or GABA levels following a 15-mg/kg intraperitoneal injection of NaHS. However, this dose caused a delayed decrease in the release of glutamine to 61% of the control ($p < 0.05$). At 3 μg/mL NaHS (the physiological level is around 2 μg/mL), there was a 62% decrease in the glycine level when compared to controls. Such a decline in the concentrations of an inhibitory neurotransmitter in the brainstem area can lead to unopposed excitatory events and final loss of respiratory drive.

At the LD_{99}, only 5-hydroxytryptamine (5HT) and dopamine levels were increased in the brainstem, whereas epinephrine and norepinephrine levels were increased in the hippocampus and striatum (Reiffenstein, 1989). In another study, Reiffenstein et al. (1988) found that the levels of the latter neurotransmitters together with dopamine were higher than controls in the brainstem region. It was suggested that the HS^--induced increase in catecholamine levels was due to the inhibition of monoamine oxidase (MAO) enzyme function. A similar conclusion was derived in the 1989 study where H_2S at high doses inhibited MAO in vivo as well as in vitro. Dithiothreitol at 0.1 mM was able to restore enzyme activity by over 400% (Warenycia et al., 1989).

In vivo iontophoresis of 30 to 50 nA HS^- onto hippocampal pyramidal cells blocked the spontaneous firing of these cells; however, very low doses (2 to 10 nA) gave the opposite results (i.e., the firing rate was increased). In in vitro studies using intracellular microelectrode recordings from CA1 pyramidal cells, 27 to 200 μM NaHS caused dose-dependent membrane hyperpolarizations and a decrease in membrane resistance; more hyperpolarizations were seen after washout. Similar findings were reported by Baldelli et al. (1989). The mechanism(s) of H_2S inhibition of neuronal activity in hippocampus, the site of retrograde amnesia in humans, may result from the suppression of synaptic input and direct membrane hyperpolarization. Similar mechanisms may also be operative in the brainstem region, which is the site of cardiovascular respiratory centers.

Both Reiffenstein (1989) and Kombian et al. (1988) used the sucrose gap junction technique to study the electrical properties of frog sympathetic ganglia. Nicotine (0.01 mM)-induced membrane depolarizations were not affected by NaHS; however, NaHS

significantly potentiated the muscarine-induced and epinephrine-induced hyperpolarizations when compared to controls. In addition, NaHS alone caused membrane depolarizations. The activity of the Na-K-ATPase was not directly affected by NaHS; the only change observed was potentiation upon washout.

Patch clamping of mouse neuroblastoma cells showed that NaHS, in the presence of either taurine or cysteine amino acids, resulted in the reversible inhibition of sodium channel currents (opening of these channels initiates the membrane action potential). None of these compounds had any effect by itself. Inhibition of these channels may be responsible for the loss of respiratory drive due to H_2S poisoning (Warenycia et al., 1989; Reiffenstein, 1989).

Another mechanism of H_2S toxicity is via free radical generation. Beck et al. (1981) showed that H_2S in vitro underwent rapid oxidation, production of H_2O_2, and oxygen utilization. Khan et al. (1987a) indicated that the H_2S stimulation of superoxide anion generation by xanthine oxidase and free radical production, and the direct inhibition of various free radical scavenging enzymes such as glutathione in vivo by S^{2-} and other sulfur-containing compounds, can be deleterious and cytotoxic.

8.1.7 Summary of Effects on Laboratory and Domesticated Animals

The effects of H_2S inhalation on a variety of animal species have been investigated. The types of effects across species are similar and principally involve the respiratory tract and brain. Acute exposures (e.g., 4 h or less) of rats or monkeys to levels of about 500 ppm (695 mg/cu.m) or greater were found to cause mortality. Exposure of rats to 300 ppm (417 mg/cu.m) resulted in visible stress, and 200 ppm (278 mg/cu.m) was associated with lung edema and other changes. The most pronounced clinical signs preceding death in rats and monkeys were respiratory distress and histological lesions of the respiratory tract and brain. Acute exposures of rats to sublethal levels as low as 50 ppm have resulted in decreased activities of cytochrome *c* oxidase, a respiratory chain enzyme essential for oxygen utilization at the cellular level. Severely decreased activity of cytochrome *c* oxidase was found in rats that succumbed to levels of 500 ppm (695 mg/cu.m) H_2S and greater. In mice exposed to 100 ppm (139 mg/cu.m) H_2S for 2 h at 4-day intervals, protein synthesis in brain decreased and was correlated with increasing inhibition of cytochrome *c* oxidase. Some studies with rabbits suggest that acute, repeated exposure to 100 ppm (139 mg/cu.m) H_2S

caused heart arrhythmias. It is not clear if H_2S has a direct effect on the heart or if these effects are secondary to poisoning.

A 90-day repeated inhalation study with SD and F344 rats in which an extensive neurological and histopathological examination was performed did not reveal any abnormal neurological function at the highest concentration (80 ppm; 111 mg/cu.m). The histopathological examination, which included four sections of the nasal turbinates, revealed no abnormalities compared to controls. In B6C3F1 mice, the only exposure-related histopathological lesion was inflammation of the nasal mucosa in the 80-ppm (111 mg/cu.m) group. There were no indications of adverse effects of any kind in both species at exposure levels of 10 and 30 ppm (14 and 42 mg/cu.m).

Domesticated animals may be more sensitive to H_2S than rodents. Cows exposed to 20 to 50 ppm (28 to 70 mg/m^3) exhibited lacrimation and discomfort, but no apparent effect on milk production. In cattle exposed to unknown, but lethal levels of H_2S, necrosis of the cerebral cortex was observed. More numerous clinical signs, including blindness, have been found in calves exposed continually to 20 or 150 ppm (28 or 208 mg/cu.m) for 7 days. One goat exposed to 100 ppm (139 mg/cu.m) for 19 h died; all goats exhibited eye irritation, coughing, and shivering while some goats exhibited increased heart and/or respiratory rate.

There are no studies involving long-term H_2S inhalation.

8.2 HUMAN HEALTH EFFECTS

Hydrogen sulfide (H_2S) poisoning attracted the interest of a number of research scientists during the 19th century (see the review by Mitchell and Davenport, 1924). The characteristic respiratory excitation caused by both inhalation of the gas and injections of H_2S and sodium sulfide were described by the mid-1800s. Also known was the high lethality of H_2S, its ability to cause respiratory arrest, its irritant effect, and the efficacy of removing victims from the contaminated environment and reviving them with artificial ventilation (Lehmann, 1892).

Probably the most widespread and common complaint of persons exposed to low concentrations of H_2S for short or extended periods of time are those related to odor. An extensive discussion on the psychological and esthetic aspects of odor in general, and

specifically applying to the odor of H_2S, is included in the National Research Council (1977) monograph on H_2S. Hydrogen sulfide has a lower limit for detection of odor of 0.003 to 0.02 ppm (0.004 to 0.03 mg/cu.m). At concentrations up to 30 ppm (42 mg/cu.m), H_2S has an odor like that of rotten eggs; at 30 ppm (42 mg/cu.m) the odor is sweet or sickeningly sweet. At 100 ppm (139 mg/cu.m) and above, H_2S quickly fatigues the sense of smell; at concentrations approaching 150 ppm (208 mg/cu.m), H_2S apparently abolishes odor sensation by anesthetizing the olfactory nerve (Indiana Air Pollution Control Board, 1964). People who have survived exposure to sudden, high concentrations reported either no awareness of odor at all, or a sickening sweet smell before loss of consciousness. The assumption that odor will warn of life-threatening levels of H_2S is unwarranted, since instantaneously introduced doses >150 ppm (208 mg/cu.m) are not perceived at all (Ahlborg, 1951).

Ruth (1986), in his review, indicated an odor threshold range of 0.0007 to 0.014 mg/m^3 (<1 to 10 ppb) with an irritant level of 14 mg/m^3 (10 ppm). An earlier review by Amoore and Hautala (1983) listed the odor threshold at 8 ppb (0.012 mg/m^3). Fyn-Djui (1959) reported a minimum perceptible threshold of 0.012 mg/m^3 (8 ppb). A similar odor threshold range was identified by The World Health Organization (1987).

8.2.1 Toxic Effects Associated with Acute Exposure

Acute exposures of 500 to 2,000 ppm (695 to 2,781 mg/m^3) for seconds or minutes primarily targets the nervous system, although other tissues with high oxygen demand, particularly the heart, are also affected. Symptoms include fatigue, dizziness, intense anxiety, loss of olfactory function, collapse, respiratory and cardiac failure, and death. Usually acute intoxication occurs from a single, massive exposure of 2,000 ppm (2,781 mg/m^3) or more, and unconsciousness occurs within a few seconds, without significant warning or pain. Unconsciousness, termed "knock-down" by workers, is almost immediately followed by respiratory paralysis, and after that by a short period of tonic convulsions (Yant, 1930). The heart continues to beat for several minutes. Death occurs unless the victim is removed from the contaminated area and artificial ventilation is immediately initiated. Pettigrew (1976) reports that 26 persons died from exposure to unspecified concentrations of H_2S between October 1, 1974, and April 28, 1976, in the high-sulfur oil fields of Wyoming and Texas. At times, victims exposed to less massive

concentrations will recover spontaneously provided they have been removed from contamination. Other instances of fatalities due to H_2S overexposure were reported by Campanya et al. (1989), Osbern and Crapo (1981), Sanz et al. (1990), and McDonald and McIntosh (1951).

If the victim is not removed from the gaseous environment and given artificial ventilation, spontaneous recovery of ventilation may not occur and death may ensue. Even if ventilation does resume, asphyxia will eventually occur with continued exposure if the victim remains in the contaminated environment. Animal data indicate that this is due to inactivation of cellular respiration, specifically the reversible inhibition of cytochrome *c* oxidase, as described previously. According to Haggard (1921), breathing is never spontaneously restored after respiratory paralysis occurs from H_2S exposure, and death from asphyxia will ensue. Rescuers must know that a self-contained breathing apparatus is absolutely vital if contaminated areas are to be entered. Many potential rescuers have succumbed, together with victims of H_2S exposure who might have been saved, because they were unaware of the lethality and rapid, overwhelming action of this toxic gas (Kleinfeld et al., 1964; Adelson and Sunshine, 1966; Simson and Simpson, 1971; Smith and Gosselin, 1979). Occasionally there are some lingering effects, such as nystagmus and disturbances of equilibrium, suggesting ototoxic effects, and changes in gait, speech, or arm movement, suggesting motor involvement. Changes in electrocardiogram (ECG) and myocardial infarction have been reported; these persistent effects may result from prolonged hypoxia rather than direct exposure to H_2S.

Lethal H_2S poisoning exerts its effects directly on the nervous system. If the concentration of the gas is sufficiently high, the respiratory center of the brain ceases functioning and breathing stops. At lower concentrations (between 500 and 1,000 ppm; 695 and 1,390 mg/m^3), the respiration controls in the carotid body are stimulated, and hyperpnea, followed by apnea, results from the instigation of the normal autonomic reflex. Asphyxiation from H_2S results on the cellular level as the gas inhibits cytochrome *c* oxidase and prevents the utilization of oxygen by cells in a manner similar to the action of HCN. Only the uncombined, unoxidized form of the gas in the bloodstream exerts these effects. Hydrogen sulfide is not considered to be a cumulative poison because it is rapidly oxidized to harmless sulfates, which can be readily eliminated from the body. Hence, its respiratory/

asphyxiation role occurs only at higher concentrations, where the effect is rapid and often fatal.

Instances of permanent neurological damage resulting from acute poisoning have been described (Aufdermaur and Toenz, 1970; Matsuo et al., 1979; Arnold et al., 1985). Included among the signs are prolonged coma, convulsions, increased tonus with extensor spasms, and negative Babinski's sign (Matsuo et al., 1979). Fatigue, somnolence, headache, irritability, insomnia, anxiety, poor memory, loss of olfactory function, and loss of libido were reported in recovered victims (Ahlborg, 1951; Poda, 1966; Arnold et al., 1985; Illinois Institute for Environmental Quality, 1974). Also described are changes in gait, nystagmus, vertigo, and other indications of toxicity to the eighth cranial nerve (vestibulocochlear nerve) and its associated CNS structures (Ahlborg, 1951). Computerized axial tomography (CAT scan) performed on a victim of acute poisoning (Matsuo et al., 1979) and postmortem examination of brain tissue of victims suggest cereberal lesions characteristic of cerebral anoxia rather than any specific neurotoxicity by H_2S (Lund and Wieland, 1966).

Changes in heart rhythms and electrocardiograms after acute H_2S poisoning have been reported by several physicians (Drews, 1940; Krekel, 1964; Arnold et al., 1985). While cardiac muscle, like the nervous tissue, has a high oxygen demand and is highly sensitive to anoxic damage, there is a suggestion by Kosmider et al. (1967) that specific enzyme damage may result from H_2S poisoning.

Workers exposed to H_2S concentrations between 500 to 1,000 ppm (695 and 1,390 mg/m^3) exhibit a period of extremely rapid breathing or hyperpnea. From a practical standpoint, this can increase the inhaled dose of gas, resulting in increased damage. Kaipainen (1954) reported a case of a worker shoveling manure who was found unconscious and contracted convulsions. He had transient ECG abnormalities similar to myocardial infarction, dilated pupils that were responsive to light, negative Babinski signs, and blood pressure of 240/140 mmHg. Venesection was carried out at 12-h postexposure, and blood pressure was lowered to 95/80 mmHg. One day later the patient was incoherent, unable to answer questions, almost unconscious, and showed muscle spasticity. By 45 days later, the patient was normal except for dizziness. The author stated that convulsions usually occur at small doses of H_2S inhalation, and they are preceded by giddiness, accelerated breathing, and finally narcosis. No convulsions prevail at higher doses (no levels were specified).

Experience with H_2S poisoning in the fossil fuel fields of Alberta, Canada, has been reviewed for the period 1969-73 by Burnett et al. (1977) and for 1979-83 by Arnold et al. (1985). These were retrospective studies based on the files of the Compensation Board and the files of the Medical Services Branch, Worker's Health and Safety, Calgary, Alberta, Canada; therefore, only complaints for which medical attention was sought were considered. The records contained no neurological followups. Burnett et al. (1977) examined 173 cases, among which 6% fatalities occurred. In the 250 cases considered by Arnold et al. (1985), the fatality rate was 2.8% (7 cases). The symptoms of acute toxicity that emerge from all of these reports include immediate respiratory paralysis and collapse at very high concentrations (>2,000 ppm; 2,781 mg/m^3), and collapse and apnea preceded by a period of hyperpnea at sublethal concentrations (500 to 1,000 ppm; 695 to 1,390 mg/m^3). The sequelae of poisoning in victims who are resuscitated vary, probably as a result of initial effect, time and intensity of exposure, and length of anoxia to vital tissues.

Recovery from acute intoxication is usually rapid and complete. Symptoms varying in nature and severity develop soon after acute poisonings and persist for different durations. In reviewing a number of cases, Poda (1966) described a syndrome including nervousness, nausea, headache, insomnia, and a dry, nonproductive cough that lasted for 1 to 3 days. Gaitonde et al. (1987) reported a case in which a 20-mo-old child was exposed for nearly a year to measured H_2S levels of 0.6 ppm (0.83 mg/m^3) or less. Upon hospital admission, ataxia, choreoathetosis and dystonia were observed. Tomograms of the brain showed striking bilateral areas of attenuation in the basal ganglia and in some of the surrounding white matter. A repeat brain scan at 10 weeks showed complete resolution. There was no respiratory disease. No abnormalities were found upon neurophysiological investigation. After 10 weeks, ataxia resolved. A muscle biopsy specimen taken one month after presentation showed normal mitochondrial structure and function. Burnett et al. (1977) listed the frequency of complaints of 173 poisoning victims in Alberta who sought medical attention (Table 8-2). In an extension of the work of Burnett et al. (1977), Arnold et al. (1985) listed the frequency of complaints of 250 medical claims in Alberta (Table 8-3). The most frequently reported complaints include unconsciousness, nausea, vomiting, and headache.

The poisoning cases reviewed by Ahlborg (1951) differ somewhat from some of these descriptions in that sequelae of acute intoxication appeared shortly after initial acute exposure

TABLE 8-2. CLINICAL FEATURES AFTER HYDROGEN SULFIDE EXPOSURE

Feature	Observed Frequency (%)		
	At Accident Site	At Physician's Office	At Emergency Room
Loss of consciousness	74	--[a]	16
Disequilibrium	17	--	29
Nausea/vomiting	13	28	22
Headache	9	25	16
Sore throat/cough	8	9	14
Conjunctivitis	5	9	11
Weakness of extremities	4	--	4
Dyspnea	3	13	--
Convulsion	3	--	6
Pulmonary edema	--	--	20
Cyanosis	1	--	11
Hemoptysis	1	--	--

[a]Not reported.

Source: Burnett et al. (1977).

and persisted for approximately 1.5 mo. In one case, symptoms still evident after 3 years included drowsiness, fatigue, headache, lack of initiative, irritability, anxiety, poor memory, and decreased libido. These patients also displayed symptoms indicating damage to the eighth cranial nerve (vestibulocochlear), such as vertigo, nystagmus, and disturbances of equilibrium.

Some of Ahlborg's cases had been previously exposed. Other reports in which such sequelae as well as damage to other vital tissues such as the heart were recorded involved lengthy periods of anoxia due to paralyzed respiration (Kapainen, 1954; Hurwitz and Taylor, 1954; Kemper, 1966). Since H_2S is rapidly metabolized and does not persist in the body of

TABLE 8-3. CLINICAL FINDINGS RECORDED

Sign or Symptom	Frequency of Notation	Percentage
Unconsciousness	135	54.0
Headache	65	26.0
Nausea/vomiting	62	24.8
Dyspnea	57	22.8
Disequilibrium	54	21.6
Conjunctivitis	46	18.4
Sore throat/cough	41	16.4
Felt ill	31	12.4
Neuropsychological	20	8.0
Extremity weakness	19	7.6
Chest pain	18	7.2
Pulmonary edema	14	5.6
Bradycardia	10	4.0
Convulsion	5	2.0
Cyanosis	3	1.2
Hemoptysis	1	0.4

Source: Arnold et al. (1985).

recovering victims, it is generally thought that persistent neurologic or cardiac effects are the result of anoxia to these tissues rather than a specific effect of sulfide damage.

Biesold et al. (1977) performed an electron microscopic examination of several regions of lung tissue excised from a 7-year-old boy who died 24 h after being exposed to H_2S vapors from an old-fashioned farm latrine. A severe alveolar and interstitial edema of the hemorrhagic type was found. Analysis of the structural elements of the alveolar septa gave evidence of a direct toxic effect of H_2S on the endothelial and epithelial barrier of the alveoli, which permitted plasma and blood cells to infiltrate the interstitial and alveolar space. There was widespread damage to the squamous epithelium, resulting in partial denudation of the

basal membrane. Indications of endothelial gaps were found, and these were often covered with microthrombi.

At concentrations of H_2S ranging from 100 to 1,000 ppm (139 to 1,390 mg/m^3), the respiratory tract and the eyes are the target organs. Respiratory paralysis prevails as a result of stimulation of carotid body chemoreceptors by H_2S, causing hyperpnea followed by apnea. This phase of poisoning is critical and requires initiation of artificial respiration to prevent death by asphyxiation (National Research Council, 1979). The respiratory symptoms include bronchitis, rhinitis, pharyngitis and laryngitis. The eyes are seriously affected and symptoms include lacrimation, hyperemia, retro-orbital aching, blepharospasm, distorted and blurred vision, photophobia, and illusion of rainbow colors around lights. Also, conjunctivitis, keratitis, corneal ulceration, and temporary loss of vision have been reported (Illinois Institute for Environmental Quality, 1974; National Research Council, 1979). The systemic effects of H_2S poisoning are headaches, fatigue, irritability, insomnia, mild depression and gastrointestinal disturbances.

Rochat (1923) described lesions of the cornea, observed by slit-lamp illumination, of workers in a sugarbeet processing plant. Similar lesions were also seen by Barthelemy (1939) and Masure (1950) in H_2S-exposed viscose rayon workers and in workers of the gas industry (Carson, 1963). Painful soreness with severe photophobia and tears that burned "the cheeks" were the symptoms reported by Howes (1944) in his investigation of an outbreak of eye problems in a tannery. Nesswetha (1969) described the progression of lesions of the eye in his review of thousands of H_2S exposure histories of viscose rayon workers. In this review, coexposure to CS_2 was suggested to play an important role by lowering the ocular sensitization threshold to H_2S. Slight, grayish opacity with petechial stippling of the superficial cell layer of the cornea can be observed upon slit lamp examination. The lesions are due to swelling and blistering of the epithelial cells rather than to cellular infiltration. As the injury progresses, vacuoles form in the cells, which burst and produce epithelial defects that spread and join to form larger and very painful ulcers on the corneal surface. Concomitant with the progress of the corneal keratitis is an inflammation of the conjunctiva, which becomes reddened. The lesions generally heal without permanent damage, except in very extreme exposures in which the erosion of the corneal surface can leave scars. Injury to the eyes is generally restricted to the cornea and conjunctiva. Nesswetha reported that

severity of damage increased with H_2S exposure concentration. Subjective symptoms most commonly described are fogging or blurring of vision, the perception of colored, rainbow-like rings around lights, tearing, sensation of foreign bodies in the eye, photophobia, pain in and behind the eyes, everted eyelids, and blepharospasm. All the above-named authors agree that ocular problems are the earliest symptoms observed in subchronic H_2S exposure, and that they appear before any complaints of respiratory difficulties are made.

Exposure to H_2S can cause loss of the corneal reflex and anesthetize the surface of the eye, so that pain and irritation may not be immediately felt upon exposure. Vision is often affected first, with changes ranging from perceived halos or rainbows around lights to "blue" and blurred vision. Damage to the conjunctiva and corneal epithelium (apparently reversible, except with repeated insult) results in "sore eye" or "gas eye," an intensely painful manifestation of inflammation that occurs after the initial loss of sensation passes, which is accompanied by visual changes. In severe form, actual ulceration of the cornea occurs, leading to scar formation and permanent impairment of vision. An instance of H_2S keratoconjunctivitis was described by Luck and Kaye (1989).

Not only are the mucous membranes of the eye affected, but H_2S can also affect the respiratory tract; effects include bronchitis, rhinitis, pharyngitis, and laryngitis (Yant, 1930; Barthelemy, 1939; Milby, 1962; Arnold et al., 1985).

Acute health effects associated with a release, over a 2-day period in 1987, of sulfur compounds was assessed by a symptom prevalence questionnaire (Haahtela et al., 1992). A questionnaire was administered to residents in 29 households 10 days after high exposure to H_2S was measured. Symptom prevalence was compared to that reported when the same households were administered the questionnaire about 4 mo later. Forty-five individuals responded to both questionnaires, and the only significant finding was an increased incidence of breathlessness. Levels were as high as 0.1 ppb (0.135 $\mu g/m^3$). The 24-h averages for the 2-day exposure were 0.02 and 0.03 ppb (0.035 and 0.043 mg/m^3). It is difficult to attribute H_2S as the cause of breathlessness. Mesityl oxide, a sensory irritant, was also reported to be present in the air because of its odor. Levels were not measured. The SO_2 concentration was about 3 $\mu g/m^3$ both during exposure and long after exposure had ceased.

At concentrations lower than the odor threshold value of H_2S, Ryazanov (1962) reported subtle responses such as alterations in the rate and amplitude of respiration,

contraction of vocal cord and bronchial muscles, variation in vascular smooth muscle tone, change in optical chronaxy, change in light sensitivity of dark-adapted eyes, inability of the cerebrum to maintain an assimilated rhythm, and establishment of an electrocortical reflex that could lead to reduction in CNS performance. However, the significance of these findings are unclear as this study has not been replicated.

Vicas and Green (1989) reported the accidental H_2S poisoning of three workers. Patient A, a 32-year-old male, opened a valve and collapsed unconscious. Patient B, a 24-year-old male, collapsed while attempting to rescue him. Patient C, a 34-year-old male, tried to assist the other two and collapsed. Patient B exhibited marked central cyanosis and pulmonary edema; he became asystolic and was pronounced dead on arrival at the hospital. At autopsy, brain sulfide levels were elevated and changes were consistent with hypoxia.

Patient A was given oxygen and ventilatory support. He developed seizures, became comatose, and died on the seventh day. Patient C showed progressive neurologic improvement and was discharged with some impairment of visual geometric detail.

Bhambhani and Single (1991) evaluated the effect of exercise (bicycle ergometer) on oxygen uptake and lactic acid production in volunteers. Sixteen males were randomly exposed to 0, 0.5, 2.0, and 5.0 ppm (0, 0.7, 2.8, and 6.9 mg/m^3) H_2S on four separate occasions. Only exposure to 5 ppm (6.9 mg/m^3) H_2S resulted in a significantly higher maximum oxygen uptake. This concentration also resulted in a significant increase in lactic acid production, but did not affect exercise capacity.

8.2.2 Epidemiological Studies

The National Research Council (1979) defines chronic intoxication in terms of the effects observed as a result of intermittent exposure to low to intermediate concentrations of H_2S in the range of 50 to 100 ppm (70 to 140 mg/m^3). The Illinois Institute for Environmental Quality (1974) describes chronic poisoning as a prolonged exhibition of symptoms, which results either from an extended single exposure or repeated, short exposures that do not produce symptoms typical of acute or subchronic poisoning. The symptoms generally are described as lingering, behavioral, and neurasthenic in nature and include fatigue, lack of initiative, mental depression, inability to concentrate, and abnormal peripheral reflexes indicative of CNS depression. Other symptoms include local irritation of the eyes

and respiratory tract, bradycardia, cold sweats, chills, gastrointestinal disturbances, sleep disorders, and headaches (Vigil, 1979).

At concentrations between 10 and 20 ppm (14 to 28 mg/m^3), exposure over time may cause irritation of the mucous membranes of the respiratory tract and the eyes. Still unresolved, however, is the debate concerning whether or not "chronic poisoning" exists as a pathologic entity or is a subjective response to an obnoxious odorant. It is also not clear if the reported signs and symptoms result from continuous low-level exposure or occur from damage done by isolated (and usually unmeasured) peak high-level exposure. Confounding factors include the following: all occupational studies performed with low-level, chronic exposure also involve exposure to other toxic gases such as SO_2, carbon disulfide (CS_2), mercaptans, sulfuric acid mist, and mixtures of volatile organic compounds that individually, or in aggregate, elicit similar complaints; the working conditions (e.g., night work, high humidity, and temperature) present many variables.

Ahlborg (1951) studied five cases in the shale oil industry thought to involve chronic H_2S poisoning. The frequency of neurasthenic symptoms such as loss of appetite, poor memory, dizziness, irritability, itching, headache, and fatigue was greater among the group of exposed workers. The author could not determine whether these symptoms resulted from the H_2S exposure or from the stressful environment.

Similar symptoms were reported by Barthelemy (1939) and Rubin and Arieff (1945) in studies in the viscose rayon industry. These workers were exposed to mixtures in which CS_2 predominated, but which also contained H_2S. These researchers also could not separate the indicated symptoms from work stress, nor could they attribute them to H_2S exposure exclusively. Glebova (1950) reported that infants who were exposed to H_2S emanating from their mother's clothing during breastfeeding showed a spectrum of signs and symptoms. The mothers worked in an artificial silk factory where they were exposed to H_2S and CS_2. When the mothers were moved away from the H_2S-contaminated areas, their infants' symptoms cleared. Concentrations of 0.02 to 0.04 ppm (0.028 to 0.055 mg/m^3) H_2S were measured during breastfeeding times. No attempts to measure CS_2 were made. Affected babies showed poor or retarded development, low weight gain, and listlessness. Some also showed lack of animation, anemia, paleness, regurgitation after feedings, and gastrointestinal distress. Susceptibility to infectious disease was also increased. The methods in this Russian study

were not clearly delineated, and no control population comparisons were made. The effects described were not adequately related to H_2S exposure, and effects from other toxic agents, work conditions, or other confounding factors were not ruled out. Consequently, attribution of observed effects to H_2S should be viewed with strong reservations.

Jappinen et al. (1990) evaluated respiratory function in a cohort of 26 male pulp mill workers exposed daily to H_2S at levels "usually below" 10 ppm (14 mg/m^3). Most measurements were between 2 and 7 ppm (2.8 and 9.7 mg/m^3) with a range of 1 to 11 ppm (1.4 to 15.3 mg/m^3). The mean duration of exposure was not reported. It appears that spirograms were obtained before daily exposure and 30 min after workplace exposure. Results were compared to predicted values from the Finnish general population. Although no significant changes on pulmonary function or bronchial responsiveness (as measured by histamine challenge) were observed, this study design has low power to detect alterations of pulmonary function parameters and should not be construed as a negative finding. The investigators also exposed 10 asthmatics (3 men and 7 women) in a laboratory exposure setting in which individuals were exposed to 2 ppm (2.8 mg/m^3) H_2S for 30 min. Subjects rapidly became accustomed to the odor, and 3 of 10 subjects complained of headache after exposure. There were no significant effects on pulmonary function parameters and no clinical symptoms were observed.

There are some data that suggest that exposure to H_2S and organic sulfides may lead to increases in cardiovascular and coronary mortality. Such an association was reported by Jappinen and Tola (1990) in male pulp mill workers. In this cohort (4,179 person-years), the overall mortality was slightly increased over that expected. Cardiovascular and coronary death were significantly elevated over expected deaths (standard mortality ratio = 150). In those with an exposure >5 years and with a follow-up period of >15 years, there were 22 observed cardiovascular deaths (12.7 expected) and 14 observed coronary deaths (8.7 expected). Differences in smoking habits were reported not to explain these findings, although the proportion of smokers was 80% higher than in other cohorts examined.

Kangas et al. (1984) investigated the results of H_2S, methyl mercaptan, and dimethyl disulfide exposure in 10 different cellulose mills in Finland. Concentrations ranged from 0 to 20 ppm (0 to 27.8 mg/m^3) H_2S, 0 to 15 ppm methyl mercaptan, and 0 to 1.5 ppm dimethyl disulfide; SO_2 concentrations reached 20 ppm in some locations. Exposed workers

reported headaches and decreased ability to concentrate more often than matched controls. Sick leave was also used more frequently among the exposed groups than among controls.

Ferris et al. (1979), Chan-Yeung et al. (1980), and Higashi et al. (1983) examined respiratory effects in workers in a single pulp and paper mill in the United States, in a single mill in Canada, and in 18 viscose rayon plants in Japan, respectively. Ferris et al. found no significant mortality or morbidity for respiratory symptoms or illness in his study. No increases in respiratory symptoms were found by Chan-Yeung et al. (1980), and Higashi et al. (1983) did not detect increases in respiratory symptoms or decreases in pulmonary function in their study populations. It should be noted that the workers in these studies were exposed to a mixture of potentially hazardous compounds. The levels of H_2S measured in these exposures were very low: <4 ppm (<5.6 mg/m^3) (Ferris et al., 1979); <0.2 ppm (<0.28 mg/m^3), with mean of 0.05 ppm (0.07 mg/m^3) (Chan-Yeung et al., 1980); and an average of 3 ppm (4.2 mg/m^3) (0.3 to 7.8 ppm, range) (Higashi et al., 1983).

In contrast to the above studies, Dales et al. (1989) found increased respiratory symptoms in the younger vs. the older population living in a 300-mi^2 area downwind from natural gas resources. Symptoms included irritation and inflammation the respiratory mucosa. These natural gas plants may contain high levels of H_2S. However, it is not possible to determine if H_2S solely contributed to these findings since sulfur oxide particulate matter was present. The authors concluded that attention should be placed on health effects induced by chronic low-level exposure to H_2S and other sulfurated compounds.

Tenhunen et al. (1983) investigated the effect of worker exposure to H_2S and methyl mercaptan on heme synthesis (heme forms part of the hemoglobin complex). Venous blood was collected from 17 workers in pulp production where the 8-h time-weighted average H_2S concentrations ranged from 0.05 to 5.2 ppm (0.07 to 7.2 mg/m^3), with methylmercaptan ranging from 0.7 to >1.0 ppm TWA and dimethyl sulfide ranging from 0.03 to 3.2 ppm. Enzymes in the heme synthesis pathway (α-amino-levulinic acid synthetase and heme synthetase) showed decreased activities in eight and six cases, respectively. Erythrocyte protoporphyrin (a precursor of heme) was decreased in seven cases. None of the workers had clinical anemia. The authors attributed these changes to H_2S exposure, but were not able to establish whether repeated peak or continuous low-level exposure occurred. No unusual complaints were recorded for any workers in the test or control groups.

Dysfunction of the vestibular portion of the vestibulocochlear nerve and its associated CNS connections has also been reported in some cases of exposure to H_2S. Characteristic symptoms include dizziness, loss of equilibrium, nystagmus, and disturbances of gait or movement; such symptoms occur as a result of exposure to concentrations of 2,500 ppm (700 mg/m^3) H_2S (Poda, 1966; Arnold et al., 1985). Exposure to H_2S has been associated with falls causing secondary injury and death, and may be attributed, in part, to this neurologic effect (Arnold et al., 1985).

8.2.3 Summary of Human Health Effects

At sufficiently high concentrations (>1,000 ppm; 1,390 mg/cu.m), H_2S is rapidly fatal to humans, causing respiratory paralysis and apparent inhibition of cellular respiration. At levels between 500 and 1,000 ppm (695 and 1,390 mg/cu.m), a period of rapid breathing (hyperpnea) is followed by cessation of breathing (apnea) and death. Damage to organs and the nervous system can result from the anoxia caused by the depression of cellular metabolism at levels above 250 ppm. At lower concentrations (50 to 100 ppm; 70 to 139 mg/cu.m), the immediate and prolonged effects are irritation with inflammation of mucous membranes, particularly of the eye and the respiratory tract. Pneumonitis can result in pulmonary edema and can be a threat to life. Though ambient concentrations tend to be below those considered harmful to human health, no long-term, low-level epidemiological studies have been performed to determine whether H_2S causes pulmonary changes similar to those caused by other irritant gases such as oxides of nitrogen and sulfur. At very low concentrations, offensiveness of odor, with mostly subjective reactions to stench, is the dominant effect. Also, neurological symptions such as visual hallucination, and short-term memory loss have been recently reported at levels thought to be not harmful. More studies are required to assess H_2S toxicity at very low concentrations. Essentially, no human health data and practically no experimental data on long-term exposures at low levels exist. No epidemiological studies relating to cancer, teratogenesis, or reproductive effects have been performed.

TABLE 8-4. EFFECTS OF EXPOSURE IN HUMANS AT VARIOUS CONCENTRATIONS IN AIR

Clinical Effect	Level of Hydrogen Sulfide ppm	Level of Hydrogen Sulfide mg/m^3	Reference
Odor perception threshold	0.003-0.02	0.004-0.028	Indiana Air Pollution Control Board (1964)
Offensive odor of rotten eggs	<30	<42	Ahlborg (1951)
Offensive odor (sickening sweet)	>30	>42	
Occupational Exposure Limit (O.E.L.)	10 *10	14 14	National Research Council (1977)
Serious eye injury	50-100	70-140	National Research Council (1977)
Olfactory paralysis	150-200	210-350	National Research Council (1977)
Pulmonary edema, threat to life	300-500	420-700	National Research Council (1977)
Strong nervous stimulation of respiration	500-1,000	700-1,400	National Research Council (1977)
Respiratory paralysis, immediate collapse, death	1,000-2,000	1,400-2,800	National Research Council (1977)

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9. CARCINOGENICITY

No long-term chronic studies for carcinogenic effects have been done with hydrogen sulfide (H_2S). Weisburger et al. (1981) conducted a long-term bioassay of the toxicity and cancer-causing potential of a number of industrial chemicals, including sodium sulfide. Sodium bisulfide was administered by gavage to Charles River-CD rats (26 males and 26 females per treatment group) at doses of 9 or 18 mg/kg, in the presence and absence of a 1% thyroid extract (to guard against possible thyroid gland impairment by sulfide). Doses were administered twice a week for 56 weeks and two to three times a week for the remaining 22 weeks. After the 78 weeks of treatment, the animals were observed for 26 weeks and then sacrificed. No statistically significant evidence of carcinogenicity was found in the treatment groups, although the low survivability in groups treated with thyroid extract made the results ambiguous. The dose ranges tested for sodium bisulfide alone, which caused some lethality in males at the low dose at 52 weeks, appeared to achieve the maximum tolerated dose. However, an insufficient number of animals survived treatment, which precludes definitive judgments about carcinogenic potential. Because of the lack of adequate animal test data, this compound is placed in Category D, based on the weight-of-evidence criteria in the U.S. Environmental Protection Agency's Carcinogen Risk Assessment Guidelines issued in August 1986. A Category D ranking means that the available data are inadequate to assess a chemical's carcinogenic potential.

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10. MUTAGENICITY

Using the Ames test with *Salmonella typhimurium* TA 1535, Gocke et al. (1981) found evidence of weak mutagenicity as shown by the number of revertants to wild type for this mutant strain of bacteria, which grows only in the absence of histidine. Addition of the S-9 microsomal fraction from the liver of Aroclor-pretreated rats abolished the effect. Since only a single tester strain was used and cytotoxic records were not provided, and since there may have been confounding effects introduced by different growth media, it cannot be unequivocally stated that evidence of mutagenicity by H_2S exists.

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11. REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

No data on human developmental effects of inhaled H_2S were found, but based on the limited information available in animals, H_2S appears to have potential to alter normal developmental processes.

Saillenfait et al. (1989) exposed pregnant Sprague-Dawley (SD) rats (7 to 9/group) to 0, 50, 100, or 150 ppm (0, 70, 139, or 208 mg/m^3) H_2S 6 h/day during Gestational Days 6 to 20. Maternal body weight gain was significantly reduced at 150 ppm (208 mg/m^3) and fetal body weight was slightly (4 to 7%) reduced in all exposed groups. In dams exposed to 100 or 150 ppm, reduced absolute weight gain and increased implantations and increased live fetuses were observed. No external anomalies were observed in any of the treatment groups. In a follow-up experiment, 23 pregnant females were exposed to 100 ppm (139 mg/m^3) for 6 h/day on Gestation Days 6 to 20. Fetal weights, number of live and dead fetuses, number of implantation sites and resorptions and external malformation were recorded. No maternal toxicity or adverse effects on the developing embryo or fetus was observed. Twenty litters (278 fetuses) were examined for anomalies.

Studies by Hannah and colleagues suggest that H_2S has the potential to alter normal CNS patterns in the developing fetus. Roth and Hannah (1989) exposed pregnant SD rats to 75 ppm (104 mg/m^3) H_2S for 7 h/day from Gestational Day 5 to Postnatal Day 21. Randomly selected pups (8 exposed and 8 control) were sacrificed at Postnatal Days 7, 14, and 21. Population densities of Purkinje and granule cells in the cerebellum were quantitated on Days 7 and 14. There was a 20% increase in the density of surviving Purkinje cells along the primary fissure, but no significant change in the mean number of granule cells. Exposure to H_2S also produced alterations in amino acid levels in the cerebellum. Brain levels of aspartate, glutamate, taurine, and GABA were significantly reduced below control levels by Postnatal Day 21. At Postnatal Day 7, taurine was elevated at 125% of control values. The consequence of these alterations are unknown. In Purkinje cells from the Day 21 group, alterations in both dendritic architecture and growth process was observed.

When pups from SD rats exposed to 50 ppm (70 mg/m^3) H_2S for 7 h/day from Gestational Day 6 to Postpartum Day 21 were examined, brain levels of taurine were elevated above controls at Day 7 but had returned to normal by Postpartum Day 21 (Hannah et al., 1990). At exposure levels of 20 and 50 ppm (28 and 70 mg/m^3) H_2S during gestation and postpartum, pups from SD rats exhibited severe alterations in the architecture and growth characterizes (i.e., nonrandom) of the Purkinje cell dendritic fields.

Hayden et al. (1990) also evaluated the effects of H_2S exposure on development of SD rats. Pregnant rats were exposed to H_2S concentrations of 0, 20, 50, or 75 ppm (0, 28, 70, or 104 mg/m^3) from Gestational Day 6 to Postpartum Day 21. Developmental and reproductive data were generated from 6 maternal rats in the 50 and 75 ppm (70 and 104 mg/m^3) groups and 12 rats in the 20 ppm group. In the control group, 24 maternal rats were evaluated. Culling took place in maternal rats at Postpartum Day 1. There was no treatment-related effect on maternal body weight gain (8 to 15 rats/treatment group). The most significant observation in dams was a treatment-related increase in parturition time over matched controls. Liver cholesterol was significantly elevated in dams from 75 ppm at Postpartum Day 21. There were no treatment-related effects on litter size, viability, sex ratio, eyelid opening, or surface righting. Neonatal pup development parameters were monitored on Postpartum Days 1, 7, 14, and 21. At Day 1, litters were culled to reduce litter size to 12 pups. At 20 ppm (28 mg/m^3), hair development and pinna detachment was significantly accelerated. Hair development in the 50 ppm (70 mg/m^3), but not 75 ppm (104 mg/m^3), also was accelerated.

Andrew et al (1980) investigated the effect of exposure of 220 ppm (306 mg/m^3) H_2S on spermatogenesis in Wistar rats. A group of 10 rats was exposed for 3 h/day for 7 days. Controls were exposed to air and a positive control was dosed with triethylenemelamine. Parameters evaluated in females included fertility, corpora lutea, total implants, and dead implants. There were no effects of exposure on these parameters.

These investigators also examined the effect of H_2S exposure on prenatal development. Pregnant Wistar rats were exposed to 220 ppm (306 mg/m^3) for 3 h/day, 5 days/week, either throughout gestation (1 to 18 days) or during a portion of organogenesis (day 7 to 11 or day 12 to 16). At day 21, animals were examined for dead implants, fetal malformations, and growth retardation. There was no evidence of maternal toxicity or embryotoxicity.

There was a high (23%) incidence of wavy ribs in the group exposed throughout gestation. It was not clear to the authors if it was solely due to treatment or was genetic in origin for this particular group of animals.

There have been no two-generation studies which have examined the role of H_2S on brain development and CNS functioning.

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12. CONCENTRATION-RESPONSE ASSESSMENT

The toxicological data base has been reviewed and an inhalation reference concentration (RfC) was verified by the U.S. Environmental Protection Agency (EPA) RfD/RfC Work Group on June 21, 1990. The documentation is available via the Integrated Risk Information System (IRIS) (U.S. Environmental Protection Agency, 1991). The Integrated Risk Information System is an on-line data base containing EPA risk assessment results and regulatory information. An RfC is defined as an estimate, with uncertainty spanning perhaps an order of magnitude of a daily exposure to the human population (including sensitive subgroups) which is likely to be without adverse effects during a lifetime (U.S. Environmental Protection Agency, 1990). The derivation of the RfC is based on a complete review of the toxicological literature and encompasses adjustments for exposure duration and dosimetry and utilizes uncertainty factors account for specific extrapolations between the population in which the effect was observed and the human population. The critical, usually the most sensitive, effect is the focus of the RfC derivation and for this effect the no-observed-adverse-effect level (NOAEL), or lowest-observed-adverse-effect level (LOAEL) if a NOAEL is not available, is identified. Detailed discussion concerning these issues can be found in U.S. Environmental Protection Agency, 1990.

The RfC for H_2S is 9E-4 mg/m^3 and was derived from the NOAEL for inflammation of the nasal mucosa in mice (Toxigenics, 1983c). Details concerning this critical effect and other aspects of the study are discussed in Chapter 8. Since the RfC may change due to evaluation of additional data, the reader is referred to IRIS for the most current information regarding the RfC for H_2S.

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