III. BIOLOGIC EFFECTS OF EXPOSURE

Extent of Exposure

The thiols constitute a group of aliphatic and aromatic organic compounds characterized by the presence of sulfhydryl (-SH) groups. The word "mercaptans" is derived from the Latin "corpus mercurium captans" [1], meaning entity seizing mercury, mercaptans being so named because the hydrogen of the -SH group is easily replaced by mercury or other heavy metals to form compounds called mercaptides [2]. The terms thiol, mercapto, and sulfhydryl are used interchangeably, with thiol being the preferred term to refer to the sulfur-containing analog of the hydroxyl group that characterizes alcohols [3].

The thiols considered in this document are those monofunctional primary thiols containing one free -SH group and no other functional group; specifically, the 14 n-alkane thiols ($C_nH_{2n+1}SH$ where n = 1,2,...12, 16, 18), one aliphatic cyclic thiol (cyclohexanethiol), and one aromatic thiol (benzenethiol). The selection of these compounds was based on a preliminary survey of the industrial importance of thiols, the number of persons occupationally exposed to thiols, and the biologic effects of thiols. The physical and chemical properties of these thiols are presented in Tables XII-1 and XII-2. Synonyms for the 16 thiols are listed in Table XII-3.

Naturally occurring thiols exist in all living systems. Most of the thiols in living cells are contributed by the amino acid cysteine and the tripeptide glutathione. Cysteine is an intrinsic structural component of proteins and thus is the precursor of reactive thiols in proteins. The major nonprotein thiol in cells is glutathione, which occurs ubiquitously in living systems [4]. Thiols and disulfides occur together in cells and react both spontaneously and catalytically as oxidation-reduction (redox) reagents in various electron transport systems, as catalytic components of enzymes, as reactive groups of coenzymes, and as reactants in cellular detoxification systems [4,5]. The enzymatic reduction of disulfides that takes place in the living systems occurs through disulfide reductases. A glutathione reductase requiring reduced nicotinamide adenosine dinucleotide phosphate (NADPH) as hydrogen donor is present in plant and animal cells, and there is accumulating evidence that the reduction of disulfide groups is brought about by a transhydrogenation reaction with glutathione [6]. The thiol-disulfide exchange reaction is, in fact, a most important unifying concept that dominates the biochemistry of thiols and disulfides in living systems and forms the subject of an extensive literature [6-8]. Considerable information also is available on the chemistry, biochemistry, and analytical determination of thiols [7,9-11].

The most important biologic effects of the thiols appear to be those of high concentrations on neurons [12-15] and obnoxious odor at ppb to ppm concentrations [16]. Methanethiol is a gas at room temperature, and the other thiols are liquids. The C_1-C_6 n-alkane thiols have relatively high vapor pressures at ambient temperatures. Their boiling points range between 6.2 and 151 C at 760 mmHg. On the other hand, the C_7-C_{12}, C_{16}, and C_{18} n-alkane thiols, cyclohexanethiol, and benzenethiol have relatively lower vapor pressures, with boiling points above 151 C. The C_1-C_6 alkane thiols and benzenethiol give rise to perceptions of obnoxious odors at much lower concentrations than do the other thiols. For the most part, thiols are "insoluble" in water but soluble in bases, alcohol, and ether. They act as weak acids in chemical reactions. Thiols form insoluble mercaptides with a variety of metal cations [17], including those of mercury, lead, silver, copper, and cadmium, and undergo oxidation (in vitro) to yield disulfides.

Methanethiol and ethanethiol occur naturally in the "sour" gas of West Texas, in coal tar, and in petroleum distillates [3]. Methanethiol at ppb levels is detected in foods and vegetables [18] and is a major contributor to normal and abnormal human mouth odor [19-22]. Onions contain 1-propanethiol [23], and 1-butanethiol occurs in the secretions of skunks [3,24].

The most important industrial method for the manufacture of thiols involves the reaction of hydrogen sulfide with olefins or alcohols, at various temperatures and pressures, in combination with a variety of catalysts and promoters (acids, bases, peroxides, and metal sulfates). Primary thiols are produced in the presence of influences, such as UV radiation, that generate free radicals. Other methods for their synthesis include the reaction of alkyl halides or sulfates with metallic hydrosulfides, the hydrolysis of thiol esters with alkali, and the reduction of alkyl and aryl disulfides and alkyl sulfonyl chlorides.

Methanethiol is used extensively in the manufacture of methionine [25]. The lower molecular weight alkane thiols and benzenethiol are intermediates in the synthesis of pharmaceuticals and pesticidal chemicals (herbicides, insecticides, defoliants, fungicides, miticides, and nematocides) [17]. Because of their penetrating, disagreeable odor at concentrations as low as 0.02 ppb [26-28] (see Odor Threshold Studies), ethane-, propane-, and butanethiol are employed as warning agents for gas leaks in mines, underground gas pipes, and refrigeration units. The higher molecular weight alkane thiols are used in the preparation of tin derivatives for use as thermal stabilizers for polyvinyl chloride (PVC) resins, particularly in PVC pipe. They are also used as modifiers and chain-length control agents in emulsion-polymerization systems for the large-scale manufacture of synthetic rubbers of the butadiene and acrylate types [29-32]. The higher alkane thiols are used in froth flotation procedures to increase the yield of copper ore [17]. Thiols are also used as inhibitors of oxidation in

pickling of steel sheets, for improving the quality of molding powders, and as anticorrosion agents, industrial surfactants [33], vulcanization accelerators, rodenticides, repellants for flies [17], and depilatories for removing hair from hides [34]. They are used also in hair-waving preparations [35] and in moisture proofing of Cellophane [17]. Other uses of individual thiols are described in Table III-1.

The major routes of exposure to thiols in the workplace are by inhalation of vapor and aerosols and by skin absorption. The likelihood of such exposures is greatest during the handling, transfer, and sampling of thiols. Exposure may also occur during maintenance operations and repair of equipment; on entry into tanks, vessels, or other confined spaces; and during emergencies or when nonroutine procedures are used. Although eye exposure rarely occurs in the occupational environment, this potential exposure route should not be underestimated.

The total production in 1976 of "methane-, ethane-, propane-, butane-, octane-, nonane-, decane-, hexadecane-, and miscellaneous thiols and other hydrocarbon derivatives (from petroleum and natural gas by chemical conversion)" was 264,797,000 pounds according to the US International Trade Commission [36]. Sales of n- and tert-dodecanethiol as polymerization regulators totaled 7,698,000 pounds in 1975 [37]. No other published production data were found.

NIOSH estimates that 178,860 employees are potentially exposed to thiols in the United States. This estimate, based on the National Occupational Hazard Survey, is nearly 50% greater than the number of workers exposed to 6 of the 16 thiols for which estimates of the numbers exposed are given in Table III-1.

Historical Reports

The first member of the monofunctional organic thiol class was synthesized in 1834 by Zeise as reported by Liebig [1]. Zeise heated barium sulfide saturated with hydrogen sulfide in a retort with calcium ethyl sulfate to yield ethanethiol. He suggested the name mercaptan for this class of compounds because ethanethiol had the unusual property of being able to remove mercury from solution as a crystalline salt.

In 1886, Fischer and Penzoldt [38] demonstrated the human olfactory threshold by waving a cloth containing various quantities of ethanethiol about a classroom. The students easily detected odors from an amount as low as "1/460,000,000 mg" (2.17 pg) of ethanethiol.

Pichler [14], in 1918, reported on the accidental exposure to ethanethiol vapor of 28 male and 2 female high school students 16-18 years old. The classroom was connected by a door to a storeroom for chemicals.

TABLE III-1

INDUSTRIAL USE AND OCCUPATIONAL EXPOSURE PROFILES FOR 16 THIOLS

Thiol	Uses	Estimated No. of US Workers
Methanethiol	Chemical intermediate, especially methionine synthesis; catalyst modifier; jet fuel additives; fungicides	19,140
Ethanethiol	Liquid propane gas odorant; adhesive stabilizer; pesticide intermediate; solvent; intermediate and starting materials, plastics and antioxidants; "sulfonal" related	23,130
1-Propanethiol	Pesticides; gas odorant	18,180
1-Butanethiol	Solvent; gas odorant; pesticide intermediates, rubber chemicals, oil additives; polymerization regulator	19,410
1-Pentanethiol	Intermediate in the synthesis of organosulfur compounds	-*
1-Hexanethiol	Chemical intermediates; antioxidant, white oil; synthetic rubber processing	-
1-Heptanethiol	Froth flotation	-
1-Octanethiol	Polymerization conditioner; organic synthesis	18,660
1-Nonanethiol	See 1-octanethiol	-
1-Decanethiol	"	-
1-Undecanethiol	"	-
1-Dodecanethiol	Stabilizer, pyrethrum-DDT aerosols; pharmaceuticals; insecticides; fungicides; nonionic detergents; synthetic rubber processing; froth flotation agents for metal refining, particularly for copper ores	21,150

TABLE III-1 (CONTINUED)

INDUSTRIAL USE AND OCCUPATIONAL EXPOSURE PROFILES FOR 16 THIOLS

Thiol	Uses	Estimated No. of US Workers
1-Hexadecanethiol	See 1-octanethiol; tarnish-preventing agents	-
1-Octadecanethiol	See 1-hexadecanethiol	-
Cyclohexanethiol	Chemical intermediate; pesticides; flavoring agents; synthetic rubber processing	-
Benzenethiol	Chemical intermediate, primarily for pesticides; pharmaceutical intermediate; amber dyes	-

*Figures not available

Adapted from National Occupational Hazard Survey (NOHS), 1972-74

The students were disturbed during morning classes by a stench emanating from the adjacent room, and instruction was discontinued after 1 hour. Ten students, including the 2 girls, experienced dull headache, general discomfort, and abdominal pain; three students vomited and had diarrhea. By afternoon, they apparently felt well and slept normally that night. Instruction was resumed in the same room the next day for 3 hours. Although both the classroom and the chemical storeroom were well ventilated, eight of the students who had been affected the previous day again developed headaches, but to a lesser degree. Two of these students stayed away from school for a few days.

Physical examination of one of the students revealed some undescribed changes about the eyes, and, although his liver was palpable, his spleen was not [14]. Protein, erythrocytes, and a few leukocytes found in the urine at that time disappeared within 5-6 weeks; no epithelial cells or casts were observed. The chemical responsible was identified as ethanethiol, about 3 g of which had vaporized in the 325-cu m rooms (approximate concentration 4 ppm or 9 mg/cu m).

Between 1954 and 1956, investigations showed that ethanethiol and closely related compounds had striking antitubercular activity when tested in mice, guinea pigs, and rabbits [39-42]. Only those compounds producing ethanethiol in vivo appeared to be active in that they prolonged the survival time of the test animals injected subcutaneously (sc) or fed orally with a preparation of a strain of human tubercle bacilli. The specificity was related to the presence of the ethyl moiety, and the extent of such activity was altered by the nature of absorption and degradation of the compound [42]. The reduced antitubercular activity of ethanethiol when tested in vitro suggested that a metabolite of ethanethiol might have been the active compound. Kushner et al [41], who studied a series of thiol pyrazinoates for "antitubercular activity," found that the ethyl derivative was significantly active. However, during testing for antitubercular activity in mice, the authors found that animals given this compound, as well as ethanethiol, had an enlarged and cyanotic spleen.

Davies and Driver [43], in 1958, also showed that ethanethiol at a concentration of 10 µg/ml inhibited the growth of tubercle bacilli in monocytes derived from peritoneal exudates of normal guinea pigs, as well as in human monocytes obtained from a leukemia patient. Methanethiol showed no antitubercular activity and, in fact, partially antagonized the effect of ethanethiol. However, diethyldisulfide did have antitubercular activity.

Effects on Humans

The reports described in this section relate to the effects of exposures to methane-, ethane-, and butanethiol resulting from accidents and to human odor threshold studies on C_1-C_6 n-alkane thiols and benzenethiol. Additionally, some controlled experiments that determined the effects of exposure to ethanethiol are also reported.

Shults et al [12], in 1970, gave a detailed description of the methanethiol poisoning of a 53-year-old black male laborer, who, as a result of accidental exposure, manifested acute, severe hemolytic anemia, methemoglobinemia, and deep coma before his death 28 days after the accident. The immediate cause of death was stated to be a massive embolus that occluded both main pulmonary arteries.

The man's job had involved the salvaging of metal cylinders of the type used for storage of gas under pressure [12]. On the day of overexposure, the man apparently had emptied several tanks containing the thiol and had refilled them with water according to instructions. No indication was given that the man used protective equipment in his job. After an hour, he was found in a sitting position, unconscious, against a tank; on the asphalt pavement near him was a vaporizing liquid. The man was admitted to a hospital in a comatose state. His breath and clothes emitted a very strong, obnoxious odor. His health prior to the poisoning episode was reportedly good. On admission, the man had tachycardia (120/minute), a labile blood pressure (188/90-230/130 mmHg), and decerebrate rigidity with suppression of response to painful stimuli and of the deep tendon reflexes.

Within the first few days following admission, evidence of severe intravascular hemolysis appeared [12]. Hemoglobinuria began on the 3rd day and was marked by the 4th day. Profound anemia developed by the 7th day (hemoglobin 5.0 g% and hematocrit 16%) and was accompanied by a concentration of bilirubin in the serum (3.5 mg/100 ml), more than twice the upper limit of normal. After a tranfusion, there was no further evidence of hemolysis, and the concentration of hemoglobin in the patient's blood actually increased during the following days. Decerebrate rigidity accompanied by random myoclonic jerks continued. The patient developed a fever (39.4 C) on the 22nd day of hospitalization and, despite antibiotic and supportive treatment, died of a sudden hypotensive crisis on the 28th day. The apparent immediate cause of death, disclosed at autopsy, was a massive embolus occluding both main pulmonary arteries. The mechanism by which methanethiol caused cerebral dysfunction was not understood. The origin of the pulmonary embolus was not explained by the authors. Erythrocytes obtained on the day before the patient died gave evidence of some deficiency of glucose-6-phosphate dehydrogenase, which the authors considered may have played a part in the hemolytic phenomena in this case. It should be recognized, however, that the percent incidence of glucose-6-phosphate dehydrogenase deficiency is higher in the American Negro than in other ethnic groups [44].

In 1968, Gobbato and Terribile [15] described an episode of acute butanethiol intoxication in seven workers 25-34 years old. These individuals worked in a pilot plant in Italy where polymerization tests with acrylic resins were conducted.

In the course of these tests, the individuals mistakenly used about 1 kg of n-butanethiol, mislabeled as octanethiol, as a stabilizer and

antioxidant for an unstated amount of acrylic resin in an autoclave [15]. The exposure occurred when the workers opened the autoclave in a routine discharge operation. The intense characteristic thiol odor was noted, and the exposure lasted for about an hour. The autoclave apparently had been opened at a temperature of 70-80 C, instead of at the prescribed 50-60 C. Gas chromatographic analysis of an air sample taken 3 hours after the accident revealed that butanethiol rather than octanethiol had been the offending chemical. The exact concentration of butanethiol in the air during the exposure was not known but was surmised to have been between 50 (184.38 mg/cu m) and 500 (1,843.84 mg/cu m) ppm, based on published odor threshold data and the fact that the workers tolerated the odor for about 60 minutes before manifesting signs and proclaiming symptoms of toxicity. Intoxication was severe in one worker, mild in six. All seven workers had asthenia, muscular weakness, and malaise; six had sweating, nausea, retching, and headache; three had neck pains, dizziness, slight inebriation, or confusion; two showed anxiety, agitation, and drowsiness; and one had disturbed vision. One of the individuals lapsed into a coma.

On admission to the hospital, all seven displayed flushing of the face, sweating, increased rate of breathing, and obvious mydriasis [15]. Six of the patients recovered within a day but were held for observation for the next 3 days in the hospital. The most seriously affected patient, a 25-year-old man who remained unconscious for 20 minutes immediately after the exposure, suffered profound weakness, dizziness, nausea and vomiting, drowsiness, and depression.

Gobbato and Terribile [15] stressed the dominance of neurologic effects over the minimal renal or hepatic signs in this episode of acute butanethiol poisoning. In retrospect, they recommended that the then current maximum allowable concentration (MAC) of 10 ppm be respected and that respiratory protection be provided in cases of extended exposure above the MAC.

In 1941, Cristescu [13] described a case of mixed thiol poisoning at an oil refinery in Ploesti, Rumania. A workman descended into a pit to empty a trap containing condensate from a line through which methane-, ethane-, and other volatile thiols from a cracking process passed in transit to a burning stack. Refinery rules for this operation required the use of a gas mask, as well as the presence of a second person to observe from outside the pit, but neither of these regulations was followed. Two hours later, the man was found unconscious at the bottom of the pit sitting on a chair, his head bent over his chest. He was quickly hospitalized.

The patient was admitted in a comatose state, exhaling a very strong odor [13]. His face, lips, and limbs were cold and cyanotic. Generalized tonic contractions were accompanied by trismus and periodic convulsions. His cyanosis disappeared after 5 hours, and he regained consciousness 8 hours after his admission but did not remember when or why he had entered

the pit. Cristescu concluded that the 2-hour exposure to methane- and ethanethiols and others at a "high (but unknown) concentration" led to the following signs in the patient: loss of consciousness followed by amnesia, coma, cyanosis of the face and extremities, skeletal muscle contractions, trismus and clonic movements, paresis of bronchial muscles (as evidenced by rhonchi), fever, leukocytosis, lung abscess, and exhalation of a strong, characteristic odor; urinary abnormalities and erythrocytic changes were not observed. The general health of the worker was improved after he was immediately removed from the contaminated surroundings and placed under medical supervision. After his 2nd day of hospitalization, he was released in good health; however, 2 weeks after release, he returned to the hospital suffering from a severe cough. Medical examination revealed an abscess of his lungs, and treatment was begun immediately. After 2 weeks of treatment, the patient's general state of health improved, and he left the hospital completely cured.

Shibata [45], in 1966, reported the effects of experimental inhalation of ethanethiol on the respiratory and circulatory functions of three men. The inhaled and exhaled gases were analyzed for thiol content by gas chromatography.

For 20 minutes, two subjects inhaled ethanethiol at 50 ppm (127.12 mg/cu m), and one subject inhaled it at 112 ppm (284.58 mg/cu m) [45]. Their frequency of respiration, pulse rate, and blood pressure were measured either continuously or at prescribed intervals for 10 minutes prior to exposure. These measurements continued during the period of inhalation of ethanethiol; a recovery period followed. Exhaled air was sampled every 2 minutes for estimation of the concentration of ethanethiol. To analyze exhaled air for thiol, two subjects inhaled ethanethiol for 35-60 minutes; during this time 5-ml samples of inhaled and exhaled air were collected at known intervals. Average tidal volume was obtained by dividing the average minute volume of expiration by the breathing rate.

In one of the two subjects who inhaled 50 ppm (127.12 mg/cu m) ethanethiol, the breathing frequency decreased as soon as the inhalation started and returned to the preinhalation level following termination of inhalation [45]. The second subject's breathing rate did not change. The breathing rate of the subject exposed at 112 ppm (284.58 mg/cu m) became slightly irregular and decreased slightly. The minute volume of expiration tended to increase in all subjects. The tidal volume usually increased markedly; in one subject exposed at 50 ppm, this change was observed during the initial period of exposure only. The pulse rate showed a slight increase in only one subject inhaling 50 ppm. There were no electrocardiographic abnormalities, and no effect on blood pressure was observed. The only subjective response to ethanethiol was the recognition of the odor during the first few inhalations after the experiment was started; the odor apparently was not objectionable later. Nothing else was mentioned by the subjects during or after exposure to the gas. This study

established that with exposure to ethanethiol at 50-112 ppm, some physiologic changes occurred and the olfactory apparatus became fatigued within minutes of exposure.

The thiol concentration in the exhaled air during the experimental period was 20-40% of that inhaled, with no constant tendency to increase or decrease [45]. In the opinion of the author, 60-80% of the inhaled thiol was absorbed into the blood from the lungs at almost a constant rate. The retention of thiol in the respiratory apparatus, calculated from the minute volume and the thiol concentrations in the inhaled and exhaled air, was 1.2 µliter/minute.

Bagramian et al [46] published an abstract in 1976 on the chromosomal aberrations observed in 11 workers employed by a factory producing latex designated LNT-1 Latex, which was used in the manufacture of footwear. The workers were exposed to a mixture of airborne chloroprene (2-7 mg/cu m), dodecanethiol (1-2.5 mg/cu m), and ammonia (4-10 mg/cu m) during an unspecified length of time. Five workers from a shoe factory served as controls. Lymphocytes from peripheral blood from each individual were cultured, and 100-200 cells were examined at the metaphase stage of cell division. Chromatid breaks were observed more frequently in cells from the workers exposed to the mixture of vapors than in those from the controls. Chromosomal breaks were observed less frequently than chromatid breaks. Other chromosomal lesions, such as rings, dicentrics, and exchanges, were not observed. In the opinion of the authors, humans exposed for a long term to airborne chloroprene, dodecanethiol, and ammonia, in concentrations significantly lower than MAC's for these substances, develop increased chromosomal aberrations.

The effects on humans of inhalation exposures to three thiols and a mixture of two of these compounds are summarized in Table III-2 [12-15,45].

Odor Threshold Studies

The characteristic obnoxious odor of thiols, especially those of lower molecular weight, has been recognized since the initial synthesis of ethanethiol in 1834 [1]. The application of this property for use as a warning agent in industry has been a subject of considerable investigation.

Katz and Talbert [16], in 1930, described an extensive study on the odor thresholds of various substances including certain thiols in humans. Subjects were exposed to odorous substances at various concentrations; in addition to the determination of intensities of odor, nasal and eye irritations were noted. A series of 74 measurements was made with 55 chemicals, among which were methanethiol, ethanethiol, 1-propanethiol, 1-butanethiol, and benzenethiol. An odorometer was used [47] for determining the amount of thiol volatilized and for diluting the vapors with air to various concentrations ranging from 1 part in 10 to 1 part in 1,013. The concentration of a thiol at which odors could be detected was

TABLE III-2

EFFECTS ON HUMANS OF ACUTE INHALATION EXPOSURES TO THIOLS

Thiol	No. Exposed	Duration of Exposure	Concentration	Effects	Reference
Methanethiol	1 M	Up to 8 hr/d for 5 d; approx 1 hr on 1 day	Unknown low; unknown high for the 1 hr	Unconsciousness, offensive odor, coma, pulmonary and neurologic changes, intravascular hemolysis, hemolytic anemia, methemoglobinemia, and hemoglobinuria, death on d 28 (pulmonary artery emboli)	12
Mixed thiols (methane/ethane)	"	2 hr	Unknown high	Unconsciousness, coma, cyanosis, muscular contractions with trismus and clonic movements, exhalation of a strong odor, leukocytosis, lung abscess, recovery	13
Ethanethiol	"	20 min	112 ppm	Breathing rate slightly irregular and decreased, increase in minute volume of expiration, olfactory fatigue	45
"	2 M	"	50 ppm	Slight decrease in breathing rate during inhalation, increase in minute volume of expiration, olfactory fatigue	45
"	28 M, 2 F	1 hr	4 ppm (est)	Varying headaches, gastric discomfort urine changes in about 50%, recovery (6-8 hr)	14
Butanethiol	7 M	"	50-500 ppm	Eye disturbances, gastric discomfort, muscular discomfort, headache, nausea, mydriasis	15

measured by weight loss of the contents of the vaporizer and the total volume of air with which the thiol was mixed.

Six observers, including the operator of the odorometer, were normally employed to test intensities of odor and irritation [16]. Fourteen observers were used for tests with butanethiol that extended for 3 days. The test results are given in Table III-3.

No significant nasal or eye irritation was observed from alkane thiols [16]. However, benzenethiol caused a choking sensation in the throat, mucosal irritation, and headache. The odor of methanethiol was described by the participants as that of decayed cabbage, onions, and garlic; of ethanethiol as that of decayed cabbage, very disagreeable; of 1-propanethiol as that of onions, disagreeable; of 1-butanethiol as skunklike, disagreeable; and of benzenethiol as very disagreeable, repulsive, persistent (eye, nose, and throat irritation immediately after exposure; eyes irritated for hours). The single common physiologic response observed for all these thiols was nausea on exposure at a sufficiently high concentration. Benzenethiol also caused headache in some observers.

Perceiving the need to consider the obnoxious odor of thiols in the formulation of standards for air purity in industrial premises, Blinova [48], in 1965, reported olfactory threshold values for ethanethiol, propanethiol, and butanethiol. The experimental procedure involved having volunteers inhale for 1 minute through a gas mask connected to a 1,000-liter chamber in which a known concentration of thiol had been established. An individual threshold was established for each volunteer. During the intervals between experiments, the chamber was purged of thiols by UV radiation from a quartz lamp and by flushing with air. Nine individuals participated in the ethanethiol experiment that involved a total of 211 tests. The olfactory threshold (minimum perceptible concentration) ranged from 0.6×10^{-5} to 0.3×10^{-4} mg/liter. The maximum imperceptible concentration ranged from 0.5×10^{-5} to 0.2×10^{-4} mg/liter. Ten individuals participated in the propanethiol study in which a total of 208 tests was performed. The minimum perceptible concentration ranged from 0.7×10^{-5} to 0.3×10^{-4} mg/liter, and the maximum imperceptible concentration ranged from 0.6×10^{-5} to 0.2×10^{-4} mg/liter. Ten individuals participated in a total of 220 tests with butanethiol. The range of minimum perceptible concentration was from 0.5×10^{-5} to 0.4×10^{-4} mg/liter. Thus, similar threshold concentration values were obtained for ethane-, propane-, and butanethiol.

Precise information on the olfactory threshold for ethanethiol was acquired by exposing volunteers to ethanethiol at various concentrations below what the author [48] called the resorptive effect, by which he may have meant the dose inducing some symptom or sign of systemic toxicity. The volunteers inhaled the vapor continuously for 3 hours/day for 5-10 days

TABLE III-3

ODOR INTENSITY OF THIOLS IN HUMANS

Thiol	Degrees of Odor Intensity Vs Thiol Concentration in ppm*											
	0		1		2		3		4		5	
Methanethiol	0.003000	(0.0059)	0.04100	(0.081)	0.570	(1.1)	7.90	(16)	110	(220)	1,500	(3,000)
Ethanethiol**	0.000021	(0.000053)	0.00097	(0.0025)	0.045	(0.11)	2.10	(5.3)	97	(250)	4,500	(11,000)
	0.000006	(0.000015)	0.00026	(0.00066)	0.011	(0.028)	0.49	(1.2)	21	(53)	920	(2,300)
1-Propanethiol	0.000110	(0.00034)	0.00160	(0.005)	0.024	(0.075)	0.36	(1.1)	05.4	(17)	81	(250)
1-Butanethiol**	0.002700	(0.0099)	0.04800	(0.19)	0.840	(3.1)	15.00	(55)	260	(960)	4,600	(17,000)
	0.000045	(0.00017)	0.00100	(0.0037)	0.022	(0.081)	0.50	(1.8)	11	(40)	250	(920)
Benzenethiol	0.000005	(0.000023)	0.00025	(0.0012)	0.014	(0.063)	0.72	(3.2)	38	(170)	2,000	(9,000)

*0 = no odor, 1 = detectable, 2 = faint, 3 = quite noticeable, 4 = strong, 5 = very strong;
numbers in parentheses = concentration in mg/cu m
**Results of two tests are presented for ethanethiol and butanethiol.

Adapted from reference 16

from a special device, which was not described. The pulse and respiration rates and blood pressures of the subjects were measured, as were the fatigue response (by an unexplained method), the sensitivity of the olfactory analyzer, and the response of the taste analyzer to sweet and bitter substances. The chronaxie of the visual apparatus of the eye was also measured by use of what was described as an electronic pulsed stimulator that applied weak electric discharges to the eyeball. These measurements were made before and after each exposure to an experimental mixture. The state of fatigue was also estimated during 5 minutes in each of the 1st, 2nd, and 3rd hours of inhaling a mixture. The volunteers were subjected also to a control experiment in which they inhaled pure air. The tests produced no significant changes other than decreases in blood pressure and pulse rate that were considered physiologic responses to forced rest.

In the first experiment with ethanethiol, three volunteers were exposed to the compound at 10 mg/cu m (3.94 ppm) [48]. One of the volunteers, a woman, had a uniform response throughout the 10-day test with no tendency toward either intensification or habituation. Observations on the two men were made, therefore, for only 5 days. The woman's olfactory threshold rose from 6.2 ±0.26 to 12 ±0.18 ml. The corresponding shifts for the two men were from 9.4 ±2.7 to 11.0 ±1.8 ml and from 6.0 ±0.9 to 16.0 ±0 ml. Thus, weakened olfactory responses to ethanethiol were observed after 3-hour inhalation exposures. No changes in taste were reported. The rheobase of the visual apparatus lowered to some extent. For the woman the rheobase before exposure was 4.1 ±0.11 volts (V) and after exposure it was 3.6 ±0.33 V. Corresponding values for the two men were 4.8 ±0.45 V and 30 ±0.73 V before and 3.0 ±0.48 and 2.1 ±0.25 V after exposure. Error analysis indicated some fatigue.

All volunteers recorded, from the beginning of the experiment, a fairly strong smell resembling that of onions, garlic, or gasoline; the intensity of the odor lessened after about 1.5-2 hours [48]. Each person complained of such sensations as periodic nausea, irritation of the mucous membranes of the mouth and lips and, less frequently, the nose, a feeling of head heaviness, and fatigue.

In the second experiment conducted about a month later, the same individuals were exposed to ethanethiol at 1 mg/cu m (0.394 ppm) [48]. Increases in the olfactory threshold, but to a lesser extent than in the first experiment, were observed. The woman had an increase from 5.8 ±0.15 to 7.3 ±0.52 ml, and the corresponding changes for the two men were 6.7 ±0.8 to 7.5 ±0.94 ml and 7.3 ±0.25 to 11.0 ±0.45 ml. Neither a change in taste nor a lowering of the original rheobase of the eye was observed. The three subjects reported a moderate odor, resembling that of onions, that disappeared entirely halfway through the experiment, but no other unpleasant symptoms. In view of the above findings and in consideration of tests of its chronic toxicity in animals, 0.001 mg/liter was tentatively

recommended by Blinova as the maximum permissible concentration of ethanethiol for factories. This study [49] showed that the inhalation of ethanethiol led to an increase in the olfactory threshold more sensitively than it did to any other index studied. No other cumulative effects were noticed during the 5-10 days.

In 1969, Wilby [50] reported on the variability in recognition of odor thresholds by panel members in a study initiated by the Pacific Lighting System of Southern California. The 18 sulfur compounds chosen for the study on the basis of their predominant occurrence in natural gas included methanethiol, ethanethiol, 1-propanethiol, and 1-butanethiol.

The panelists chosen were not previously trained in odor threshold work [50]. All were company employees working in the same building. Comprising the panel were five men (two smokers) and four women (one smoker) aged 18-35; six men (four smokers) and seven women (four smokers) aged 36-55; and seven men (four smokers) and six women (two smokers) aged 56-66. All testing was done outdoors during clement weather when "no ambient odors" were present. A two-step dilution procedure was adopted to obtain threshold concentrations in the ppb range. First, a gas mixture of highly purified methane containing a few ppm of a thiol was confined at 200 psig in a specially constructed and cleaned 1.7-cu ft stainless steel pressure vessel and allowed to equilibrate for 24 hours. The gas mixture was then analyzed for the thiol by hydrogenation followed by estimation of H_2S by the methylene blue method and by gas chromatography. The latter procedure permitted detection of oxidation of the thiol to a disulfide. Second, dilution to the olfactory thresholds was accomplished with special odorometers. For each test, a series of concentrations, in increments of $10^{0.2}$, was presented to the panel in random order. The author estimated that the overall accuracy of estimation of the concentration was ±30%. The panelists walked in single file past three odorometers, pausing at each to take one or two breaths and to note on their test cards whether they detected an odor. The threshold concentration was defined as the lowest concentration the respondent could smell consistently, not necessarily the lowest concentration reported. The ratio of highest to lowest odor threshold concentration was determined for each panelist as a measure of the range of response of the panelists. The odor thresholds were presented in histograms for the different compounds. The histograms yielded estimates for the median and mean threshold concentrations, which are given in Table III-4.

Methanethiol and ethanethiol are metabolites of the human body and are excreted in the breath of normal subjects [20,22] and in higher concentrations in the breath as well as the urine [21] of patients suffering from advanced liver disease [22]. In 1975, Solis-Gaffar et al [20] published levels of methanethiol (expressed as elemental sulfur) in the early morning oral breath of normal subjects ranging from 23.4 to 33.9 ng S/ml. In 1955, Challenger and Walshe [21] established the presence of

TABLE III-4

VARIATION IN ODOR THRESHOLDS OF SELECTED THIOLS

Thiol	No. of Observers	Concentration Median (ppb)	Concentration Mean (ppb)	Standard Deviation	Coefficient of Variation	Highest:Lowest Odor Threshold (± SD)
Methane-thiol	34	0.80	0.99	0.71	0.72	3.15 (3.02)
Ethane-thiol	33	0.32	0.40	0.26	0.65	2.80 (1.62)
Propane-thiol	35	0.75	1.20	1.20	0.98	3.20 (2.85)
Butane-thiol	35	0.62	0.72	0.57	0.79	3.25 (2.24)

*Adapted from reference 50

methanethiol in the urine of a patient with fetor hepaticus ("liver breath") by precipitating it as the characteristic mercury mercaptide.

In 1970, Chen et al [22] reported the use of gas chromatography to measure the concentrations of methanethiol, ethanethiol, and dimethyl sulfide in the breath of normal subjects, both fasting and following ingestion of methionine, and of patients with cirrhosis of the liver or in hepatic coma. Methanethiol and ethanethiol levels in the breath of seven normal fasting subjects ranged from 0.1 to 1.3 ng/liter and 1.1 to 12.3 ng/liter, respectively. After daily ingestion of 8-12 g of methionine for 7 days, six subjects showed an average 1.5-fold increase in the concentrations of both methanethiol and ethanethiol in their breaths.

In 10 cirrhotic patients, the concentrations of methanethiol and ethanethiol in their breaths averaged 4.4 and 11.5 ng/liter respectively [22]. Methanethiol constituted on the average about 28% of the total thiols exhaled by these patients as compared with 12% in the controls. After ingesting methionine, the cirrhotic patients had slight increases in the exhaled concentrations of thiols, the percent increase in that of methanethiol being somewhat greater than in that of ethanethiol. The concentrations of ethanethiol did not increase appreciably in the breaths of the cirrhotic patients following ingestion of methionine. The intensity of the breath odor in cirrhotic patients after methionine administration was unrelated to the concentration of thiols but was directly related to the concentration of dimethyl sulfide, which ranged from 33.2 to 0.683 ng/liter in seven patients. The authors believed that the excess methionine fed to these patients was metabolized by intestinal bacteria to form dimethyl sulfide that bypassed the impaired liver to be excreted by the lungs.

The effects of thiols on humans in odor threshold studies are summarized in Table III-5.

Epidemiologic Studies

No report of an epidemiologic study of a population of workers exposed to thiols was found in the literature.

Animal Toxicity

(a) Acute Toxicity

The literature contains information on the acute toxicity of methanethiol, ethanethiol, propanethiol, butanethiol, pentanethiol, hexanethiol, and benzenethiol, but little published information was found on the acute toxicity of the other thiols (heptanethiol through octadecanethiol and cyclohexanethiol).

TABLE III-5

EFFECTS ON HUMANS OF THIOLS IN ODOR THRESHOLD STUDIES

Thiol	No. Exposed	No. or Duration of Exposures	Concentration ppm	Concentration mg/cu m	Effects	Reference
Methanethiol	6	1 inhalation	7.9	15.5	Nauseating odor (cabbage, onion, garlic)	16
"	6	"	41.0*	0.081	Very faint odor	16
"	6	"	3.0*	0.0059	No odor	16
"	34 M and F	1 or 2 inhalations in 3 sequential exposures	Mean ratio 3.15 (SD 3.02)		Highest to lowest odor threshold	50
Ethanethiol	6	1 inhalation	0.49	1.2	Easily noticed, nauseating odor	16
"	6	"	0.26*	0.00066	Very faint odor	16
"	6	"	0.006*	0.000015	No odor	16
"	18 M 17 F	1 or 2 inhalations in 3 sequential exposures	Mean ratio 2.80 (SD 1.62)		Highest to lowest odor threshold	50
"	1 M	20 min	112	284	Breathing rate slightly irregular and decreased, increase in minute vol of expiration, olfactory fatigue	45
"	2 M	"	50	127	Slight increase in breathing rate during inhalation, increase in minute volume of expiration, olfactory fatigue	45
"	9	1-min exhalation x 211 tests	Minimum 10.8-2.2*	0.03-0.006	Perceptible concentration	48
			Maximum 7.2-1.8*	0.02-0.005	Imperceptible concentration	
"	1 F	3 hr/d for 10 d	4	10	Nauseating odor (onion, garlic, gasoline), olfactory fatigue, mucosal irritation	48
"	"	3 hr/d for 10 d, 1 mo after above exposure	0.4	1.0	None	48

TABLE III-5 (CONTINUED)

EFFECTS ON HUMANS OF THIOLS IN ODOR THRESHOLD STUDIES

Thiol	No. Exposed	No. or Duration of Exposures	Concentration ppm	Concentration mg/cu m	Effects	Reference
Ethanethiol	2 M	3 hr/d for 5 d	4.0	10	Nauseating odor (onion, garlic, gasoline); olfactory fatigue; mucosal irritation	48
"	"	3 hr/d for 5 d, 1 mo after above exposure	4.0	10	Same as above but less pronounced	48
"	"	3 hr/d for 5 d	0.4	1.0	None	48
"	"	3 hr/d for 5 d, 1 mo after above exposure	0.4	1.0	"	48
Propanethiol	6	1 inhalation	0.36	1.12	Easily noticed, nauseating odor (onion)	16
"	6	"	1.6*	0.005	Very faint	16
"	6	"	0.11*	0.00034	No odor	16
"	18 M 17 F	1 or 2 inhalations in 3 sequential exposures	Mean ratio 3.2 (SD 2.85)		Highest to lowest odor threshold	50
"	10	1-min inhalation x 208	Minimum 9.6-2.2*	0.03-0.007	Perceptible concentration	48
			Maximum 6.4-1.9*	0.02-0.006	Imperceptible concentration	
Butanethiol	14	1 inhalation	0.5	1.85	Nauseating odor	16
"	14	"	1.00*	0.0037	Very faint odor	16
"	14	"	0.045*	0.00017	No odor	16
"	18 M 17 F	1 or 2 inhalations in 3 sequential exposures	Mean ratio 3.25 (SD 2.24)		Highest to lowest odor threshold	50
"	10	1-min inhalation X 220	Minimum 10.83-1.89*	0.04-0.007	Perceptible concentration	48
			Maximum 5.4-1.3	0.02-0.005	Imperceptible concentration	
Benzenethiol	6	1 inhalation	0.72	3.244	Headache, irritation (throat, eyes, nose), putrid odor	16
"	6	"	0.26*	0.0012	Very faint odor	16
"	6	"	0.005*	0.000022	No odor	16

*ppb

(1) Methanethiol through Hexanethiol; Benzenethiol

The inhalation toxicity of methanethiol was determined by Selyuzhitskii [51] in experiments with mice and rats. The details of exposure were not stated. The 2-hour LC_{50} for mice was 6.53 (5.67-7.5) mg/cu m. The 4-hour LC_{50} for rats was 8.87 (7.64-10.29) mg/cu m. The oral LD_{50} value for methanethiol in mice was 60.67 (52.3-64.31) mg/kg. Overall, the lack of data to support the statements made in the text and the paucity of details about experimental procedures diminish the utility of this report.

In 1978, a report [52] was published on the inhalation toxicity of methanethiol in white rats. Ninety rats were divided into 9 groups, each containing 5 males and 5 females. Each group was placed in a custom-built 75-liter glass chamber prior to a 4-hour exposure period. Eight groups of rats were exposed to methanethiol at eight concentrations ranging from 400 to 800 ppm (788 to 1,576 mg/cu m). The remaining group of rats was sham exposed to check for mortality arising from conditions other than actual gas exposure. After exposure, the rats were separated by sex and observed for the subsequent 14 days. Gross pathologic observations were made on the 2-week survivors as well as on those that died.

The calculated LC_{50} value for methanethiol was 675 ppm (1,338 mg/cu m), with apparent 95% confidence limits of 643-709 ppm (1,265-1,395 mg/cu m) [52]. No gross pathologic changes were observed in the survivors or in those that died. The author stressed that the results are relevant to the understanding of the acute effects and are not applicable to toxic effects produced by chronic exposure to low doses because the mechanism of chronic toxic effects is different from those of acute lethality.

Ljunggren and Norberg [53], in 1943, reported on the inhalation toxicity of methanethiol in white female rats weighing 90-130 g. Methanethiol was introduced into a chamber that had a 7.6-liter capacity and into which one rat was placed. The chamber was then hermetically sealed. At the end of the 30- to 35-minute exposure, the maximum calculated carbon dioxide concentration was 1.8% by volume, which the authors concluded would not affect the experiment. The concentrations at which methanethiol was tested ranged from 500 to 10,000 ppm (985 to 19,700 mg/cu m). Apparently, only one rat was exposed to methanethiol at each of four concentrations. Surviving animals were killed by decapitation after a 24-hour observation period, and their lungs were prepared for microscopic examination.

No physiologic or pathologic changes were observed in the rat exposed to methanethiol at 500 ppm (985 mg/cu m) for 30 minutes [53]. The rat exposed at 700 ppm (1,379 mg/cu m) "appeared tired" during exposure but recovered quickly on removal from the chamber. After 30 minutes, the rat exposed at 1,500 ppm (2,955 mg/cu m) could rise on its legs "only

momentarily" but recovered within 5 minutes after removal from the chamber. Microscopic examination of the lungs of this rat revealed thickened alveolar walls and a hemorrhagic exudate within the alveoli. The rat exposed at 10,000 ppm (19,700 mg/cu m) went into convulsions after 1 minute and died within 14 minutes. Necropsy disclosed small hemorrhagic areas in the lungs, and microscopic examination revealed areas in the alveoli filled with erythrocytes and serous fluid. The evidence suggests that methanethiol, at high concentrations, is a central nervous system (CNS) depressant causing paralysis of the locomotor muscles and irritation of the mucous membranes.

In a 1960 report on the inhalation toxicity of methanethiol in white mice, Horiguchi [54] suggested that methanethiol is a CNS toxicant. Groups of 10 mice (13-17 g) were exposed to methanethiol for up to 4 hours at each of the following concentrations" 1,200 (2,364 mg/cu m), 1,300 (2,561 mg/cu m), 1,500 (2,955 mg/cu m), 1,600 (3,152 mg/cu m), 1,800 (3,546 mg/cu m), and 2,200 ppm (4,334 mg/cu m). Nine mice made up a 2,000-ppm (3,940 mg/cu m) exposure group. Signs of methanethiol poisoning preceding death and the number of deaths occurring during the test were recorded.

The calculated LD_{50} value for methanethiol was 1,664 ppm (3,278 mg/cu m), with apparent 95% confidence limits of 1,577-1,757 ppm (3,107-3,461 mg/cu m) [54]. If signs of toxic effect were present, they consisted of paralysis of all four limbs and convulsions. The initially increased, and later suddenly decreased, respiratory rates in the mice that died indicated to Horiguchi paralysis of the respiratory centers in these animals. Within 10-60 minutes after the first evidence of limb paralysis and general convulsions, all mice manifesting these effects were dead. Approximately 20% of the surviving animals exhibited increased respiratory rates during exposure.

The animals were necropsied either immediately after death or following a 4-hour observation period after the end of the exposure [54]. Minor congestion was found in the lungs, liver, kidneys, and spleen of all mice. With exposure at increasing concentrations of methanethiol, the air content of the lungs decreased, and inflammation was found in the nasal mucosae and lungs. Microscopic examination of fixed and stained sections of organs from mice exposed to methanethiol at 1,200, 1,600, and 2,200 ppm (2,364, 3,152, and 4,334 mg/cu m) revealed fatty degeneration of the liver and congestion of the kidneys and lungs in proportion to the concentration of methanethiol; in addition, hyperemia of the lungs, with some edema perivascularly and within alveoli, and petechiae of the respiratory tract were found in mice exposed to methanethiol at 2,200 ppm (4,334 mg/cu m). No changes were found in their hearts, digestive organs, or brains.

In 1974, Zieve et al [55] reported on the "coma-producing" properties of methanethiol and ethanethiol. Male rats, weighing 285-325 g, were exposed singly for up to 15 minutes to methanethiol or ethanethiol at

concentrations of approximately 600-2,200 ppm (1,182-4,334 mg/cu m) or 27,000-38,000 ppm (68,580-96,520 mg/cu m), respectively. Three to eight rats were exposed at each concentration. For static exposures, the desired thiol concentrations were achieved by injecting the required amount of thiol through a rubber septum in the lid of the chamber into which the rat had been placed. Occasional analysis of chamber air showed that actual concentrations varied by 5% at a 1%-by-volume (10,000 ppm) concentration and by less than 25% at the 0.1%-by-volume (1,000 ppm) concentration. For the determination of dose-response relationships, the animals were observed until they had completely lost the righting reflex or until they had been exposed to the thiol for 15 minutes. The concentration of thiol in the blood was determined after a 4-minute exposure.

The thiol concentration at which 50% of the rats lost their righting reflex was 1,600 ppm (3,152 mg/cu m) for methanethiol and 33,000 ppm (83,820 mg/cu m) for ethanethiol [55]. The rats went through a brief excitement period before they became "groggy and lethargic," and then their righting reflex was lost within the next few minutes. The duration of the phase of excitement and beginning depression varied inversely with the concentration of the thiol inhaled. Methanethiol at 2,000 ppm (3,940 mg/cu m) caused all rats to lose their righting reflex, whereas 1,200 ppm (2,364 mg/cu m) was the highest concentration at which no animal lost the righting reflex. If the animals were removed from the chamber immediately after losing the righting reflex, "consciousness" was regained within 30 minutes. How consciousness was determined was not stated.

The ratio of the concentration of thiol in the blood to that inhaled was determined [55]. Rats exposed to methanethiol at an air concentration of 0.066 millimole/liter had blood levels of the substance that ranged from 0 to 0.5 millimole methanethiol/ml blood. For ethanethiol, a mean concentration of 200 nmol/ml blood was attained in 11 of 15 rats exposed to the substance at an air concentration of 1.32 millimoles/liter. From the data presented, no dose-response relationship could be determined.

Three metabolic studies relating to CNS toxicity [49,56,57] suggest that prolonged exposure and consequent increased concentrations of methanethiol in the brain may affect the brain Na^+,K^+-ATPase system. Foster et al [56] reported a study of the effect of methanethiol and ethanethiol on the Na^+,K^+-ATPase system in rat brains. When concentrations of methanethiol ranging from 0.19 µmol (9.12 µg) to 1.9 µmol (91.2 µg) were added to the system in vitro, 46-74% inhibition of Na^+,K^+-ATPase was produced. The dose-response relationship was characterized as not linear. The concentrations of methanethiol and ethanethiol required to cause 44-45% inhibition were 0.19 µmol and 0.21 µmol, respectively.

In a detailed examination of the kinetics of inhibition of the Na^+,K^+-ATPase system, Quarfoth et al [49], in 1976, indicated that

methanethiol caused a 50% inhibition of rat brain ATPase at a 0.1 mM thiol concentration and that the inhibition increased to 72% at 1.0 mM. Complete inhibition was not achieved. Inhibition was not time dependent, did not increase during 30 minutes, and was determined to be completely reversible when the concentration of the thiol was decreased by dilution of the assay medium.

Pashchenko [57], in 1969, studied in vivo the effect of methanethiol on rat Mg^{++}-ATPase activity. When rats inhaled methanethiol at 2,030-2,538 ppm (4,000-5,000 mg/cu m) for an unspecified period, brain ATPase activity decreased by 15%. When rats inhaled methanethiol at 203-305 ppm (400-600 mg/cu m) for 3 hours/day for 3 weeks, the ATPase activity of brain, lungs, and spleen decreased by 15, 13 and 28%, respectively. However, when the animals were exposed for a longer, unspecified time, the ATPase activities of brain and spleen increased by probably insignificant amounts (5 and 12%, respectively). These results indicate that exposure to methanethiol alters Mg^{++}-ATPase activity.

Shibata [58], in 1966, reported the effect of ethanethiol on respiratory function in six 3-kg male rabbits that inhaled ethanethiol at 10, 100, and 1,000 ppm (25, 254, and 2,540 mg/cu m) for approximately 20 minutes through masks. Both the breathing rate, which was measured by observed movements of the thorax, and the minute expiratory volume, as measured by wet spirometry were monitored throughout the exposure period. The tidal volume then was calculated by dividing the minute expiratory volume by the breathing rate.

All indicators of respiratory function, except the breathing rate, of the rabbits exposed to ethanethiol at 1,000 ppm, returned to control levels by the end of the 35-minute postexposure observation period [58]. The breathing rate of the rabbits exposed at 1,000 ppm remained depressed. For all three concentrations of ethanethiol, a negative correlation was found between the breathing rate and tidal volume during inhalation.

In 1958, Fairchild and Stokinger [59] extensively reported on the acute toxicity of ethanethiol, propanethiol, butanethiol, hexanethiol, and benzenethiol in rats and mice and on the toxicity of benzenethiol in rabbits. Male animals were exposed to the thiols by intraperitoneal (ip) injection, oral intubation, inhalation, and cutaneous application. Rats, weighing an average of 180-220 g, were exposed to the various thiols by each of the four routes of administration. Mice, weighing 25-28 g, were exposed to thiol vapors by inhalation. The experimental groups consisted of from 5 to 10 animals, except that 3 groups of only 2 rabbits each, of unspecified weight, were exposed to benzenethiol applied to the skin on an area of the back from which the hair had been clipped. The dosages administered by each exposure route differed by a factor of either 1.26 or 2.0 in a geometric series. For the ip and oral administration, cumulative mortality was determined on days 1, 2, 3, 5, 10, and 15. In most cases,

the LD_{50} and LC_{50} values were calculated for days 1, 2, and 15. The mortalities of rats and rabbits were closely observed during the 72 hours following a single application of benzenethiol to the skin. Animals were killed either immediately after an experiment or after a 2-week to 1-month observation period.

In the inhalation experiments, groups of 5 rats and 10 mice were exposed for 4 hours [59]. The accuracy of the sampling technique and analytical procedure was such that the recovery was found to be within 2% of the calculated amount taken. For ip and oral administration, the aliphatic thiols were given undiluted. Benzenethiol was administered orally as an 8% V/V solution in ethanol and ip as a 5% V/V solution in ethanol. For estimation of absorption from the skin's surface, areas of skin approximately 3 cm square, or 6 x 10 cm, of the upper midbacks of rats and rabbits, respectively, were clipped as close to the skin as possible without causing abrasions. Measured amounts of undiluted benzenethiol were dropped on the clipped areas of the skin.

Mice were more susceptible to 4-hour inhalation exposures of the various thiols than were rats [59]. Except for benzenethiol, the thiols were approximately twice as toxic to mice as to rats on the basis of comparisons of 15-day postinhalation LC_{50} values. The incidence of delayed toxicity was higher in rats than in mice, but delayed mortality was very marked in both rats and mice exposed to benzenethiol. Table III-6 presents the 4-hour LC_{50} data for rats and mice.

The characteristic signs of toxicity found with maximum sublethal and lethal concentrations of thiols were increased breathing rate and restlessness (hyperactivity in mice), uncoordinated movement and staggering gait, muscular weakness, partial skeletal muscle paralysis beginning in the hind limbs, light to severe cyanosis, tolerance of the prone position, and apparently mild to heavy sedation [59].

When administered in single ip doses to rats, as by other routes of administration, ethanethiol was the most toxic of the alkane thiols tested, being surpassed in toxicity by benzenethiol alone. The data are presented in Table III-7.

Fairchild and Stokinger [59] observed that, to some extent, delayed toxicity with intermittent mortality was found for all thiols. The 24-hour ip LD_{50}'s were approximately 1.5-2 times the respective 15-day values. Much of the delayed toxicity occurred subsequent to the 48-hour postinjection period. For all thiols tested, the appearance of the following toxic effects in rats was fairly consistent: restlessness, increased respiratory activity, incoordination, muscular weakness, skeletal muscle paralysis (in most cases), mild to heavy cyanosis, lethargy or sedation or both, respiratory depression followed by coma, and death after lethal doses. Muscle paralysis, when present, affected the hind limbs first. All the aliphatic thiols were found to be apparent central

TABLE III-6

LC_{50} VALUES FOR RATS AND MICE AFTER 4-HOUR INHALATION EXPOSURES TO VAPORS OF SELECTED THIOLS

Thiol	Analyzed Concentration (ppm)	LC_{50} (95% Confidence Limits in ppm)					
		Rats (range of body wt: 180-220 g)			Mice (range of body wt: 25-2 g)		
		24 hr	48 hr	15 d	24 hr	48 hr	15 d
Ethanethiol	2,600-5,125	4,870 (4,783-4,957)	4,565 (4,448-4,682)	4,420 (4,299-4,541)	-	2,770 (2,661-2,879)	-
Propanethiol	3,050-11,260	-	7,300 (Estimate)	-	-	4,950 (Estimate)	4,010 (Estimate)
Butanethiol	2,150-6,000	4,460 (4,132-4,786)	4,280 (3,959-4,601)	4,020 (3,656-4,384)	2,950 (2,824-3,076)	-	2,500 (2,437-2,563)
Hexanethiol	220-1,475	1,200 (1,115-1,285)	1,145 (1,044-1,236)	1,080 (930-1,230)	610 (548-672)	550 (487-613)	528 (470-586)
Benzenethiol	20-132	-	59 (50.7-67.3)	33 (29.6-36.4)	47 (43.4-50.6)	35.5 (32.4-38.6)	28 (24.8-31.2)

Adapted from reference 59

TABLE III-7

LD_{50} VALUES FOR RATS AFTER SINGLE IP DOSES OF SELECTED THIOLS

Thiol	Dose Range* (mg/kg)	LD_{50} (95% Confidence Limits in mg/kg) Day 1	Day 2	Day 15	Day of Last Death
Ethanethiol	105-1,680	450 (359-564)	420 (331-532)	226 (180-283)	5-10
Propanethiol	209-1,672	1,028 (781-1,298)	780 (556-1,096)	515 (390-679)	3-5
Butanethiol	209-1,672	679 (515-896)	-	399 (257-619)	5-10
Hexanethiol	212-1,696	600 (405-887)	-	396 (282-556)	10-15
Benzenethiol	6.7-108.00	25.2 (17.9-35.4)	-	9.8 (7.0-13.7)	5-10

*Altered by factors of 2 (ethanethiol) or of 1.26 (remaining thiols) in geometric series; 5-10 animals at each dose

Adapted from reference 59

depressants, the degree of depression ranging from mild stupor to heavy sedation. Benzenethiol caused only slight sedation.

The acute toxicity data for rats following single oral doses of selected thiols are presented in Table III-8.

The oral toxicity of the thiols was considerably lower than that found after ip injection [59]. Delayed toxic effects, although evident with the alkane thiols, were not seen as frequently in the orally dosed rats as in those injected ip; none was reported for rats given benzenethiol. The signs of acute oral toxicity were essentially the same as those seen after ip administration. The characteristic central depressant action of the alkane thiols was evident, particularly in rats given hexanethiol.

Benzenethiol was applied to the clipped backs of rats at doses ranging from 134 to 538 mg/kg and to the backs of rabbits at doses from 67 to 269 mg/kg [59]. The cutaneous LD_{50}'s, determined 4-8 hours after application, were 300 mg/kg (95% confidence limits 236-384 mg/kg) for rats and 134 mg/kg (estimated) for rabbits. Signs of percutaneous benzenethiol toxicity were similar to those observed after ip and oral administration. Benzenethiol produced some inflammation of the skin a few hours after percutaneous application, but the redness usually disappeared within 24-48 hours after the exposure ended.

When autopsies were performed on animals dying after single ip, oral, or percutaneous doses of thiols, significant gross or microscopic tissue changes were not usually found [59]. Animals that survived near-lethal ip or oral doses of thiols and that were killed 20 days after treatment frequently exhibited microscopic changes indicative of damage to the liver and kidneys. The rats and mice that died several hours after exposure at a high concentration of a thiol vapor had mild to severe hyperemia of the trachea and lungs. Changes seen in mice exposed to thiols at moderate to near-lethal concentrations were greater than those in rats exposed at the same concentrations. Microscopic changes included those called cloudy swelling, fatty degeneration, and necrosis of the liver; capillary engorgement, patchy edema, and occasional hemorrhage in the lungs; and mild to moderate cloudy swelling in the kidneys. Rats had a high incidence of acute pneumonia, and alveolar wall breakage was consistently found in those exposed to benzenethiol.

To determine whether there was a true increase of pneumonia as a result of benzenethiol exposure, the authors exposed 7-10 rats with existing pulmonary infection at "low" concentrations of the thiol for 3 consecutive days [59]. Histologic examination of sections of lung revealed that benzenethiol exacerbated the latent respiratory infection, whereas in nine control rats the latent pulmonary infection had not been activated. Benzenethiol was the only thiol that caused microscopic changes after exposure at low concentrations.

TABLE III-8

LD_{50} VALUES FOR RATS AFTER SINGLE ORAL DOSES OF SELECTED THIOLS

Thiol	Dose Range* (mg/kg)	LD_{50} (95% Confidence Limits in mg/kg) Day 1	Day 2	Day 15	Day of Last Death
Ethanethiol	210-3,360	1,034 (667-1,603)	-	682 (517-900)	10-15
Propanethiol	1,327-3,344	2,362 (2,014-2,770)	2,055 (1,836-2,300)	1,790 (1,632-1,963)	5-10
Butanethiol	1,093-3,344	2,575 (2,145-3,090)	1,683 (1,346-2,105)	1,500 (1,244-1,809)	7
Hexanethiol	848-2,137	-	1,580 (1,347-1,855)	1,254 (1,084-1,451)	3-5
Benzenethiol	21.6-172.5	46.2 (29.8-71.6)	-	-	1

*Altered by factors of 2 (ethanethiol, benzenethiol) or 1.26 (remaining thiols) in a geometric series; five animals at each dose

Adapted from reference 59

In summary, the general signs of acute thiol poisoning exhibited by mice, rats, and rabbits were central depression and respiratory paralysis, with death caused by respiratory failure [59]. Certain thiols were associated with a high incidence of delayed mortality. All the thiols were central depressants, the degree ranging from mild stupor to heavy sedation. Inhalation, ip, and oral administration, at relatively high levels, caused liver and kidney damage. Inhalation of thiols at high concentrations caused slight to severe irritation of the respiratory tract, the degree being dependent on the thiol. Some rats exposed to benzenethiol developed pneumonia, possibly as a result of activation of latent pulmonary infection. Benzenethiol, administered by all routes, also caused significant microscopic changes at low levels of exposure.

Carpenter et al [60] performed range-finding assays, published in 1949, on acute vapor toxicity. Male and female albino rats weighing 100-150 g were exposed to pentanethiol in a 9-liter desiccator. The authors assumed that the calculated pentanethiol concentration was probably slightly higher than an analytically estimated one would have been. Six rats were exposed to pentanethiol at 2,000 ppm (8,520 mg/cu m) for 4 hours. Two, three, or four (exact number not stated) of six rats died during the 14-day observation period. On the basis of these results, the authors concluded that the degree of hazard associated with exposure to pentanethiol is moderate. As mentioned earlier [59], the LC_{50} values on the 15th day following single 4-hour inhalation exposures of rats to butanethiol and hexanethiol were 4,020 ppm (14,834 mg/cu m) and 1,080 ppm (5,227 mg/cu m), respectively. Pentanethiol would thus appear to rank between butanethiol and hexanethiol in inhalation toxicity.

(2) Heptanethiol through Dodecanethiol; Cyclohexanethiol

The intravenous (iv) toxicity of the following thiols was determined in mice: propane-, butane-, hexane-, heptane-, octane-, nonane-, decane-, undecane-, dodecane-, hexadecane-, octadecane-, and cyclohexanethiol (WW Wannamaker, written communication, December 1977). The LD_{50} values for the aliphatic thiols were all greater than 316 mg/kg. The LD_{50} value for cyclohexanethiol was estimated to be 316 mg/kg because one of two mice injected at this concentration died. When present, the signs of toxicity generally were rapid breathing, hunched posture, and decreased activity.

The acute toxicity of dodecanethiol and of a mixture of C_7 through C_{11} thiols to mice and rats was studied by Gizhlaryan [61] in 1966. The intragastric LD_{50} of dodecanethiol was 4,225 mg/kg (range 3,069-5,381) for the mouse, but a dose of 7,000 mg/kg caused no deaths in rats. When a mixture of C_7 through C_{11} thiols was administered, the LD_{50} for rats was 3,300 mg/kg (range 2,842-3,758) and the LD_{50} for mice was 2,025 mg/kg (range 1,579-2,471). Experimental details were not provided. The author indicated that the inhalation of unstated concentrations of dodecanethiol,

or the thiol mixture, for 2 hours caused no lethal effects. Dodecanethiol and the thiol mixture applied to the skin at concentrations of 3.4 ±0.1 mg/liter and 2.9 ±0.12 mg/liter, respectively, caused marked local effects, as well as general poisoning, in both rats and mice; no further information was provided. This study indicates that the higher molecular weight thiols have a low toxicity in comparison with the lower molecular weight thiols.

(3) Ocular Effects

Fairchild and Stokinger [59], in 1958, reported results of tests on the ocular toxicity of ethane-, propane-, butane-, hexane-, or benzenethiol in rabbits. One-tenth of a milliliter of each undiluted thiol was instilled into the conjunctival sac of the right eye of a male rabbit; the whole left eye served as a control. Propanethiol and benzenethiol were later instilled into the control eye, which was washed copiously with water approximately 5 seconds after instillation. Ocular reactions were observed with a hand slit lamp.

Ethanethiol, propanethiol, and butanethiol caused slight to moderate irritation [59]. Hexanethiol was not irritating. Propanethiol was the only alkane thiol that caused an irritation observable 48 hours after exposure ended. Heavy discharge and severe redness of the palpebral conjunctivae were evident at the 24- through 96-hour observations, and chemosis appeared at 48 hours. The same signs of irritation were seen in eyes washed with water after instillation of propanethiol, but, in each case, the conditions gradually improved and disappeared by day 8 after the exposure.

Benzenethiol produced not only severe irritation of the conjunctivae but also injured the cornea [59]. The instillation of undiluted benzenethiol into the conjunctival sac produced moderate to severe redness, chemosis, and a discharge that continued for 3-4 days. The conjunctivae gradually returned to normal by the 16th day. All three benzenethiol-exposed rabbits developed diffuse corneal opacities involving three-quarters of the cornea by the 4th day after instillation. In one, this condition subsided within 3 days, and no sign of injury persisted. In the other two rabbits, the corneal opacities gradually increased in density until the 16th and 19th days, when opalescent areas covered the iris. All evidence of corneal injury disappeared within 2 months following instillation.

The injury caused by benzenethiol seemed to be increased in rabbits whose eyes had been flushed with water approximately 5 seconds after instillation of the thiol [59]. Signs of conjunctival irritation were evident in two such rabbits until the 16th and 21st days after the instillation. By approximately 3 weeks after treatment, opalescent areas of the cornea completely covered the iris in one rabbit, whereas the other had slightly less corneal injury. These conditions persisted for several

weeks before recovery began, but recovery was complete after 3-4 months. The authors found that, if the eye was flushed with 0.5% silver nitrate solution immediately after thiol instillation and then with copious amounts of water to remove the visible silver-sulfhydryl precipitate, both the irritation and corneal injury caused by benzenethiol were minimized. Depilation frequently occurred in areas around and below the eyes, being caused apparently by contact with benzenethiol. Depilation was prominent at 5-6 days after exposure and lasted from 2-3.5 weeks.

(b) Subchronic Toxicity

(1) Methanethiol through Hexanethiol; Benzenethiol

Horiguchi [54], in 1960, reported on the toxicity of methanethiol to mice. Eleven male white mice, weighing 13-17 g, were exposed at 300 ppm (591 mg/cu m) for 2 hours/day, 3 days/week, for 2 months. Horiguchi's methods of exposure were described in Acute Toxicity. All 11 mice were dead after 25 exposures, 6 or more having died after 15 exposures.

In 1972, Selyuzhitskii [51] described the effects in rats of methanethiol inhalation. White rats, in groups of 15 each, were exposed to methanethiol 6 hours/day for 6 months at 0.51, 0.05, and 0.0003 ppm (1.0 ±0.003, 0.1 ±0.002, and 0.0005 ±0.00004 mg/cu m).

Rats that inhaled methanethiol at 0.51 ppm (1.0 mg/cu m) exhibited a reduced growth rate, an increase in the ratio of heart weight to body weight and changes in the distribution of corticosteroids in adrenal tissue [51]. Electroencephalographic (EEG) recordings from rats exposed 6 hours/day for 6 months showed that these animals perceived a concentration of methanethiol of 0.5 µg/cu m but were not distressed by it; a concentration of 5 µg/cu m reportedly caused desynchronization of the EEG, persistent disturbance of breathing, and irregular heartbeats. A concentration of 0.1 mg/cu m caused a number of alterations in the biochemistry of the rats, including increased concentrations of carbon dioxide, SH, cholesterol, and lactic and pyruvic acids in the blood. A concentration of 1 mg/cu m decreased the rate of growth of the rats, increased the relative weight of the heart, and altered the distribution of corticosteroids in adrenal tissues. The effects of the oral administration of methanethiol to mice were not discussed.

Sandage [62] studied physiologic changes in male mice, rats, and monkeys continuously exposed to methanethiol for 90 days. The animals were kept in large, insulated exposure chambers, where the rate of air turnover was 10% of the chamber volume per minute. Methanethiol in cylinders equipped with regulator valves was used to maintain the methanethiol in the chamber at 50 ±5 ppm (98 ±9.8 mg/cu m). Mean body weights for the 10 monkeys, 50 rats, and 100 mice making up the experimental group were 1.7 kg, 175 g, and 25 g, respectively. Various clinical studies were performed

before, and at 30-day intervals during, exposure. Approximately 50% of the animals surviving the 90-day exposure were subjected to a stress test in which the swimming time for the control animals was 45 minutes. The stress-tested animals were necropsied shortly after the stress tests. The remaining animals were observed for 2 weeks and then necropsied. Tissues from the heart, lungs, liver, kidneys, and brain were examined microscopically. The data were summarized with statements that certain changes were statistically significant (95% confidence limits) but without actual values.

Monkeys had a 40% mortality during the exposure period [62]. The experimental monkeys lost significant amounts of weight. However, in the swimming stress tests, they were able to hold their heads above water longer than the control animals. Urinalyses gave normal results. When compared with preexposure values, the only measured index in blood that was significantly altered in the exposed monkeys was the concentration of sodium, which was increased. When compared with control animals kept for 90 days, monkeys exposed to methanethiol showed increases in blood cholinesterase and alkaline phosphatase activity. When necropsies were performed on the monkeys, six were found to have lung changes and two were found to have brain changes consisting of a small softening in the cortex of the right parietal lobe of one monkey and of the left frontal lobe of the other; three of the monkeys had no microscopic changes. Most of the pulmonary effects consisted of mild to moderate edema, often associated with either vascular congestion or an accumulation of polymorphonuclear leukocytes or both. None of the lesions reported accounted for the 40% mortality found in monkeys.

A number of hematologic changes were seen in rats exposed to methanethiol [62]. When compared with values determined before the experiment, reticulocyte counts, hematocrit, hemoglobin concentration, mean corpuscular volume, and mean corpuscular hemoglobin values were significantly elevated. When experimental values were compared with the values from the control animals kept for 90 days, there were significant decreases in erthyrocyte and platelet counts and hemoglobin concentration and significant increases in leukocyte and reticulocyte counts. The differences observed in the rats (the 10% mortality, the stress-test results, and weight changes) were not considered statistically significant when compared with those of the control group. In rats, the findings at necropsy were limited to unspecified lung changes in 16% of the animals; 84% showed no organ changes. The changes observed in the blood could have resulted from the stress to which the animals were subjected. The effect of methanethiol on the rats was considered slight by the investigator.

Mice exposed to methanethiol at 50 ppm (98 mg/cu m) had a variety of hematologic and other effects [62]. When compared with preexposure values, the erythrocyte count was decreased and the leukocyte and platelet counts and the mean corpuscular volume were increased in the exposed mice. In

comparison with the control group at 90 days, the experimental group had significantly decreased erythrocyte counts and mean corpuscular hemoglobin concentrations; significant increases were found in the reticulocyte and platelet counts, the mean corpuscular volume, and the concentration of urobilinogen. Mortality at 43% was statistically significant. Methanethiol-exposed mice also demonstrated shorter stress-test swimming times. At necropsy, the percentages of mice showing cellular changes in the various organs were tallied, but not described, as follows: liver, 75%; lung, 26%; kidney, 22%; and heart, 1%. Persistent hepatitis was found in the mice, and there were a "few cases" of bronchopneumonia and pyogenic abscesses of the liver and lungs. The 43% mortality of the mice was most probably caused by hepatitis, pneumonia, and lung and liver abscesses. Methanethiol may have contributed to the morbidity and mortality.

The subchronic toxicity of ethanethiol for rats and rabbits was studied by Wada [63]. Ethanethiol was prepared as either a 10 or 30% solution in peanut oil and injected into groups of rats and rabbits. One group of rats and rabbits was injected sc with 0.01 ml/kg of ethanethiol daily. In another group, some of the rats were injected with 0.09 ml/kg of ethanethiol daily, and others were injected with 0.09 ml/kg of ethanethiol every other day. In one more group, rabbits were injected initially with 0.03 ml/kg of ethanethiol every other day. After an unspecified period of time, the injected volume was changed to 0.01 ml/kg of ethanethiol. The injections were continued for 1 year or until the animal died.

All animals developed localized necrosis at the site of injection, with rabbits generally developing more serious effects [63]. The intensity of the necrosis increased in proportion with the concentration injected. When injection ceased, the necrotic tissue was gradually replaced with scar tissue. Rabbits injected with 0.01 ml/kg of ethanethiol either daily or every other day had reductions in red blood cell counts (RBC's) and hemoglobin. Leukocyte and reticulocyte counts both increased. The degree of change generally was related to the dose. The most marked microscopic changes in both rats and rabbits were found in the spleen. Findings there included hyperemia and dilation of sinusoids, deposits of hemosiderin, fibrosis, red blood cell destruction by white blood cells, and hematopoiesis. Some of these changes were seen also in the control animals. Microscopic changes in the liver, lungs, kidney, testes, and ovaries were minor and were possibly incidental to the debility caused by the injection. The author concluded that many of the red blood cells were destroyed, as reflected in the splenic changes, but offered no further explanation. This and the previous study [62] both indicate that subchronic exposure to methanethiol or ethanethiol may lead to a reduction in the erythrocycte count.

Shibata [58] reported in 1966 on the effects of inhalation of ethanethiol on the blood picture, urinary volume, sulfate excretion, and body weight of rabbits. Four male rabbits each inhaled 10 liters/day of

air containing ethanethiol at 1,000 ppm (2,540 mg/cu m) for 20 minutes/day through masks on their muzzles. Midway through the 10-day experimental period, the animals were given 1 day of rest; the total inhalation period was thus 9 days. Urinary sulfates, blood cell counts, urine amounts, and body weights of the rabbits were determined on days 3, 5, 7, 9, 11, and 13.

Although shortly after the start of the study urinary sulfates were considerably elevated in two of the four exposed rabbits, they had returned to control levels by day 11 [58]. The author concluded that, in general, the rabbits exposed to ethanethiol had no significant increase in urinary sulfates above control values. In the experimental group, the WBC decreased slightly after 5-7 days of exposure to ethanethiol but returned to the control levels by day 11. Erythrocyte counts, urinary excretory volumes, and body weight gains were similar in both the experimental and control groups. Thus, the inhalation of ethanethiol at 1,000 ppm (2,540 mg/cu m) during 20 minutes/day for 9 days did not adversely affect these variables in the rabbits.

In 1975, Szabo and Reynolds [64] as a part of their survey of compounds having a two-carbon atom skeleton for possible ulcerogenic effects, included tests on ethanethiol and butanethiol in rats. Two of five female rats given oral dosages of butanethiol at 20 mg/100 g thrice daily on the 1st and 2nd day and at 40 mg/100 g on the 3rd and 4th day developed adrenal necrosis, apparently in the cortex. The degree of necrosis was not described. None of the rats developed duodenal ulcers. None of the rats given a similar dosage schedule of ethanethiol for only 3 days developed either adrenal necrosis or duodenal ulcers. No other studies have indicated these types of changes.

Fairchild and Stokinger [59], in 1958, reported a study on the subchronic toxicity of benzenethiol in rats. Six male rats, weighing 180-220 g, were injected ip with nine doses of 3.5 mg/kg benzenethiol, ie, one-third the ip LD_{50}, as a 2% V/V solution in ethanol for 3 weeks. One rat died on the 7th day. The remaining rats had no significant weight loss, and no signs of cumulative toxicity were noted. When the rats were necropsied, only minor lesions were found. The repeated irritation of ip injections apparently caused a fibrous thickening of the splenic capsule. Enlargement of the spleen was found in most of the rats, and hyperemia of the adrenal medulla was found in all rats. Some rats had a mild degree of what was called at that time cloudy swelling in the tubules of the kidneys, with hyaline casts in the lumina. These pathologic changes are similar to those noted following acute exposure to benzenethiol, as described previously.

(2) Heptanethiol through Octadecanethiol; Cyclohexanethiol

Gage [65] reported in 1970 on the subchronic inhalation toxicity of many industrial chemicals, including dodecanethiol, in rats. Two male and two female rats, with an average weight of 200 g, were exposed to a

"nearly saturated atmosphere" of dodecanethiol for up to 6 hours/day, 5 days/week, for 4 weeks (a total of 20 exposures). During the 20 exposures, no signs of toxicity were noted. After the last exposure, urine was collected overnight. The following day, samples of lungs, liver, kidneys, spleen, and adrenals were collected for microscopic examination. The heart, jejunum, ileum, and thymus were also assayed for some of the chemicals studied; whether these organs were taken from the rats exposed to dodecanethiol was not stated. Microscopic examination of these tissues revealed all to be normal.

The subchronic inhalation toxicity of dodecanethiol and of a mixture of C_7 through C_{11} thiols to rats was reported by Gizhlaryan [61] in 1966. Thirty-two male and female rats inhaled air saturated with thiols from a 750-liter chamber, 4 times/week for 5.5 months (length of daily exposure unspecified). The saturation level of dodecanethiol was 3,400 mg/cu m (411 ppm) and that for the thiol mixture was 2,900 mg/cu m. No changes in body weight, oxygen consumption, ability of the CNS to summate threshold pulses, blood catalase level, erythrocyte count, -SH group content in the hemolysate, liver function as indicated by hippuric acid excretion in urine after a test dose of benzoic acid, and the duration of hexobarbitol-induced sleep were found after 2 months of exposure to either dodecanethiol or the C_7 through C_{11} thiol mixture. After 5.5 months of exposure, a suppression of body weight gain was accompanied by slight changes in oxygen uptake by the red blood cells and in oxygen usage by the tissues, a slightly increased leukocytosis, a 50% decrease in the functioning of the adrenals as indicated by the number of eosinophils following sc administration of ACTH, reduced liver function as indicated by urinary hippuric acid output following a preliminary loading with sodium benzoate and the duration of hexobarbital-induced sleep. The liver, spleen, brain, and kidneys, but not the blood (hemolysate and serum), of rats exposed to either dodecanethiol or the C_7 through C_{11} thiol mixture had small increases in sulfhydryl content. The ratio of organ weight to body weight was unchanged.

Microscopic examination of the organs of exposed animals showed vascular congestion in all organs, with hemorrhages in the lungs and adrenal medulla, mild bronchitis, slight inflammation of the renal tubules, myocardial fibrosis, slight fatty infiltration of the liver, depletion of lipid in the adrenal cortex, and slight edema of the brain [61]. The author concluded that both dodecanethiol and the C_7 to C_{11} thiol mixture were of low toxicity and that they presented only a slight hazard to industrial workers but noted that the experimental information suggested the possibility of rare cases of low-level chronic intoxications in workers.

(c) Skin Effects

Cirstea [66], in 1972, reported on the contact-sensitizing capacities of butanethiol, octanethiol, and dodecanethiol in guinea pigs. The flanks

of five albino guinea pigs (300-500 g) of either sex were depilated manually 24 hours before commencement of the daily application of 0.2 ml of a 20% solution of each thiol in acetone for 10 days, or until signs of contact dermatitis were evident. Erythema, induration, and eczematous crusts were considered positive indications of contact dermatitis. One month after the final application of the thiol solution, the opposite flank of the animal was shaved and painted with the solution if the animal had exhibited contact dermatitis during the 10-day period of application.

None of the thiols tested had a primary irritating effect as judged by the absence of local changes within 48 hours after the first application [66]. If signs of dermatitis developed within 3 days or more after the first application, the author considered the thiol to have exhibited contact-sensitizing ability. Ordinarily, at least 5 days are considered necessary for development of a sensitization response. The severity of the response was graded according to the intensity of the reaction and the time that had elapsed between the first application and appearance of dermatologic signs. With respect to contact sensitization, dodecanethiol was rated "intense," octanethiol as "moderate," and butanethiol as "absent or negative." When animals were painted with octanethiol and dodecanethiol only once, about half the animals developed dermatitis; when animals were painted again on the opposite flank 1 month later, signs of sensitivity appeared within 24 hours, as compared with control animals (3-5 days). The duration of the dermatitis was not stated, however.

Brooks et al [67], in 1957, reported some effects of a number of compounds, including octanethiol, dodecanethiol, and octadecanethiol, on mouse skin. Male albino mice 7-10 weeks old were used in the tests. Either pure liquids or solutions in ether were applied in 0.2-ml quantities to a shaved area on the backs of mice either on the 1st, 3rd, and 5th days of the experiment or on 6 days over a 2-week period. The skin was removed on the 6th or the 14th day and cut into 1.5- x 2-cm sample patches. The epidermis was isolated from the dermis, the epidermal patches were dried and weighed, and cholesterol and delta-7-cholestenol (D7-cholestenol) in the epidermal patches were determined. The results are shown in Table III-9.

The application of undiluted octadecanethiol to mouse skin produced within 6 days degeneration of the sebaceous glands, hyperplasia of the follicles, hyperkeratinization of the epidermal surface and the follicles, and, as shown in Table III-9, a decrease in the delta-7-cholestenol concentration in the skin and an increase in that of cholesterol [67]. These changes are similar to those found after dermal application of methylcholanthrene that eventually induced dermal carcinomas. The authors suggested, therefore, that prolonged contact of undiluted octadecanethiol with human skin may result in cancer.

Although no evidence was found in the available literature suggesting that thiols affect human skin, the animal data presented, although not

TABLE III-9

EFFECTS OF HIGHER MOLECULAR WEIGHT ALKANE THIOLS ON MOUSE SKIN

Thiol	Total Dose (mg)	Epidermal Weight (mg/sq cm)	Cholesterol Level (µg/sq cm)	D7-Cholestenol Level (µg/sq cm)	Epidermal Thickness (cells)	Sebaceous Glands	Hair Follicles
Control	-	2.4	51	50	2-3	-	-
Octane-thiol	3	2.2	46	48	2-3	-	-
	500	-	-	Normal	-	Normal	-
Dodecane-thiol	3	6.1	101	46	4-5	"	Elongated and swollen
	500	-	-	Normal	-	"	
Octadecane-thiol	3	4.2	78	23	4-5	Atrophied slightly	Normal
	6	2.5	51	23	3-4	Normal	Elongated
	500	19.3	291	30	8-10	Absent	Hyperplasia and hyperkeratinization

Adapted from reference 67

conclusive, indicate that a delayed dermatitis is possible. The effects on animals of acute and subchronic exposures to thiols are summarized in Tables III-10 and III-11 [51,53-55,58-62,64-67].

Bagramian and associates [46] and Bagramian and Babaian [68] reported on the mutagenic potential of dodecanethiol, chloroprene, and ammonia in rats. Six to eight white rats, weighing 180-250 g, inhaled a combination of chloroprene, dodecanethiol, and ammonia for up to 4 months. Chromosomal aberrations in bone marrow cells in both the anaphase and telophase stages of cell division were determined using acetocarmine. A relative increase in the number of chromosomal aberrations in the exposed animals over that in the control animals was observed in both experiments. In one experiment [68], after approximately 24 hours of inhalation of chloroprene (1.96 ±1.04 mg/cu m), dodecanethiol (5.02 ±1.96 mg/cu m), and ammonia (19.8 mg/cu m), the production of abnormal chromosomal aberrations increased from 5.5% in the controls to 8.8% in the test animals. After weekly exposure to chloroprene, dodecanethiol, and ammonia at the above concentrations for 4 months, the number of chromosomal aberrations was 11.1% above that of the control animals ($P < 0.05$).

In another experiment [46], at the end of 120 days of exposure (daily exposure period not specified) to chloroprene (0.89 ± 0.9 mg/cu m), dodecanethiol (0.12 ±0.03 mg/cu m), and ammonia (2.07 ±0.27 mg/cu m), the test group had 10.1% chromosomal aberrations, whereas the control group had 5.3% ($P = 0.01$). The increase in aberrations consisted mainly of an increase in the number of chromosomal fragments.

The mutagenic studies on Drosophila by Garrett and Fuerst [69] mention methanethiol as one of the six gases investigated. No experimental evidence was presented other than that treatment with this thiol resulted in an LD_{100} at a flowrate of 22 ml/minute.

Metabolic Studies

In their 1953 report, Canellakis and Tarver [70] showed that methanethiol was rapidly oxidized in the rat, yielding carbon dioxide and sulfate. A series of four experiments was carried out. In the first, one 180-g male rat was given 0.3 mg ^{14}C-labeled methanethiol ip, and the distributions of ^{14}C in tissues and excretory products were determined. The exhaled methanethiol and carbon dioxide were trapped and separated by reaction with isatin in concentrated sulfuric acid. During the 1st hour following injection, 29.2% of the dose was excreted as ^{14}C-labeled carbon dioxide, and 6.4% was exhaled as volatile sulfur-containing compounds. During the 2nd hour, no ^{14}C-methanethiol was detected in the exhaled air, and no further determinations were made of exhaled ^{14}C-labeled methanethiol. The exhalation of ^{14}C-labeled carbon dioxide was as follows:

TABLE III-10

SUMMARY OF EFFECTS ON ANIMALS OF SINGLE EXPOSURES TO THIOLS

Thiol	Species	No. of Animals	Duration and/or Route of Exposure	Concentration or Dose	Effects	Reference
Methanethiol	Rat	1	30-35 min, respiratory	500-10,000 ppm (985-19,700 mg/cu m)	At 10,000 ppm (19,700 mg/cu m), neurologic and respiratory changes, lung changes, death (14 min)	53
"	"	3-8	up to 15 min, respiratory	600-2,200 ppm (1,182-4,334 mg/cu m)	At 1,600 ppm (3,152 mg/cu m), 50% lost righting reflex	55
"	Mouse	10	up to 4 hr, respiratory	1,200-2,200 ppm (2,364-4,334 mg/cu m)	LC_{50} = 1,664 ppm (3,278 mg/cu m); neurologic and liver changes	54
Ethanethiol	Rat	5 or 6	4 hr, respiratory	2,600-5,125 ppm (6,606-13,021 mg/cu m)	LC_{50} = 4,870 ppm (12,370 mg/cu m) at 24 hr, 4,420 ppm (11,227 mg/cu m) at d 15; some latent toxicity	59
"	"	3-8	up to 15 min, respiratory	27,000-38,000 ppm (68,603-96,552 mg/cu m)	At 33,000 ppm (83,820 mg/cu m), 50% lost righting reflex	55
"	"	5	oral	210-3,360 mg/kg	LD_{50} = 1,034 mg/kg at d 1, and 682 mg/kg at d 15; some latent toxicity	59
"	"	5 or 10	ip	105-1,680 mg/kg	LD_{50} = 450 mg/kg at d 1, and 226 mg/kg at d 15; some latent toxicity	59
"	Mouse	10	4 hr, respiratory	2,600-4,832 ppm (6,606-12,277 mg/cu m)	LC_{50} = 2,770 ppm (7,038 mg/cu m) at 48 hr	59
"	Rabbit	2	20 min, respiratory	1,000 ppm (2,540 mg/cu m) 100 ppm (254 mg/cu m) 10 ppm (25 mg/cu m)	Respiratory change Temporary respiratory changes Brief respiratory fluctuations	58
Propanethiol	Rat	6	4 hr, respiratory	3,050-11,260 ppm (9,499-35,066 mg/cu m)	Estimated 48-hr LC_{50} = 7,300 (22,734 mg/cu m)	59
"	"	5	oral	1,327-3,344 mg/kg	LD_{50} = 2,362 mg/kg at d 1, 1,790 mg/kg at d 15; some delayed toxicity	59
"	"	5	ip	209-1,672 mg/kg	LD_{50} = 1,028 mg/kg at d 1, 515 mg/kg at d 15; delayed toxicity	59
"	Mouse	20	4 hr, respiratory	3,050-11,260 ppm (9,499-35,066 mg/cu m)	Estimated LC_{50} = 4,950 ppm (15,415 mg/cu m) at 48 hr, 4,010 ppm (12,488 mg/cu m) at d 15	59
Butanethiol	Rat	5	"	2,150-6,000 ppm (7,929-22,126 mg/cu m)	LC_{50} = 4,460 ppm (16,447 mg/cu m) at 24 hr, and 4,020 ppm (14,824 mg/cu m) at d 15	59

TABLE III-10 (CONTINUED)

SUMMARY OF EFFECTS ON ANIMALS OF SINGLE EXPOSURES TO THIOLS

Thiol	Species	No. of Animals	Duration and/or Route of Exposure	Concentration or Dose	Effects	Reference
Butanethiol	Rat	5	oral	1,093-3,344 mg/kg	LD_{50} = 2,575 mg/kg at d 1, 1,500 mg/kg at d 15; some delayed toxicity	59
"	"	5	ip	209-1,672 mg/kg	LD_{50} = 679 mg/kg at d 1, 399 mg/kg at d 15; delayed toxicity	59
"	Mouse	10 or 12	"	2,150-6,000 ppm (7,929-22,126 mg/cu m)	LC_{50} = 2,950 ppm, (10,879 mg/cu m) at 24 hr, 2,500 ppm (9,219 mg/cu m) at d 15	59
Pentanethiol	Rat	6	4 hr, respiratory	2,000 ppm (8,520 mg/cu m)	Mortality in 2-4 of 6 by d 14	60
Hexanethiol	"	5 or 6	"	456-1,475 ppm (2,205-7,131 mg/cu m)	LC_{50} = 1,200 ppm (5,808 mg/cu m) at 24 hr, 1,080 ppm (5,227 mg/cu m) at d 15	59
"	"	5	oral	848-2,137 mg/kg	LD_{50} = 1,580 mg/kg at d 2, 1,254 mg/kg at d 15; some delayed toxicity	59
"	"	5	ip	212-1,696 mg/kg	LD_{50} = 600 mg/kg at d 1, and 396 mg/kg at d 15; delayed toxicity	59
"	Mouse	10 or 12	4 hr, respiratory	220-1,475 ppm (1,064-7,131 mg/cu m)	LC_{50} = 610 ppm (2,950 mg/cu m) at 24 hr, 528 ppm (2,553 mg/cu m) at d 15	59
Thiols (C_3, C_4, C_{6-12}, C_{16}, C_{18})	"	2	iv	31.6-316 mg/kg	All LD_{50}'s > 316 mg/kg	*
Thiols (mixture C_7-C_{11})	Rat	Unspecified	intragastric	Unspecified	LD_{50} = 3,300 mg/kg	61
"	Mouse	"	"	"	LD_{50} = 2,025 mg/kg	61
Dodecanethiol	Rat	"	"	"	7,000 mg/kg caused no deaths	61
"	Mouse	"	"	"	LD_{50} = 4,225 mg/kg	61
Cyclohexanethiol	"	2	iv	31.6-316 mg/kg	LD_{50} approximately 316 mg/kg	*
"	"	10	cutaneous	100 mg/kg	Median lethal time 50 min	*
Benzenethiol	Rat	5, 6, or 10	4 hr, respiratory	20-132 ppm (90-595 mg/cu m)	LC_{50} = 59 ppm (266 mg/cu m) at 48 hr, 33 ppm (149 mg/cu m) at d 15; latent toxicity	59
"	"	5	oral	21.6-172.5 mg/kg	LD_{50} = 46.2 mg/kg at d 1	59

TABLE III-10 (CONTINUED)

SUMMARY OF EFFECTS ON ANIMALS OF SINGLE EXPOSURES TO THIOLS

Thiol	Species	No. of Animals	Duration and/or Route of Exposure	Concentration or Dose	Effects	Reference
Benzenethiol	Rat	5	ip	6.7-108.0 mg/kg	LD_{50} = 25.2 mg/kg at d 1, 9.8 mg/kg at d 15; latent toxicity	59
"	"	5 or 10	cutaneous	134-538 mg/kg	LD_{50} = 300 mg/kg at 4-8 hr	59
"	Mouse	10	4 hr, respiratory	20-79 ppm (90-356 mg/cu m)	LC_{50} = 47 ppm (212 mg/cu m) at 24 hr, 28 ppm (126 mg/cu m) at d 15; latent toxicity	59
"	Rabbit	3	cutaneous	67-269 mg/kg	Estimated LD_{50} = 134 mg/kg	59

* WW Wannamaker III, written communication, December 1977

TABLE III-11

SUMMARY OF EFFECTS ON ANIMALS OF SUBCHRONIC EXPOSURES TO THIOLS

Thiol	Species	No. of Animals	Duration and/or Route of Exposure	Concentration or Dose	Effects	Reference
Methanethiol	Rat	15	2 hr/d x 6 mo, respiratory	0.003-0.5 ppm (0.006-0.985 mg/cu m)	At 0.5 ppm (1 mg/cu m) reduced growth rate; heart and adrenal changes	51
"	"	50	90 d, respiratory	50 ppm (.45 mg/cu m)	Mortality 10%; blood changes; lung changes	62
"	Mouse	11	2 hr/d, 3 d/wk for 2 mo, respiratory	300 ppm (590.72 mg/cu m)	Mortality 100% after 25 exposures	54
"	"	100	90 d, respiratory	50 ppm (.45 mg/cu m)	Mortality 43%; blood changes; lung, liver, and kidney changes	62
"	Rhesus monkey	10	"	"	Mortality 40%; significant body weight loss; lung and brain changes	62
Ethanethiol	Rabbit	4	10 d* respiratory	1,000 ppm (2,543.31 mg/cu m)	No urine or blood changes	58
Propanethiol	"	1	ocular	0.1 ml	Severe eye irritation	59
1-Butanethiol	Rat	5	oral	20 mg/100 g, 3/d, d 1-2 40 mg/100 g, 3/d, d 3-4	Cortical or medullary changes	64
"	Guinea pig	5	10 d, cutaneous	0.04 ml/d	No dermal changes	66
1-Octanethiol	"	5	"	"	Moderated contact sensitization	66
"	Mouse	**	5 d, cutaneous	500 or 3 mg	No dermal changes	67
Thiols (mixture C_7-C_{11})	Rat	32	4 d/wk x 5.5 mo, respiratory	2,900 mg/cu m	Slightly reduced growth rate, slight decrease in liver and adrenal function	61
1-Dodecanethiol	"	32	"	411 ppm (3,400 mg/cu m) saturated atm	"	61
"	"	4	6 hr/d, 5 d/wk x 4 wk, respiratory		No changes	65
"	Mouse	**	5 d, cutaneous	500 mg	Changes in epidermis and hair follicles	67
"	"	"	"	3 mg	Changes in epidermis	67
1-Dodecanethiol	Guinea pig	5	10 d, cutaneous	0.04 ml/d	Intense contact sensitization	66
1-Octadecanethiol	Mouse	**	5 d, cutaneous	500 mg	Moderate to severe changes in epidermis, hair follicles, sebaceous glands	67
"	"	"	"	3 mg	Slight changes in epidermis and sebaceous glands	67
"	"	"	13 d, cutaneous	6 mg	Slight to moderate changes in epidermis and hair follicles	67
Benzenethiol	Rat	6	3 wk, ip	3.5 mg/kg, 3 x/wk	Mortality of 1 at d 7; no other changes	59
"	Rabbit	3	ocular	0.1 ml	Severe eye changes	59

*Animals not exposed on d 4 or 5
**Unspecified

Fraction of Dose Excreted (%)	Period (hr)
29.2	0-1
6.2	1-2
3.8	2-4
1.6	4-6

Extrapolation of the curve constructed with these values indicates that the excretion of isotopic carbon through the lungs as carbon dioxide would become essentially zero between 6 and 7 hours after the injection [70]. This means that about 60% of the methyl moiety of methanethiol must be used in some other way within the body than by oxidation to carbon dioxide. When analyzed, the tissues contained considerable amounts of radioactive label, in counts/minute/mg tissue as follows: liver, 17.8; kidneys, 11.4; spleen, 9.8; lungs, 11.5; testes, 8.5; plasma protein, 22.7; erythrocytes, 0; muscle, 2.2; and intestinal mucosa, 16.7. Canellakis and Tarver concluded that all the ^{14}C-methanethiol may have been oxidized to a one-carbon fragment and sulfate by the 6th hour after its administration.

In the second experiment, one 200-g male rat was given 1.4 mg ^{14}C-methanethiol ip in nine fractions at 1-hour intervals [70]. The animal was killed 2 hours after the last dose; the liver proteins were isolated, and the amino acid contents were identified and determined by column radiography. Most of the activity was present in the serine peak. A small fraction of the methanethiol appeared as the sulfoxide, approximately in the same location as alanine. Two other small peaks, which could not be identified, also appeared. The authors thought that one of these peaks may have been due to aspartic acid. Very little radioactivity was found in the cystine peak; this may have been due to loss during isolation of the amino acids. Differing amounts of radioactivity were found in tissue choline, creatine, and serine.

For the third experiment, one 200-mg male rat was given a total of 2 mg of ^{35}S-labeled methanethiol in four ip doses, given at 2-hour intervals; 92% of the administered dose was excreted in the urine within 8 hours [70]. Of the total 1.33-mg dose of sulfur, 33.7% was excreted as total sulfur; 96.7% of this sulfur was in the form of sulfates, of which 90.5% was inorganic sulfate. There appeared to be no relationship between the methanethiol oxidized to carbon dioxide (experiment 1) and the sulfate appearing in the urine (experiment 3). The authors attributed this to the fact that in one case methanethiol was given as a single dose, and in the other the dose was given during an 8-hour period.

The authors [70] concluded that both the carbon and the sulfur of methanethiol were rapidly oxidized to carbon dioxide and sulfate, respectively. The methyl group of methanethiol was also incorporated into serine at the beta-carbon and into the methyl groups of methionine, choline, and creatine. It is presumably these labeled methyl groups that

account for the radioactivity found in the tissues that were sampled for activity.

McBain and Menn [71] studied the metabolic products excreted in the urine of adult rats fed ^{35}S-labeled benzenethiol, methylphenyl sulfide (MPS), and methylphenyl sulfone ($MPSO_2$) at doses of 6 mg/kg, 2.5 mg/kg, and 2.5 mg/kg, respectively. One hour after administration, excreted urine was extracted with benzene and the aqueous layer was acidified with sulfuric acid and extracted with ether. The benzene- and water-soluble products were analyzed by thin-layer chromatography and gas-liquid chromatography. Urine from rats not fed the above compounds was treated with labeled thiols to detect any in vitro decomposition products. The only benzene-soluble product isolated from the urine was $MPSO_2$. The water-soluble products appeared to consist of p- and o-hydroxy $MPSO_2$ in all cases. In the control urine, 30% of the added benzenethiol was converted to diphenyl disulfide (DPDS), and no $MPSO_2$ was recovered. Based on these findings, it appeared that ^{35}S-labeled benzenethiol readily underwent S-methylation in vivo followed by oxidation to MPS and then to $MPSO_2$. Similarly, guinea pigs and mice dosed with ethanethiol excreted ethylmethyl sulfone [72].

Snow [72] studied the kinetics of distribution of radioactivity in the tissues and excreta of mice and guinea pigs injected sc with ^{35}S-labeled diethyl disulfide and ethyl ^{35}S-thiobenzoate, two derivatives of ethanethiol with antitubercular activity. About 50% of the radioactivity was excreted in the urine of the guinea pig in 10 hours. Considerable radioactivity was present in the breath and very little in the feces. Inorganic sulfate contained about 80-90% of the radioactivity in urine, and organic sulfur metabolites accounted for about 10-20% of the radioactivity. Two organic sulfur metabolites were detected; only one of them, ethyl methyl sulfone, was identified.

Bremer and Greenberg [73] identified an enzyme in the microsomal fraction of several tissues of the rat and in the liver of mammals (mice, rats, rabbits, guinea pigs, cattle, and sheep) capable of catalyzing the transfer of a methyl group from S-adenosylmethionine to produce nonphysiologic sulfhydryl compounds that included methanethiol. With S-adenosylethionine, a transethylation was found to take place.

These investigators [73] showed that the incubation of sodium sulfide, S-adenosylmethionine, and rat liver microsomal protein in tris-HCl buffer, pH 8.0, for 2 hours, led to the formation of methanethiol and dimethyl sulfide. This was demonstrated by acidifying the reaction mixture after incubation and capturing the liberated gas in mercuric chloride solution. The authors surmised that methanethiol and dimethyl sulfide could well be metabolic products of hydrogen sulfide in vivo. They further argued that the increased formation of methanethiol in liver disease could be due to blockage of the oxidation of cysteine to sulfate and taurine, leading to an increase in hydrogen sulfide formation. This investigation suggested a

mechanism for the in vivo formation of methanethiol under normal metabolic conditions.

The limited information found on the metabolism of alkane thiols and benzenethiol, as shown in Figure III-1, has led NIOSH to construct the following scheme:

(a) The sulfur atom of thiols is metabolized by oxidation and is excreted almost entirely as urinary inorganic sulfate, as in the case of methanethiol [70], or for the major part, as in the cases of ethanethiol [72] and benzenethiol [71]. In addition, methylation of the thiol followed by oxidation leads to excretion of some of the sulfur as the sulfone of the methylated thiol. This metabolic pathway seems to become more important in the thiols of larger molecular weight.

(b) The sulfur atom of the thiol group is not incorporated into the cysteine or methionine sulfur in mammals [70].

(c) The carbon atom of methanethiol is eliminated to a significant extent as respiratory CO_2 and is found in the beta-carbon of serine and in a methyl group of methionine, choline, and creatine, as indicated by the study by Canellakis and Tarver [70].

(d) Although thiols are easily oxidized in vitro to disulfides, no evidence was found demonstrating the conversion of thiols to their corresponding disulfides in vivo. It is possible that the thiols, as well as disulfides, are maintained in vivo in a reduced state. Such a reaction may be brought about by transhydrogenation [6,7] via a thiol-disulfide system such as the one mediated by glutathione reductase. Thiols in a reduced state could then be either oxidized directly or methylated first by an enzyme, such as the one catalyzing the transfer of the methyl group of S-adenosyl methionine [73], and subsequently oxidized. Snow's study [72] of the metabolism of diethyl disulfide serves as an example.

(e) Oxidation of dimethyl sulfide to dimethyl sulfone was suggested by a study [76] in which dimethyl sulfide administered orally or injected into rats did not lead to the urinary excretion of excess inorganic sulfate.

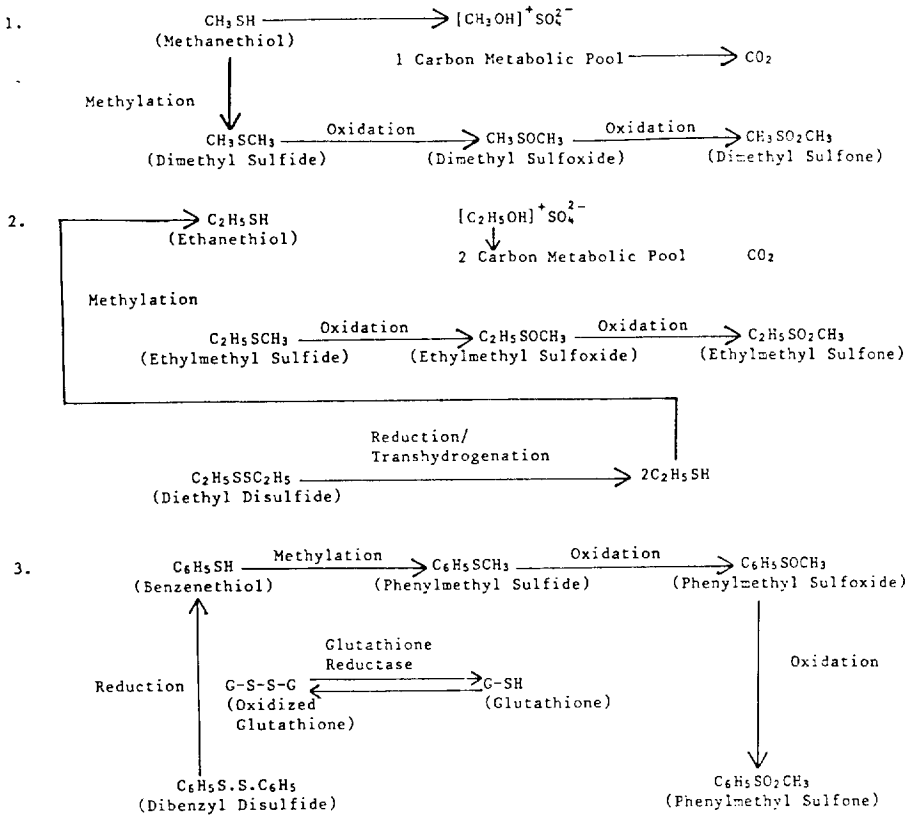
(f) Although enzymatic sulfhydryl-disulfide interchange leading to the formation of mixed sulfides may occur between substances containing -SH groups in the cell [74,77], no studies designed to evaluate the importance of these reactions and their relationship to the toxicities of the alkane thiols, cyclohexanethiol, or benzenethiol were located.

Correlation of Exposure and Effect

The data on the effects of exposure of humans and experimental animals to thiols demonstrate the following dose-effect relationship: Acute

FIGURE III-1

METABOLISM OF THIOLS AND SULFIDES



Adapted from references 5,6,8,70-72,74-76

exposure at high concentrations of thiols in humans [12-15] as well as in mice, rats, and rabbits [51,53,54,58,59] caused narcosis, apparent decerebrate rigidity, bronchospasm, uremia, hematuria, and proteinuria. The available human studies on exposure to methane-, ethane-, and butanethiols are on essentially short-term exposures designed to measure odor thresholds. Data indicate that human exposure to the lower molecular weight thiols (C_1-C_6) can produce severe CNS depression [12-14]. A worker exposed to methanethiol at extremely high concentrations died, without recovering from coma, approximately 1 month following exposure [12]. Another worker exposed to both methanethiol and ethanethiol at high concentrations for 2 hours was also found in a comatose condition [13].

In an experimental odor threshold study [48], inhalation of ethanethiol at 0.01 mg/liter (4 ppm) for 3 hours/day for 5-10 days, caused some rise in olfactory threshold and fatigue, periodic nausea, irritation of mucous membranes, and head heaviness. Inhalation of ethanethiol at 0.001 mg/liter (0.4 ppm) caused relatively less increase in the olfactory threshold and no other of the signs mentioned above.

In another controlled experiment to determine the effects of exposure to ethanethiol at 50 ppm (120 mg/cu m) and 112 ppm (270 mg/cu m) for 20 minutes, the investigator [45] observed no significant adverse effects in three volunteers. There were changes in respiratory frequency, which returned to normal after exposure ended. In contrast, an accidental exposure of high school students to ethanethiol at an estimated concentration of 3.6 ppm (9 mg/cu m) for 1 hour produced general symptoms of headache, discomfort, and abdominal pain and vomiting and diarrhea [14]. Recovery was apparently complete in a few hours. The above complaints could have resulted from mass hysteria and anxiety rather than from the specific effects of the thiol. Ten persons complained only of headache. One of the students studied in detail had unspecified changes around the eyes, a palpable liver, a spleen described as nonpalpable, and protein and red and white blood cells in the urine.

Signs and symptoms of CNS toxicity occurred in seven workers exposed to butanethiol for 1 hour at an estimated concentration of 50-500 ppm (180-1,800 mg/cu m) [15]. The workers exhibited asthenia, muscular weakness, and malaise. When humans were exposed to methane-, ethane-, and butanethiols to establish odor thresholds, odors in the concentration range of 0.5-7.6 ppm were noticed quite readily.

The LC_{50} and LD_{50} data on mice and rats for ethanethiol, propanethiol, butanethiol, hexanethiol, and benzenethiol as reported by Fairchild and Stokinger [59] suggest that there is similarity in toxicity among the n-alkane thiols via the ip and oral routes of administration. However, via the inhalation route hexanethiol was four and five times as toxic as ethanethiol to rats and mice, respectively. The 48-hour LC_{50} of ethanethiol after a 4-hour inhalation period was 2,770 ppm. Horiguchi [54]

reported a LC_{50} of 1,664 ppm (3,261 mg/cu m) for methanethiol in mice exposed for up to 24 hours. Carpenter et al [60] reported that 33-67% of the rats exposed to pentanethiol at 2,000 ppm for 4 hours died within 15 days. Taken together, these results indicate that methane-, ethane-, propane-, butane-, pentane-, and hexanethiols may be grouped together on the basis of acute toxicities. Furthermore, the intragastric LD_{50} values for higher molecular weight n-alkane thiols (C_7-C_{12}) are in the same range [61] as the LD_{50} values found for the lower molecular weight n-alkane thiols in rats [59].

The toxicity of inhaled benzenethiol was 77- and 78-fold greater than that of ethanethiol in mice and rats, respectively [59]. By oral and ip administrations to rats, the toxicity of benzenethiol was 22- and 23-fold greater, respectively, than that for ethanethiol. The ratios of LC_{50} and LD_{50} values for these species suggest that benzenethiol is from 22- to 78-fold as toxic as ethanethiol, depending upon the route of exposure. Instillation of benzenethiol (0.1 ml in the conjunctival sac) into the conjunctival sacs of rabbits caused severe irritation, corneal injury lasting 3 weeks to 2 months, and depilation of skin around the orbit when the exposed eye was washed with water.

Several subchronic toxicity studies have been conducted on the C_1-C_6 alkane thiols [54,58,62]. Exposures to methanethiol at 300 ppm for 2 hours/day, 3 days/week for 2 months killed 50% of mice after 15 exposures, and 100% after 25 exposures [54]. Inhalation exposures of monkeys, rats, and mice to methanethiol at 50 ppm continuously for 90 days caused 40% mortality in monkeys, 10% mortality in rats, and 43% mortality in mice [62]. Subjecting rabbits to inhalation of ethanethiol at 1,000 ppm (2,541 mg/cu m) for 9 days produced no notable effects [58].

The intragastric LD_{50} values for mice and rats administered a mixture of C_7 through C_{11} thiols were 2,025 mg/kg and 3,300 mg/kg, respectively [61]. The intragastric LD_{50} value for mice administered dodecanethiol was 4,225 mg/kg, and an intragastric dose of 7,000 mg/kg caused no deaths in rats. Gage [65] exposed rats to air saturated with dodecanethiol for up to 6 hours/day, 5 days/week for 4 weeks and found no signs or microscopic evidence of toxicity. Rats exposed to dodecanethiol at the saturation level of 3,400 mg/cu m or to a mixture of C_7 through C_{11} thiols four times weekly for 2 months showed no signs of toxicity [61]. After 5.5 months of exposure, there was a small decrement of weight increase, a slight degree of leukocytosis, a 50% decrease in the functioning of the adrenals as measured by the number of eosinophils released following the sc administration of adrenocorticotropic hormone, and reduced liver function as demonstrated by both the amount of hippuric acid produced following a preliminary loading with sodium benzoate and the duration of hexobarbital-induced sleep.

The only available toxicity data on cyclohexanethiol suggested that the iv LD_{50} for mice is approximately 316 mg/kg (WW Wannamaker III, written

communication, December 1977). The application of 100 mg/kg cyclohexanethiol to the skin of mice resulted in the death of two out of two animals in 24 hours. Higher molecular weight alkane thiols (C_7-C_{12}, C_{16}, C_{18}) and cyclohexanethiol do not appear to represent a substantial inhalation hazard; the main effect is on the skin [61,66,67].

No information on the acute dermal or subchronic effects of thiols in humans was found. However, when 0.04 ml of octanethiol was applied daily for 10 days to the depilated flanks of guinea pigs, there was no irritation of the skin, but contact sensitization resulted [66]. A total dose of 3 mg of dodecanethiol applied to the clipped backs of mice for 1 week caused an increase in the dry weight of the epidermis, an increase in the concentration of epidermal cholesterol, and a possible decrease in the amount of epidermal delta-7-cholestenol [67]. Microscopic examination of the skin revealed slight thickening of the epidermis, and the hair follicles were elongated and swollen. The sebaceous glands were described as normal. When a total dose of 500 mg was applied to the skin of mice, the epidermal delta-7-cholestenol concentration was not affected, and the sebaceous glands were not damaged. Cyclohexanethiol (WW Wannamaker III, written communication, December 1977) when applied to the skin of mice at a dose of 100 mg/kg killed all the animals in 24 hours. No other data on skin sensitization in animals were found.

In summary, all thiols behave as weak acids, their chemical reactivity being due essentially to the -SH group. The predominant biologic effect of exposure to thiol vapors is on the CNS. Toxicity via the inhalation route of administration is of importance in the case of the C_1-C_6 group of alkane thiols and the dermal route in the case of C_7-C_{12}, C_{16}, and C_{18} alkane thiols and cyclohexanethiol, the former group being more volatile than the latter. Such a distinction, however, does not apply when ocular exposure is considered. Benzenethiol is the most toxic of all the thiols included in this document. It is pertinent to recognize that all the thiols have strong odor that constitute a nuisance at concentrations far lower than those at which they cause signs and symptoms of toxicity. In general, the low molecular weight thiols have a more obnoxious odor than the high molecular weight thiols at comparable concentrations. On the basis of similarity in the toxicity, the n-alkane thiols C_1-C_{12}, C_{16}, and C_{18} and cyclohexanethiol can be considered together as a group whereas benzenethiol needs to be considered separately because of its relatively higher toxicity.

Carcinogenicity, Mutagenicity, Teratogenicity and Effects on Reproduction

No data on teratogenicity or effects on reproduction have been found. There are, however, a few reports dealing with the mutagenic and carcinogenic potential of thiols.

Bagramian and associates [46] and Bagramian and Babaian [68] reported on the mutagenic potential of dodecanethiol admixed with chloroprene and

ammonia. Rats were exposed to the combined vapors of the three substances. These three substances are components of "LNT-1 Latex." The incidence of aberrations was found to be increased in experimental rats compared with that in the controls. Eleven workers employed in a "LNT-1 Latex" factory were also studied [46]. The peripheral blood lymphocytes obtained from each individual were cultured and examined. More chromatid breaks were found in the lymphocytes of these individuals than in those of a group of five employees from a shoe factory who served as controls.

Based on the information presented in these studies it is difficult to assess the mutagenic potential of dodecanethiol, since the experimental animals as well as workers were exposed simultaneously to chloroprene, a compound that reportedly causes chromosomal aberrations [78]. Furthermore, in the human study [46], insufficient numbers of cells were examined and the control group used in the study was not clearly defined.

The application of 500 mg of undiluted 1-octadecanethiol to the shaved backs of mice resulted in epidermal changes similar to those found after applications of doses of methylcholanthrene known to produce tumors [67]. This response is not considered to be necessarily indicative of carcinogenesis. Such observations leave unsettled the carcinogenicity of octadecanethiol. No other indication that thiols may be carcinogenic has been found in the literature.

Garrett and Fuerst [69] reported that all six gases, including methanethiol, in their study were significantly mutagenic in Drosophila. However, no data on mutagenicity by methanethiol per se were presented in the paper.