III. BIOLOGIC EFFECTS OF EXPOSURE

Extent of Exposure

Benzyl chloride ($C_6H_5CH_2Cl$), molecular weight 126.58, is a colorless to light-yellow liquid with a pungent odor [1,2]. It is a strong lacrimator and has an irritating effect on mucous membranes. Benzyl chloride is lipid soluble [3] and is miscible in all proportions with alcohol, chloroform, and ether [4]. Benzyl chloride is considered a moderately volatile liquid, with vapor pressures of 1 mmHg at 22 C, 10 mmHg at 60.8 C, and 100 mmHg at 114.2 C [5]. Some of the important physical and chemical properties of benzyl chloride are listed in Table XI-1.

Benzyl chloride is a commercially important alkylating agent that reacts readily with both organic and inorganic nucleophilic agents and so may also be expected to react with such nucleophiles as amines, carbohydrates, lipids, proteins, ribonucleic acids, and deoxyribonucleic acids in biologic systems. Benzyl chloride reacts rapidly with sulfhydryl groups [6] and binds to the epsilon-amino group of lysine in collagen [7]. Benzyl chloride decomposes relatively slowly in the presence of water. The half-life for the hydrolysis of benzyl chloride, at pH 7 and 25 C, is 15 hours [8]. At 60 C, the rate of hydrolysis is 45 times as fast as at 25 C [9].

In the United States, benzyl chloride is commercially prepared primarily by the continuous photochlorination of toluene [10]. Excess toluene is distilled off and returned to the reactor. Hydrogen chloride gas formed in the reaction is vented to another location where it is absorbed by water to produce a 30% solution that can be sold as muriatic acid. The composition of the crude product is 90-95% benzyl chloride and 5-10% benzal chloride (benzylidene chloride, $C_6H_5CHCl_2$) or benzotrichloride ($C_6H_5CCl_3$) [11]. Further distillation results in a product of greater than 99% purity [12].

Anhydrous benzyl chloride is available in both technical and refined grades [13]. Benzyl chloride undergoes Friedel-Crafts condensation reactions in the presence of such metals as iron, copper, zinc, aluminum, magnesium, and tin. The presence of moisture and/or heat favors such reactions in which the hydrogen chloride released may result in the rapid buildup of pressure in closed containers [1,5,14,15]. A stabilizer such as propylene oxide, triethylamine, or sodium carbonate often is added to inhibit these condensation and decomposition reactions by neutralizing acidity and scavenging free radicals [1,14]. Benzyl chloride is shipped in glass carboys, phenolic-lined steel or returnable nickel drums, tank cars, and tank trucks. It has an effective storage life of 2-3 months at normal temperatures.

Currently, there are four major manufacturers of benzyl chloride in the United States [16]. Domestic production increased at the relatively steady rate of 5.6% a year from 1964 to 1974 [17]. Some of the demand, especially in the textile industry, appears to be leveling so that a growth rate of 4% a year has been predicted through 1979 [17]. Total US production increased from 10.4 million pounds in 1950 to an estimated 90 million pounds in 1976 [16].

In 1972, the last year for which such figures are available from the US Tariff Commission [16], approximately 77% (as calculated by the sales figures given) of the total benzyl chloride produced was used captively by its manufacturers mainly for the preparation of butyl benzyl phthalate and benzyl alcohol [16].

At present, an estimated 60-70% of the benzyl chloride manufactured in the United States is devoted to the production of n-butyl benzyl phthalate, a plasticizer for the flexible vinyl polymers used in coatings and floor coverings [16,17]. Benzyl alcohol, the manufacture of which accounts for 10-15% of the benzyl chloride output, is used as an adjunct in the dispersion, uptake, and rate of fixation of dyes in the textile industry and to make specialized solvents. Another 10-12% of the benzyl chloride output is used to form a variety of quaternary ammonium compounds that are used as germicides, fungicides, and sanitizers and in products such as hair conditioners, wetting agents, dispersing agents, and emulsion paint preservatives. The remaining 5-10% of benzyl chloride production is used in the synthesis of such materials as benzyl acetate, benzyl cyanide, benzyl salicylate, benzyl butyrate, and benzylamines, many of which are prepared on batch or campaign bases [16]. These products are used in the pharmaceutical, perfume, flavors, and dye industries. Benzyl chloride is reported to be effective as an extreme-pressure lubricant [18,19], the lubricating property of which is attributed to a chloride film that forms on iron surfaces [18]. In other applications, benzyl chloride can undergo condensation reactions forming low molecular weight polybenzyl resins that are temperature- and pressure-stable and noncorrosive [20] and have been proposed for use as hydraulic fluids, heat transfer fluids, and heat-stable fuel. Imports of benzyl chloride derivatives in 1974 were reported to have amounted to about 1.48 million pounds, approximately 1% of the domestic market [16].

NIOSH estimates that 3,000 workers in the United States are potentially exposed to benzyl chloride. Occupations involving potential exposure to benzyl chloride are listed in Table XII-2.

Historical Reports

Wolf [21], in 1912, described his personal experience when he was exposed to benzyl chloride vapor while conducting animal experiments. He

experienced a severe burning sensation in his eyes and copious tearing when he opened the exposure chamber at the beginning and end of the experiments. These effects, present even at the nominal concentration of 160 mg/cu m, were accompanied by conjunctivitis at higher concentrations. Wolf also noted some nasal irritation. Schutte [22], during similar experimental procedures, experienced a burning sensation in his eyes on the 1st day of exposure; an inflammation of the upper respiratory mucous membranes developed, accompanied by free discharge that persisted for the remaining several weeks of the tests.

In 1919, Hill [23] of Birmingham, England, patented the use of the noxious properties of benzyl chloride to disable enemy troops. He proposed mixing 35-60 grains (2.3-3.9 g) of benzyl chloride with 1 ounce (28 g) of light magnesium carbonate to aid in its dispersion and volatilization. When packed into shells, grenades, and bombs, to be shot among the enemy, the powder, with its coating of benzyl chloride, would allegedly remain suspended in the air and expose the troops to the noxious effects of benzyl chloride. There are no reports of the actual use of this system. According to Meyer's 1926 report [24], benzyl chloride and the other benzyl halides did not play significant roles as noxious agents during World War I. The starting material, toluene, had become expensive and had a higher priority in the manufacture of trinitrotoluene. Also, because atomized benzyl chloride lacked long-lasting effects, its use as a war gas was superseded by a more potent irritant, bromobenzyl cyanide [24]. Meyer stated that benzyl chloride vapor at 85 mg/cu m was intolerable to humans. No supporting information was given. Flury and Zernick [25], referring to Meyer's work, concluded that 16 ppm (85 mg/cu m) was intolerable to man in 1 minute, although Meyer had not specified a time.

In 1929, Lallemand [26] rated benzyl chloride as rapidly toxic to the developing chicken embryo. A toxic time was defined as the duration of exposure to a saturated atmosphere of a gaseous or volatile chemical at 18 C that would arrest the development of a chicken embryo, but not of the blastoderm, when it was returned to normal incubation conditions for 48 hours. Unexposed control embryos did not develop at 18 C, but both embryonic and blastodermal development resumed after a 48-hour normal incubation. The toxic time for benzyl chloride was 5 hours.

Effects on Humans

Watrous [27], in 1947, reported some of his observations of toxic effects related to the use of certain halides as intermediates in the pharmaceutical industry. He observed some instances of conjunctivitis and upper respiratory tract irritation in a small group of workers that he described as intermittently exposed to benzyl chloride vapor (up to 500 ppm, 2,590 mg/cu m), and he ascribed a vesicant action to liquid benzyl chloride without, however, citing any examples. The duration of exposure

was not indicated. Smyth [28] in 1956, commented that "This (benzyl chloride) is a potent lacrimator irritating to eye, nose, and throat and capable of causing lung edema.... It may be inferred that the liquid causes severe corneal injury."

In the 1971 edition of the Encyclopedia of Occupational Health and Safety published by the International Labour Office (ILO), Mikhailova [29] reported that production workers exposed to benzyl chloride at 10 mg/cu m and above complained of weakness, rapid fatigue, persistent headaches, increased irritability, a hot feeling, loss of sleep and appetite, and, in some, itching skin. Medical examination of workers revealed instances of weakness, sweating, localized tremors, poor proprioception, and dermatographism. Findings of abnormally high blood bilirubin, abnormally low leukocyte counts, and nonspecific abnormalities in the serum protein levels (positive Takata-Ara and Weltmann tests) suggested to the author a disturbance of liver function. Because the number of workers, working conditions, actual range of benzyl chloride exposures, and other exposure details are unknown, the reports of signs and symptoms attributed to exposures to benzyl chloride at concentrations greater than 10 mg/cu m are questionable.

In a 1930 technical report prepared for the US Bureau of Mines, Katz and Talbert [30] listed the odor and irritation thresholds of some volatile chemicals, including benzyl chloride. In each test series, five or six persons were exposed to the vapor of commercially prepared benzyl chloride, having a boiling point of 174-180 C at 745 mmHg. The vapor concentration in the primary airstream was calculated (considering the vapor as a perfect gas) as the weight loss of the vaporizer divided by the product of air flowrate and time.

Volunteers remained in an outer ventilated room except for the few seconds of testing [30]. For the odor and nasal irritation tests, each subject lifted the cover of a small, strongly ventilated hood, placed his nose well into the nosepiece, and took one breath. He then recorded the intensities of odor and pain perceived according to separate rating scales. To test for ocular irritability, each subject held one eye open to the airstream at the nosepiece for 10 seconds. Eye irritation was recorded as pain perceived, according to the same rating scale used for nasal irritation.

Participants described the odor of benzyl chloride as "aromatic, benzene-like" [30]. They rated benzyl chloride odor as "just perceptible" at 0.21 mg/cu m, "faint" at 1.7 mg/cu m, "easily noticeable" at 13 mg/cu m, and "strong" at 110 mg/cu m. Eye irritation was rated as "just perceptible" at 41 mg/cu m, "very unpleasant" at 88 mg/cu m, "painfully strong" at 190 mg/cu m, and "intolerable" at 410 mg/cu m. Nasal irritation was rated as "just perceptible" at 180 mg/cu m.

Mikhailova [29] further stated that benzyl chloride at 160 mg/cu m was unbearably irritating, that 50-100 mg/cu m caused immediate tearing and eyelid twitching, and that 5 minutes of exposure at 6-8 mg/cu m caused a slight conjunctivitis. She stated that benzyl chloride production workers showed an increased tendency toward respiratory illnesses (similar to colds and allergic rhinitis) and dermatitis. This report did not include important information such as sampling and analytical methods, number and percentage of the workers affected, possible exposures to other chemicals, or time before onset of effects. In spite of the absence of these details, which would allow validation of the information contained in the abstract, and considering the variability involved in the subjective evaluation of pain by individuals, it appears that the ocular effects described by Mikhailova at 6-160 mg/cu m substantially agree with those of the laboratory study by Katz and Talbert [30].

Leonardos et al [31], in 1969, reported the odor recognition thresholds of benzyl chloride and 52 other chemicals. The chemicals were presented to a panel of four analysts who had been trained to recognize odor quality and character. The odor threshold was defined as the lowest concentration at which all four panelists could positively identify an odor that, in turn, could be consistently recognized at higher concentrations.

The odor threshold for benzyl chloride was determined to be 0.047 ppm (0.24 mg/cu m), or about 1/100 that of benzene and 1/50 that of toluene [31]. The authors suggested that the experimentally determined odor recognition value for benzyl chloride was probably lower than it would have been under environmental background conditions.

Many investigations concerning the metabolic fate of benzyl chloride have been conducted in animals (see Animal Toxicity). Glutathione-S-transferases, which catalyze the conjugation of benzyl chloride with glutathione and which have been reported in several animal species, have also been isolated from human liver and show activity with benzyl chloride substrate [32] (See Table III-1).

Epidemiologic Studies

No epidemiologic reports on benzyl chloride were located in the literature.

Animal Toxicity

(a) General

Back et al [33], in 1972, reported the results of a study they performed for the US Department of Transportation. Benzyl chloride was classified as a toxic agent for transportation purposes based on rat and mouse oral and inhalation toxicity data. The classification was said to be

TABLE III-1

DISTRIBUTION OF GLUTATHIONE-S-TRANSFERASES IN DIALYZED LIVER SUPERNATANTS FROM DIFFERENT SPECIES

Species	No. and Sex	Aralkyltransferase Activity (Benzyl Chloride Substrate) Relative	Measured*
Rat**	30 M,F	1.00	2.3
Dog	1 F	0.91	2.1
	1 M	0.48	1.1
Guinea pig	3 M	3.26	7.2
Mouse**	20 M	0.83	1.9
Rabbit	1 M	0.48	1.1
Ferret	2 M	0.13	0.3
Pigeon**	6 M	1.43	3.3
Hamster**	5 M	0.83	1.9
Human (post mortem)	1 F	0.22	0.5
Human (fetus)	2 M	0.30	0.7

*Micromoles thiol lost/min/g tissue
**Tissues pooled

Adapted from reference 32

applicable to any substance that had, after a single exposure, a 1-hour LC_{50} of 200-20,000 ppm (1,036-103,600 mg/cu m for benzyl chloride) or a 14-day oral LD_{50} of 50-5,000 mg/kg. The authors cited the oral LD_{50} of benzyl chloride for rats as 1,231 (95% confidence limits 1,145-1,656) mg/kg and for mice, 1,624 (1,153-2,185) mg/kg. They also reported that rats and mice survived a 1-hour exposure to benzyl chloride at 2,000 mg/cu m. No information on experimental design was given.

Mikhailova [34], in 1964, reported the acute lethal effects of benzyl chloride and of benzal chloride and benzotrichloride, which are sometimes present as contaminants of benzyl chloride. The compounds were tested in a total of 140 adult male rats and 82 white mice of unspecified sex, age, weight, and strain. An unspecified number of rats and mice were exposed for 2 hours in a 100-liter static chamber to determine the LC_{50} of each of these compounds, the vapor concentrations of which were measured spectrophotometrically. The rats were observed for 1 month and the mice for 2 weeks after exposure.

In mice, the LC_{50} values were 390 mg/cu m for benzyl chloride, 210 mg/cu m for benzal chloride, and 60 mg/cu m for benzotrichloride [34]. In rats the LC_{50} values were 740 mg/cu m for benzyl chloride, 400 mg/cu m for benzal chloride, and 150 mg/cu m for benzotrichloride. The author observed that all three compounds when inhaled at concentrations greater than 100 mg/cu m produced signs of excitement, ocular and respiratory mucosal irritation, and slowed respiration in both species. She also noted a marked hyperemia of the ears, paws, and tails of animals throughout the experiment. Only at concentrations of about 1,000 mg/cu m were there signs of such nervous system effects as unresponsiveness and slowed respiration (in both species), motor automatism (in mice), and peripheral muscle twitching (in rats).

Examination of animals that died during the study revealed respiratory tract inflammation (fibrin films, desquamation of epithelium, edema, and submucosal hemorrhages) [34]. A superimposed secondary infection was generally noted if the animals died 12-14 hours after exposure. Evidence of plethora, stasis, and blood effusions was found in all organs examined. Changes, described as both albuminoid and fatty degeneration, were found in the hepatic cells. Albuminoid degeneration of the convoluted tubular epithelium, sometimes leading to necrosis, also was reported. Unspecified degenerative changes were noted in the myocardium and brain.

Another experiment was conducted to observe recovery from the effects of single 2-hour exposures to each compound at 100 mg/cu m [34]. Groups of 10 male white rats of approximately equal weight and a 10-rat control group, which was placed in a control chamber for 2 hours, were monitored for 1 month following exposure. Body weights, general condition, thresholds of neuromuscular excitability, renal function (18-hour water loading and 24-hour protein measurements), and blood counts were determined at regular, but unspecified, intervals.

Loss of body weight was commonly noted in rats that underwent single 2-hour exposures to any of the three compounds at 100 mg/cu m [34]. Animals exposed to benzyl chloride showed a mean loss of 15 ±3 g, whereas controls had gained 11 ±2 g by the 5th day after exposure. The weights of rats exposed to benzyl chloride had returned to their initial values at 14 days and had increased above this weight by 29 3.7 g at the end of the 30-day observation period. The mean total weight gain in control rats during this time was 41 ±3.3 g. Weight losses were greater following benzal chloride exposure (18 ±3 g) and most severe after benzotrichloride (36 ±4 g) at the 5th day after exposure. No remarkable changes in renal function were found following exposure to benzyl chloride or benzal chloride, but a decrease in urine produced after water-loading was found 3 weeks after exposure to benzotrichloride. Peripheral blood cell counts and hemoglobin levels in benzyl chloride- and benzal chloride-exposed animals were essentially the same as control values, but leukocyte and erythrocyte counts were slightly lower in rats 1 month after exposure to benzotrichloride.

Mikhailova [34] concluded that the toxicities of all the three compounds were similar qualitatively but quantitatively became more severe with increasing replacement of hydrogen by chlorine on the side chain. However, since benzotrichloride, the most toxic of these three substances, was the least volatile and since the volatility and toxicity of benzal chloride were intermediate between those of benzotrichloride and benzyl chloride, the author suggested that there was a differing probability of actual inhalation absorption for each of these substances and that their net effects singly might be similar under identical environmental conditions.

The LC_{50} data obtained by Mikhailova [34] do not agree with those of Back et al [33]. Mikhailova reported 50% lethality in rats after a 2-hour exposure to benzyl chloride at 740 mg/cu m, whereas Back et al stated that all rats survived a 1-hour exposure to benzyl chloride at 2,000 mg/cu m. Since important experimental details were not provided in either report, the differences in results cannot be explained.

In 1912, Wolf [21] reported the results of single and repeated exposures of nine cats and one rabbit to benzyl chloride vapor. The ages and sexes of the animals were not specified. A stream of air was forced through sulfuric acid to remove moisture and then directed through a bottle of benzyl chloride and into the glass chamber. Another stream of fresh air was used as a diluent, and its flowrate was measured with a water wheel with a gas gauge. The loss of weight of the benzyl chloride in the bottle divided by the ventilation rate of the chamber yielded the nominal concentration of benzyl chloride in mg/liter of air.

Seven cats were exposed continuously for 8 hours at 160-3,330 mg/ cu m [21]. One rabbit also was exposed at 480 mg/cu m for 8 hours. One cat

exposed for 8 hours at 160 mg/cu m was reexposed 6 days later for 3 hours to benzyl chloride at 2,480 mg/cu m. Two additional cats were exposed for 7.0 and 7.5 hours at respective concentrations of 7,000 and 17,700 mg/cu m. All animals were observed during and after exposure until either recovery or death occurred. All animals that died were examined grossly.

Of the seven cats and one rabbit exposed for 8 hours at 160-3,330 mg/cu m, the one cat exposed at 160 mg/cu m, two cats exposed at 480 mg/cu m, and the one cat exposed at 630 mg/cu m recovered within 12-15 hours after exposure [21] . They showed little residual effect except for moderate conjunctivitis. The rabbit exposed at 480 mg/cu m recovered immediately after exposure. The cat exposed to benzyl chloride at 890 mg/cu m died on the 9th day. The cat that was originally exposed at 160 mg/cu m for 8 hours and reexposed 6 days later at 2,480 mg/cu m for 3 hours recovered after 24 hours. Both cats exposed at 3,330 mg benzyl chloride/cu m of air for 8 hours died within 24 hours. The two cats exposed at the highest concentrations of benzyl chloride, 7,000 and 17,700 mg/cu m of air for 7.0 and 7.5 hours, respectively, died within 0.5 hour after cessation of exposure.

From Wolf's [21] observations, intense local inflammation (and sequelae) of the mucous membranes of the eyes, nose, mouth, and all the tubular air passages to the pulmonary alveoli were the main effects of exposure by inhalation to benzyl chloride vapor in cats and, to a lesser extent, in the rabbit. These effects were observed at all tested concentrations; however, the severity of these reactions varied directly with the concentration and duration of exposure. The immediate results of exposure were eyeblinking, eyelid closing, tearing, salivation, sneezing, and coughing. At 630 mg/cu m, a greatly increased respiratory rate, up to three times the normal rate, was followed by a marked decrease. The respiration then became irregular. At 2,480 mg/cu m and above, the animals progressed to inactivity and marked unresponsiveness, until either fresh air was supplied or death occurred. Autopsies on cats exposed to benzyl chloride at 890 mg/cu m and above revealed markedly increased mucoid, often hemorrhagic secretions throughout the respiratory organs, with patches of edematous or hemorrhagic consolidation of the lungs, and severe pneumonia in a cat that survived for a few days after exposure. One cat had severe conjunctivitis and clouded corneas.

The two cats and one rabbit that had been previously exposed at 480 mg/cu m for 8 hours were reexposed at the same concentration and for the same duration for 5 more consecutive days [21]. The cats showed a mild to moderate conjunctivitis that, after the 1st day, became more marked with subsequent exposures. Both cats gradually developed severe coughs, one by the 2nd and the other by the 4th day, which persisted for an unspecified time beyond the exposure period. Their appetites decreased each day. One of the cats did not recover; however, no autopsy was reported. The rabbit showed signs of mild eye irritation, but not of conjunctivitis, during the

exposure period, and a perceptible reddening of its oral and nasal membranes occurred by the 6th day. Salivation, coughing, sneezing, or tearing were absent, and the rabbit maintained its appetite. These results indicate that the effects of benzyl chloride may become more severe with repeated exposure.

Using the method described by Wolf [21], Schutte [22], in 1915, reported his observations on 12 cats, 5 rabbits, and 1 dog that had been exposed to benzyl chloride at 800-23,600 mg/cu m of air for 0.5-8 hours. Initial weight, but not age or sex of the test animals, was reported. As in the acute study by Wolf [21], all animals were observed until recovery or death occurred. Autopsies were performed on the animals that died.

Two cats and one rabbit exposed at 1,100 mg/cu m for 7.5 hours died within 2 days [22]. However, one cat and one rabbit exposed at 1,500 mg/cu m for 6 hours recovered; the cat that survived developed bronchitis. The rabbit was exposed 4 days later to 9,600 mg/cu m for 2.25 hours. Two cats exposed at 2,000 mg/cu m for 0.5 hour, and one cat and one rabbit exposed at 3,900 mg/cu m of air for 2 hours, recovered rapidly except for slight conjunctivitis in one cat. All cats exposed at 5,300-9,600 mg/cu m for 2-2.5 hours died within 2 days. Two cats exposed at 23,600 mg/cu m for 0.5 hour appeared to have recovered but died within the next 3 weeks. The three rabbits exposed at 6,600-9,600 mg/cu m for 2-2.25 hours died within 10 days. The dog died within 24 hours after an 8-hour exposure to benzyl chloride at 1,900 mg/cu m. The two cats initially exposed to benzyl chloride at 2,000 mg/cu m for 0.5 hour, which, as previously stated, appeared to recover rapidly, were reexposed 5 days later at 800 mg/cu m for 0.5 hour. By the next day, their breathing was audible and labored; both died of pneumonia within 3 weeks. It appears that after initial exposure the animals became less resistant to reexposure.

Schutte's [22] observations agreed with those reported by Wolf [21]. The immediate effects of benzyl chloride were severe irritation of the eyes and, to a lesser degree, the nasal mucosa. After a 0.5-hour exposure at the lower concentrations and sooner at the higher concentrations, benzyl chloride appeared to exert a narcotic effect as indicated by the animals' passive behavior. In one cat exposed at 1,500 mg/cu m and in others exposed at higher levels, the author noted disturbances in equilibrium and occasional tremors. Corneal turbidity was reported in seven animals after inhalation exposure at 1,100-11,500 mg/cu m, but it cleared within 3 days in the cat exposed at 1,500 mg/cu m. Serious but unspecified changes were seen in the respiratory organs of the animals that died.

In a 1936 paper, Landsteiner and Jacobs [35] discussed the induction of sensitization by benzyl chloride in guinea pigs. Benzyl chloride in saline solution at a dose of 0.01 mg/animal was injected intracutaneously twice weekly for 12 weeks into an unstated number of guinea pigs. Two weeks

later, sensitization tests were conducted on these animals. The details of this test were not reported, but the procedure may be inferred from other experiments reported in the same paper [35], ie, a drop of the test solution mixed with olive oil was spread on a shaved flank of each animal. The authors noted positive effects that were not specified; it may also be inferred from related experiments that these effects included erythema and some swelling of the treated site. The authors concluded that benzyl chloride had a sensitizing capacity.

Holmberg and Malmfors [36], using Ehrlich-Landschutz diploid (ELD) ascites tumor cells, examined the cytotoxic effects of benzyl chloride at volumetric concentrations of 50 and 100 ppm in cell suspensions (1 x 10^6 cells/ml) that were incubated for up to 5 hours at 37 $\pm$1 C under constant stirring in sealed 3-ml glass tubes. The increased permeability of ELD ascites tumor cells to Lissamine green induced by organic solvents was considered to be a stage of irreversible cellular damage preceding cell death. Even at 100 ppm benzyl chloride, the proportion of dead ELD cells increased from 5.0% at 0 time to 14.5% after 5 hours of incubation, which the authors concluded was a moderate cytotoxic reaction. However, because these experiments were carried out on ascites tumor cells in vitro, the results are only suggestive of a cytotoxic effect of benzyl chloride in vivo, but may explain some of the tissue changes found in the respiratory system following exposure at high concentrations [21,22,34].

Stekol [37], in 1947, studied the growth patterns in young rats fed a diet that included various amounts of sulfur-containing amino acids and benzyl chloride. For these experiments, a basal diet was formulated that the author considered to be nutritionally complete, but with a low sulfur content. Commercially pure benzyl chloride, 0.5% by weight, was added to the basal diet for 7-10 days. This was alternated with periods of 10-21 days during which the basal diet alone or with benzyl chloride with and without supplements of l-cystine, d-cystine, dl-methionine, dl-homocystine, or taurine was given.

All animals lost weight while on the benzyl chloride diet [37]. The weight loss was alleviated, and normal growth rates were resumed when the diet was supplemented with l-cystine, dl-methionine, or dl-homocystine. Taurine and d-cystine, which are not considered cysteine sources, did not stop this weight loss. The author stated that although there was a "significant reduction of food consumption" in animals on the benzyl chloride diet, this alone could not account for the inhibition of growth that was observed. He suggested that this was due, at least in part, to interference by benzyl chloride with utilization of the sulfur-containing amino acids needed for growth. Because benzyl chloride was added directly to the diet, its conjugation with sulfur-containing amino acids may have occurred prior to its ingestion. Stekol mentioned, but did not report data on, controls with the same lowered food intake.

(b) Metabolism

One of the major excretion products following ingestion of benzyl chloride is a cysteine conjugate, benzylmercapturic acid [38-42]. Most studies of the metabolism of benzyl chloride have been concerned with aspects of the detection and the mechanism of formation of this conjugate in rats, rabbits, mice, dogs, and quinea pigs. A proposed pathway for the formation of benzylmercapturic acid from benzyl chloride is presented in Figure III-1.

Stekol [39], in 1938, described the recovery of N-acetyl-S-benzyl cysteine (benzylmercapturic acid) from dog urine following the feeding of benzyl chloride and S-benzylcysteine. In a later (1939) study [38], he also isolated benzylmercapturic acid from the urine of rats and rabbits administered benzyl chloride subcutaneously (sc) in an unspecified carrier.

Witter [40] found no increase in sulfur, and thus no increase in benzylmercapturic acid, in the urine of rabbits injected sc with 260 mg benzyl chloride suspended in 10 cc of gum tragacanth solution but did report an increase in urinary nitrogen that he considered indicative of tissue damage. However, urinary nitrogen/creatinine ratios remained within the range of control values. Open sores that developed several weeks later at injection sites suggested to Witter that benzyl chloride in gum tragacanth solution was poorly absorbed and had irritating properties when left in contact with tissue. No such irritation was reported following injection of gum tragacanth solution alone.

In a 1958 study, Bray et al [43] examined the metabolic products detectable in rabbit urine following oral administration of 200 mg benzyl chloride/kg body weight. Urine was collected for approximately 24 hours and analyzed. The ether-soluble acid fraction of the rabbit urine accounted for 86.4% of the dose administered: 49% as benzylmercapturic acid, 20% as a glycine conjugate, 0.4% as glucosiduronic acid, and 17% possibly excreted as unconjugated benzoic acid. No glucuronic acid and no ethereal sulfate were detected. Maitrya and Vyas [44], as reported in 1970, gave benzyl chloride to six rats in oral doses of 44 mg/kg body weight daily for 7 days. Urine samples were collected and analyzed for hippuric acid only, by Quick's method [45]. Of the total amount of benzyl chloride administered, 30% was excreted as hippuric acid.

Knight and Young [42], using radiochromatographic and isotope dilution methods, concluded that benzyl chloride, unlike related compounds including chlorinated benzenes, is converted directly into benzylmercapturic acid without the formation of acid-labile precursors.

Barnes et al [41] measured the rate and amount of benzylmercapturic acid formation in the urine of fasted female rats after oral administration of an aqueous suspension of 158 micromoles benzyl chloride/100 g body weight (200 mg/kg). Urine was collected from four rats until no more

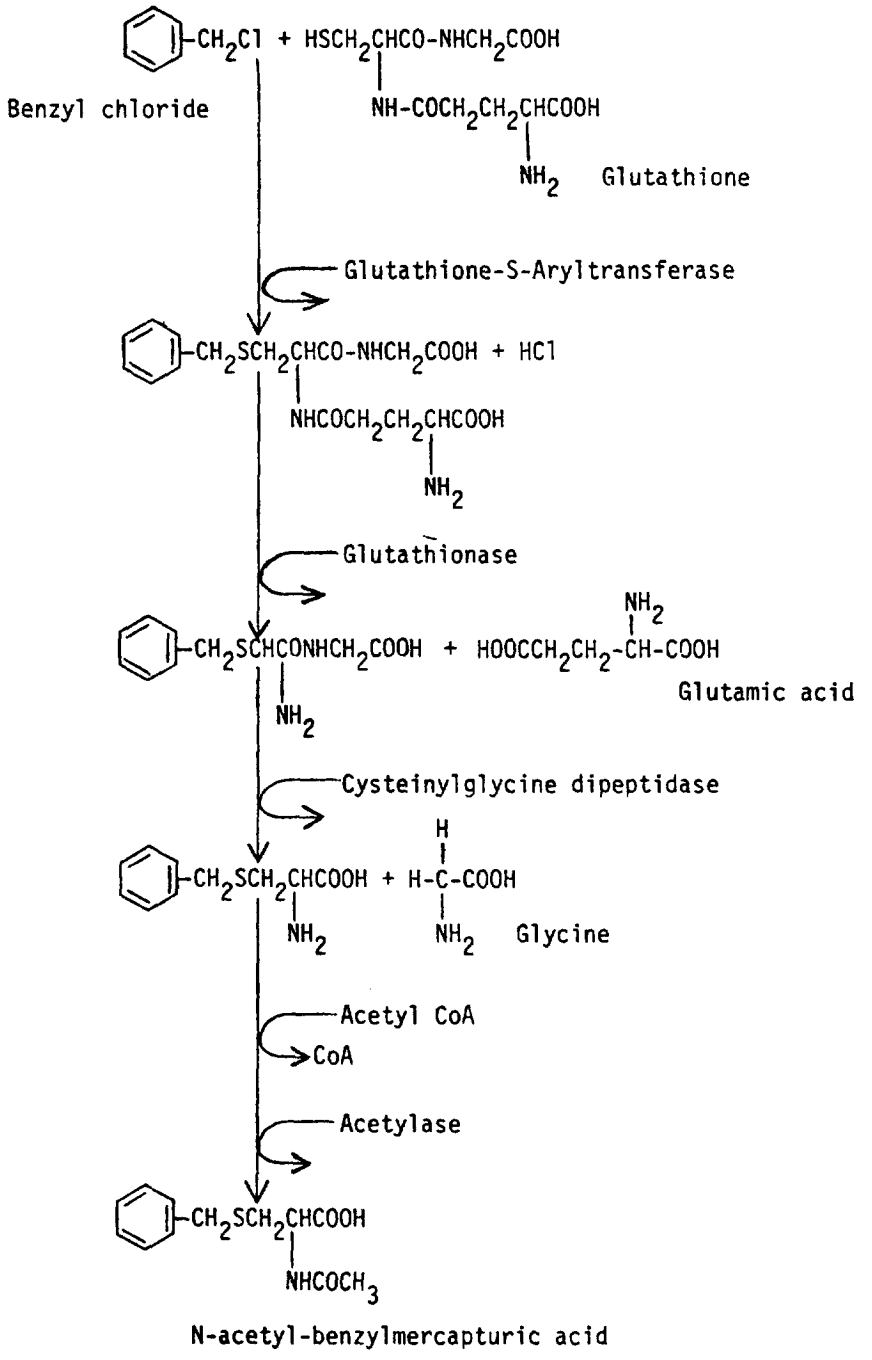

FIGURE III-1

A PROPOSED PATHWAY FOR BENZYLMERCAPTURIC ACID FORMATION FROM BENZYL CHLORIDE IN RATS*

*Adapted from reference 64

mercapturic acid could be detected (usually 24 hours). To measure the rate of mercapturic acid formation, the authors again administered six fasted rats with 158 micromoles benzyl chloride/100 g body weight. Two hours after benzyl chloride administration, the urine in the bladder of the rats was expelled by gentle pressure and discarded. At this time, and again 3 hours later, 3 ml of water was given by stomach tube. All urine produced between hours 2 and 6 was collected and pooled. Preliminary experiments conducted at 2-hour intervals showed that the rate of mercapturic acid excretion was nearly constant during this period [41].

The mean total benzylmercapturic acid excretion in the rat corresponded to 27% (range 20-32%) of the benzyl chloride administered [41]. Bray et al observed these values to be 49% in the rabbit [43] and 4% in the guinea pig [46] after similar doses of benzyl chloride. The mean maximal excretion rate during the 2-6 hour period was calculated to be 5.8 micromoles/100 g/hour (3.5-7.8), ie, 14.7 mg/kg body weight/hour. The excretion rate was almost constant during that period but began to decline slightly by the 6th hour.

Stekol [39] noted that the thiol group of benzylmercapturic acid was attached to the carbon of the side chain instead of directly to a ring carbon, as in the conjugation of other aromatics such as halogenated benzenes, anthracene, and naphthalene [47]. He suggested that the affinity of benzyl chloride for thiol groups was a common factor in the mechanism of its detoxification and of its sensitizing properties.

Stekol's [39] observations raised a question regarding the source of the thiol group in benzylmercapturic acid. In contrast to Stekol's explanation that benzyl chloride conjugated with tissue protein, Barnes et al [41] postulated that the conjugation might have been with the tripeptide, glutathione. They calculated glutathione turnover rates from maximum excretion rates of mercapturic acid and from the levels of glutathione in the rat liver before and after administration of benzyl chloride.

Glutathione levels in the liver were significantly lower in the 5-11 rats administered benzyl chloride compared with the 4-12 water-fed controls [41]. The mean values for reduced glutathione (GSH) in benzyl chloride-dosed rats were 38, 16, 20, 27, and 70 mg/100 g of liver at 0.5, 2, 4, 6, and 10 hours, respectively. The corresponding values for water-fed controls were 120, 163, 140, and 133 mg/100 g of liver (no 10-hour sample). The mean values for oxidized glutathione (GSSG) in benzyl chloride-dosed rats at 0.5, 2.0, and 4.0 hours, respectively, were 21, 4, and 15 mg/100 g of liver in the benzyl chloride-fed rats and 53, 14, and 35 mg/100 g of liver in the control rats.

The turnover rate of glutathione in the liver was calculated by an adaptation of the method of Simkin and White [48], based on the depletion

of free glycine in the liver and the excretion of benzoic acid, the precursor of hippuric acid [41]. Turnover rates were 49 mg/100 g of liver/hour for GSH, and 50 mg/100 g of liver/hour for GSH + GSSG. With the formula $t^{0.5} = 0.7$ g/v where g is the normal glutathione level of the liver (176 mg/100 g of liver) and v is the turnover rate, half-lives of 2.1-4 hours (mean 3.1) were calculated; this finding agrees well with those of other investigators [49,50].

The marked drop in the liver glutathione levels of rats administered benzyl chloride was roughly proportional to the amount of mercapturic acid formed [41]. Barnes and coworkers concluded that the rate of glutathione turnover was adequate to account for the amount of mercapturic acid formed.

The data [41] implicating liver glutathione as the source of thiol groups for mercapturic acid formation are supported by a study reported in 1967 by Suga et al [51] who determined the distribution of the enzyme that conjugates glutathione with benzyl chloride in various organs of the rat. The crude enzyme remained in the 105,000 x g liver supernatant fraction, ie, it was not microsomally bound. Activity of the enzyme was measured by its ability to accelerate the conjugation of glutathione with a benzyl chloride substrate. The reaction rate for liver enzyme with benzyl chloride was constant for 30 minutes. Rat liver had the highest glutathione-conjugating activity; the kidney had 73% and the spleen 13%, whereas the brain, intestine, lung, and heart each had 4% or less, compared with the liver.

The previous conclusion by Barnes et al [41] that glutathione turnover was sufficient to account for the thiol source for mercapturic acid synthesis was strengthened by the report of Bray et al [52] that assessed the relative importance of glutathione and protein thiol groups as conjugating agents for benzyl chloride in liver homogenates from adult female rats. The authors found a rapid drop in glutathione levels after the addition of 53 millimoles of benzyl chloride/100 g of liver. Glutathione levels (in millimole/100 g of liver) decreased in the following manner: 0.64 at 0.5 minutes, 0.21 at 2.0 minutes, 0.09 at 4.0 minutes, 0.03 at 6.0 minutes, and 0.02 at 8.0 minutes. In the same preparations, nonglutathione thiol levels averaged 1.68 millimoles/100 g of liver for the 8 minutes. Control glutathione levels remained at about 0.88 millimoles per 100 g of liver. The authors concluded that because the initial drop in glutathione was so rapid, benzyl chloride probably conjugated with glutathione rather than with general tissue protein. Beck et al [53], in 1964, also found liver glutathione levels reduced (by 50% in 2 hours) after intramuscular administration of benzyl chloride in mice.

(c) Carcinogenic and Mutagenic Effects

The first evidence of tumor production by benzyl chloride was published in a 1968 preliminary report by Preussmann [54]. In 1970, Druckrey et al

[55] published the full-text report of the study in which 12 direct alkylating agents were screened for carcinogenic activity in rats. The authors first conducted an acute toxicity (LD_{50}) experiment to determine appropriate dose levels for the chronic study. Benzyl chloride, purified by double distillation and dissolved in peanut oil, was administered sc to 100-day-old rats of 3 BD strains. The LD_{50} was determined to be 1 g/kg, with deaths occurring after 1-4 days. No information was provided on dosage or numbers of animals used in the toxicity tests. The toxic signs observed during the acute toxicity tests were said to have been similar for each of the 12 agents studied, but the results for benzyl chloride were not specifically reported. The initial toxic sign that appeared about 20 minutes after administration was lassitude. Later, in 10-20 hours, signs of lung edema with increasing labored breathing were observed in all animals. A hemorrhagic diarrhea developed. Autopsy confirmed the diagnosis of lung edema with hemorrhages. Liver damage, not further characterized, was found in many of the injected animals.

Two dose levels were then selected for the chronic study with benzyl chloride [55]. In the chronic test, 14 rats were injected sc with benzyl chloride at a dose of 40 mg/kg and 8 other rats were injected with 80 mg/kg once weekly for 51 weeks. According to the authors, these represented total doses of 2.1 and 3.9 g/kg, respectively.

After a mean induction time of 500 days, 3 of the 14 animals that had received the 40 mg/kg dose had injection-site sarcomas; and 6 of the 8 animals given the 80 mg/kg dose had larger sarcomas, most of which had metastasized to the lungs [55]. By microscopic examination of the injection-site tumors, six fibrosarcomas, two spindle cell sarcomas, and one myosarcoma were identified. Benzyl chloride-induced tumors were reported to be transplantable. It was not clear from the authors' description and table of results which types of tumors were associated with the high (80 mg/kg) doses and which with the low (40 mg/kg). It was also impossible to determine which tumors had metastasized and which were transplantable. A moderate local necrosis at the site of injection also was observed. A control group of rats injected with pure peanut oil at and above the volume used as a carrier did not develop local sarcomas.

Poirier et al [56], in 1975, reported the results of a study of the correlation between the chemical reactivity of 17 low molecular weight alkyl halides and their carcinogenic activity as measured by induction of pulmonary tumors in A/Heston strain mice. All compounds, including benzyl chloride, were more than 98% pure. Each group of 20 male and female mice, 6-8 weeks old and weighing 17-19 g, was injected intraperitoneally (ip) 3 times/week for 8 weeks at one of three dose levels of benzyl chloride solubilized in tricaprylin. Mice receiving no injections, and mice given injections of tricaprylin alone, served as negative and vehicle controls, respectively. Mice injected with two dose levels of urethane served as positive controls.

The maximum tolerated dose (MTD), defined as the maximum single dose that at least four of five mice tolerated after receiving six ip injections in 2 weeks, was determined for each chemical [56]. Three experimental groups received the MTD, or a 1:2 or 1:5 dilution of the MTD. The authors stated that because of its toxicity, fewer doses of benzyl chloride were administered than originally planned. Toxic effects were not specified. A total of 15.8 millimoles/kg (2 g/kg) benzyl chloride was given in 8 doses, 11.8 millimoles/kg (1.5 g/kg) in 12 doses, and 4.7 millimoles/kg (0.6 g/kg) in 12 doses.

Twenty-four weeks after the initial injections of benzyl chloride, the surviving mice were killed by cervical dislocation[56]. There were 8/20 survivors at the 2 g/kg, 16/20 at the 1.5 g/kg, and 15/20 at the 0.6 g/kg dose. The lungs were removed and fixed in Tellvesniczky's fluid for 3-4 days. Tumors, which appeared as pearly white nodules on lung surfaces, were counted, and some of these were examined microscopically. The average number of lung tumors/mouse injected with the 2-g/kg dose of benzyl chloride was 0.25 ±0.08. There was an average of 0.50 ±0.13 for the 1.5 g/kg and 0.26 ±0.07 for the 0.6 g/kg dose. The results after benzyl chloride injection were not significantly different, as determined by the standard Student's t-test, from either the untreated (0.21 ±0.03) or the tricaprylin-vehicle-treated (0.24 ±0.05) mice. Urethane-injected animals exhibited an average of 17.8 ±4.32 tumors/mouse at the dose level of 20 mg/mouse and 8.1 ±2.3 tumors at the dose level of 10 mg/mouse. Lungs were examined grossly and microscopically for abnormalities such as adenomatosis and inflammatory reactions, and none was reported. Except for one lymphoma and two salivary gland tumors seen in unspecified groups and described as spontaneous, no other unusual findings were cited.

According to Poirier et al [56], the absence of significant effects by benzyl chloride in mice was unexpected on the basis of known chemical activity and because of the injection-site sarcomas seen in rats by Druckrey et al [55]. Poirier et al [56] suggested that the difference may have been due to a greater metabolic inactivation of benzyl chloride in the liver by the ip route than by the sc route. Other important differences in experimental design were observed between these two investigations. The positive results reported by Druckrey et al [55] were observed in rats injected sc once a week for 51 weeks with a total dose of either 2.1 g or 3.9 g benzyl chloride/kg of body weight after a mean induction time of 500 days. In the 24-week study by Poirier et al, mice received only 8-12 ip injections during 8 weeks amounting to a total of 0.6-2.0 g of benzyl chloride/kg of body weight. Sufficient induction time for tumors may not have been allowed, although statistically significant increases in tumor induction occurred with 10 of the 17 alkyl halides tested.

McCann et al [57], in 1975, included benzyl chloride in a group of 23 chemicals that they used to evaluate two strains of Salmonella typhimurium, TA 100 and TA 98, recently derived as more sensitive indicators of

mutagenic potential. They apparently followed their standard "Ames test" procedure [58], without microsomal activation. After incubation for 48 hours at 37 C, colonies of histidine-independent revertants were counted and the results presented as revertant colonies/plate, after subtraction of spontaneous revertant values. The reported results were taken from the linear portion of the dose-response curve for each substance. Other dose levels tested and the number of plates prepared were not described.

For benzyl chloride at a dose of 2 mg/plate, 12 revertant colonies/plate were reported with test strain TA 1535, and 230 colonies/plate were reported with strain TA 100 [57]. Strain TA 1535 responds to base-pair substitution such as could occur following the alkylation of one of the bases of DNA. Strain TA 100 is made from strain TA 1535 with the addition of a plasmid (R factor) that may enhance damage to DNA by interfering with the recombinational repair system. No revertants were detected with strain TA 1538, which responds especially to frameshift mutagens. Bifunctional alkylating agents can cause DNA crosslinking, which could lead to frameshift mutations, but this would not be expected from a monofunctional alkylating agent like benzyl chloride. Twenty revertant colonies/plate were reported with strain TA 98, made from TA 1538 plus the R factor. The authors concluded that benzyl chloride was much more active as a mutagen in strain TA 100 than in its parent strain, TA 1535.

Later in 1975, these results were presented by McCann et al [59] in a tabulation of 300 chemicals, for which published reports of carcinogenicity in mammals were compared with the results of their Salmonella mutagenicity screening. In this evaluation, the authors defined "weakly mutagenic" as producing fewer than 0.10 revertants/nanomole of material tested. Benzyl chloride produced 0.02 revertants/nanomole and was thus classified as weakly mutagenic by the authors. However, inspection of the data obtained by the authors on other chemicals revealed that their "nonmutagenic" classification may range from fewer than 0.00001 revertants/nanomole to fewer than 0.25 revertants/nanomole. It appears that when benzyl chloride is tested in what is thought to be the most sensitive bacterial test system available, the results cannot be distinguished from those on "nonmutagens."

In 1976, Fluck et al [60] reported their results after testing a large number of chemicals, including benzyl chloride, in order to evaluate a bacterial system for screening potential mammalian carcinogens. The system utilized a repair enzyme-deficient (pol A-) mutant (P3478) of Escherichia coli that contained less than 1% of the DNA polymerase activity of its parent (pol A+) strain (P3110). This enzyme repairs altered DNA by excision of the defective portion of the molecule and catalyzes resynthesis of the correct sequence. The authors had postulated that chemicals that react with cellular DNA would be expected to decrease the growth rate of the mutant to a much greater extent than that of the parent.

Twenty-five µl of benzyl chloride was placed in the center well of each of two plates containing either the parent or the mutant strain [60]. Control plates of both strains were grown with ampicillin and colistin to check strain constancy and with dimethyl sulfate, which had consistently served as a (differential toxicity) positive control. All plates were incubated for 16 hours, presumably at 37 C. The zones of growth inhibition about the center wells then were measured in millimeters. A difference of 4 mm or more between parent and mutant strain was considered positive. The differential between the parent and mutant strain tested with benzyl chloride was 24 mm, ie, benzyl chloride apparently had a damaging effect on the DNA in this system.

The authors [60], however, concluded, on the basis of their data, that their test system would not be useful for routine prescreening of large numbers of compounds for carcinogenic potential. It did not distinguish between many known carcinogens and noncarcinogens, and it was not a quantitative assay in that a dose-response relationship was not seen and "potent" carcinogens did not necessarily show greater responses than did "weaker" ones.

Rosenkranz and Poirier, in a National Cancer Institute report [61] evaluating the degree of correlation between mutagenic and DNA-modifying activity in bacterial systems and carcinogenicity in animal tests, examined results of Ames and E. coli pol A tests with benzyl chloride. Salmonella strains TA 1535 and TA 1538 described above (but not the newer, more sensitive strains TA 98 and TA 100) were used in the Ames system. Each strain was exposed with and without the S-9 microsomal activation system at 10 µl benzyl chloride/plate and without activation at 5 µl/plate. In strain TA 1535, without activation, there were 43 revertants/plate at the 5 µl dose and 68 at 10 µl, contrasted with 22 revertants/control plate. The authors classified this result as "marginally positive." In both strains, control plates and those containing 10 µl of benzyl chloride and the S-9 system had 12 revertants/plate. The S-9 microsomal preparation had an inactivating effect on benzyl chloride at the 10 µl dose level, presumably because of the reaction of benzyl chloride with components of the S-9 system, probably protein.

Negative results were obtained on strain TA 1538 under the same experimental conditions. The authors noted that benzyl chloride is labile at 45 C , the temperature of top agar used in the Ames test, which could possibly have diminished its reactivity.

For the E. coli pol A assay, 10 µl benzyl chloride was added to the central disk of each plate. Each control and each dose level of benzyl chloride was tested in duplicate at least three times. The zone of inhibition was 16.3 mm in the pol A+ parent and 19.0 mm in the pol A- mutant; the difference of 2.7 mm was considered significant by the authors.

Correlation of Exposure and Effect

Occupational exposure to benzyl chloride may occur by inhalation or by contact with the eyes or skin. Benzyl chloride is a powerful lacrimator and causes intense irritation of the upper respiratory tract as well [21,22,29,30]. The capacity of liquid benzyl chloride to damage the skin has been mentioned in the literature [27,62] but is not supported by any human case reports. However, because the molecule is highly reactive and capable of covalent bonding with nucleophilic groups such as are present in protein [7], and because hydrolysis of benzyl chloride releases hydrochloric acid, the potential for skin irritation by liquid benzyl chloride is consistent with these chemical (reactivity) considerations.

Human exposures to benzyl chloride vapor at 160-23,600 mg/cu m have been reported [21,22,27,29,30]. The longest exposure duration reported was only 5 minutes [29], presumably because of the intense irritation caused by the compound at concentrations as low as 88 mg/cu m [30]. At all concentrations in the range, the primary effect was an intense burning sensation in the eyes, accompanied by profuse tearing. At higher concentrations in the range, these effects were accompanied by conjunctivitis and respiratory irritation [21,22], described by Schutte as a catarrah [22], an inflammation of the mucous membranes of the head and throat. Watrous [27] described similar effects on the conjunctiva and respiratory tract after intermittent exposure to benzyl chloride at 2,590 mg/cu m.

Katz and Talbert [30] determined the ocular and nasal irritation thresholds of benzyl chloride. Intolerable eye irritation resulted from a 10-second controlled exposure to benzyl chloride at 410 mg/cu m. The threshold for nasal irritation was determined to be 180 mg/cu m, whereas that for eye irritation was determined to be 41 mg/cu m. In a 1971 summary report, for which documentation is not available, Mikhailova [29] stated that benzyl chloride at concentrations of 6-8 mg/cu m could result in slight conjunctivitis in workers after a 5-minute exposure.

The acute toxicity of benzyl chloride may not be limited to irritant effects on the eyes, respiratory mucosa, and skin. Animal studies have indicated that benzyl chloride, when inhaled at 100-23,600 mg/cu m, can induce acute pulmonary reactions [21,22,34]. Focal pulmonary hemorrhages were observed in rats exposed to benzyl chloride for 8 hours at 3,300 mg/cu m; however, exposures at 1,100 mg/cu m for 7.5 hours resulted only in pulmonary edema [21,22]. In animals exposed to benzyl chloride at 890 mg/cu m or greater for 6.8 hours, corneal turbidity was reported; however, this condition was reversible in 3 days [22]. When benzyl chloride was inhaled for 0.5 hours or more, at concentrations greater than 800 mg/cu m, an increased mucous secretion was consistently noted. Mikhailova [34], after exposing rats and mice for 2 hours to benzyl chloride at 100 mg/cu m, found hepatic changes that were described as albuminoid and fatty

degeneration. The fatty degeneration could possibly have included some fatty infiltration. Necrosis of the kidney, presumably a result of albuminoid degeneration of the convoluted tubular epithelial cells, was also reported. Workers exposed to benzyl chloride above 100 mg/cu m would probably exhibit similar reactions and may also be susceptible to secondary pulmonary infections. Moreover, workers with preexisting lung disease may be subject to an increased risk of further pulmonary damage.

Two studies [21,22] suggested that the irritant effects of benzyl chloride vapor may be cumulative. Schutte [22] initially exposed two cats to benzyl chloride at 2,000 mg/cu m for 0.5 hours and observed only lacrimatory effects, which ceased 0.5 hour after exposure ended. Reexposing the animals 5 days later at 800 mg/cu m for 0.5 hour resulted in similar lacrimatory effects; recovery was slower, however, and the animals died 3 weeks later of what was diagnosed as pneumonia. Wolf [21], who exposed two cats to benzyl chloride at 480 mg/cu m for 8 hours/day for 6 consecutive days, observed that the irritant effects of benzyl chloride appeared sooner each day, and with increasing severity, as the exposures continued.

Skin sensitization in guinea pigs exposed to benzyl chloride was tested by Landsteiner and Jacobs [35] in 1936. The animals were injected intracutaneously with 0.01 mg benzyl chloride in saline twice weekly for 12 weeks, then allowed to rest for 2 weeks prior to topical application of a benzyl chloride/olive oil suspension, whereupon an allergic-type skin response, manifested as erythema and swelling, was elicited.

Although no thorough studies of the metabolism of benzyl chloride in humans have been located, some inferences may be drawn from the available animal studies. In these cases, animals were exposed by various routes, and metabolic products in their urine were identified. In studies conducted with rats [37,41,42,44,63,64], rabbits [38,40,42,43], dogs [39], guinea pigs [46], and mice [53], benzylmercapturic acid was found to be a urinary excretion product. The percentage of administered benzyl chloride that was recovered as the product of its conjugation with glutathione, benzylmercapturic acid, varied from 49% in rabbits to 27% in rats and 4% in guinea pigs [41,46,65]. The enzymes which catalyze this conjugation of benzyl chloride with glutathione have also been isolated from human liver and show activity with benzyl chloride as substrate [32]. Another study [66] confirmed that humans are capable of forming mercapturic acids. It seems likely, therefore, that in humans benzyl chloride is excreted as benzylmercapturic acid.

Carcinogenicity, Mutagenicity, Teratogenicity, and Effects on Reproduction

The carcinogenic potential of benzyl chloride has been examined in two animal studies [55,56]. In a rat study [55], one group of 14, and another

of 8, animals received sc injections of benzyl chloride in peanut oil once weekly for 51 weeks at respective doses of 40 and 80 mg/kg/week. After a mean induction time of 500 days, injection-site sarcomas were identified in 3 of 14 rats administered the 40 mg/kg doses and in 6 of 8 rats administered the 80 mg/kg doses. No sarcomas were found in the vehicle-treated controls. Without further description, the authors stated that of the animals injected with the 80 mg/kg doses most had metastases to the lung, but that no lung metastases occurred at the 40 mg/kg doses. The authors stated that transplantation of the tumors was successful; however, no further descriptions were furnished.

The results of this study [55] indicate that benzyl chloride administered sc in peanut oil has a carcinogenic potential in rats. The weekly dosages administered amounted to 4-8% of the LD_{50} for these animals. Local necroses occurred at the injection sites. The responses were dose related, ie, more injection-site sarcomas developed in animals given the higher dose, and lung metastases occurred only within the high-dose group.

Poirier et al [56] studied the carcinogenic activity of 17 low-molecular-weight alkyl halides including benzyl chloride. The production of lung adenomas in A/Heston strain mice was used as an indicator of the carcinogenic activity of these compounds. This strain has been shown to be sensitive to chemical carcinogens, as indicated by increased rate of lung adenoma formation. Benzyl chloride in tricaprylin was given in multiple ip injections at the MTD and at one-half and one-fifth the MTD. However, because of unspecified toxic effects the maximum dose subsequently was reduced, and all injections were given less frequently than originally planned (three times/week for 8 weeks). Total doses of 2,000 mg/kg, 1,500 mg/kg, and 600 mg/kg were administered in 8, 12, and 12 injections, respectively. All animals were killed 24 weeks after the initial injections; and lungs were removed, fixed, and examined. No significant increase in the incidence of pulmonary tumors was found in the benzyl chloride-injected mice compared with controls, probably, according to the authors, because the benzyl chloride was administered ip, enhancing the probability of metabolic deactivation of the injected material. In spite of the high rate of spontaneous tumorigenesis of the strain-A mice and the relatively short induction time, 10 of the 16 other low-molecular-weight monofunctional alkyl halides yielded positive results in this test system.

McCann et al [57] included benzyl chloride in a study in which 23 chemicals were screened for mutagenic activity in S. typhimurium strains TA 100 and TA 98, which are derived, by addition of a plasmid (R factor), from strains TA 1535 and TA 1538, respectively. For benzyl chloride at 2 mg/plate, 12 revertants/plate were reported with strain TA 1535 and 230 revertants/plate with the more sensitive TA 100. No revertants were reported with strain TA 1538, and 20 revertants/plate were reported with strain TA 98. The results of these tests were again published by McCann et

al [59] in a tabulation of the results of tests of 300 chemicals for mutagenic activity. In the report, benzyl chloride was classified as "weakly mutagenic," as defined by fewer than 0.10 revertants/nanomole. "Nonmutagenic" was not defined but appeared to range from fewer than 0.00001 revertants/nanomole to fewer than 0.25 revertants/nanomole.

Fluck et al [60], evaluating a repair enzyme-deficient E. coli test system for its potential as a carcinogen screen, included benzyl chloride as a test chemical. Benzyl chloride, 25 µl, was placed in the center well of each of two plates containing either the parent strain (pol A+) or the repair enzyme-deficient (pol A-) mutant. After 16 hours of incubation at 37 C, the difference in the zones of inhibition between the parent and mutant strains was 24 mm. This result was considered positive by the authors, who also concluded that the system was not useful as a routine prescreening test for carcinogens because it showed no dose-response relationship and could not distinguish between known carcinogens and noncarcinogens. The results of Fluck et al cannot be considered indicative of any purely mutagenic effects of benzyl chloride because differential growth may indicate effects other than specific alterations of the DNA molecule.

In a National Cancer Institute study, Rosenkranz and Poirier [61] tested several compounds, including benzyl chloride, in Salmonella and E. coli systems. Salmonella strains TA 1535 and TA 1538, described above, were tested with and without S-9 microsomal activation at 10 µl/plate, and without activation at 5 µl/plate. Without activation, there were 43 TA 1535 revertants/plate at the 5 µl dose and 68 at the 10 µl dose, compared with 12 spontaneous revertants/plate. With activation, both the control plates and those containing 10 µl of benzyl chloride had 12 revertants/plate. The S-9 microsomal preparation had an inactivating effect on benzyl chloride. This was presumed by the authors to have been caused by reaction of benzyl chloride with microsomal protein. Negative results were obtained with strain TA 1538 under the same experimental conditions. In a test system with repair enzyme-deficient E. coli, by the same authors, 10 µl of benzyl chloride added to the central disk of each plate resulted in zones of inhibition of 16.3 mm for the pol A+ parent strain, and 19 mm for the pol A- mutant. The authors proposed use of the TA 1535 and pol A tests in tandem as a screening technique for carcinogenic compounds. The data from strain TA 1535 are indicative of a dose-related effect. McCann et al [57], using benzyl chloride at 2 mg/plate, reported 12 revertants/plate. Rosenkranz and Poirier [61], using doses of 5 and 10 µl (5.5 and 11.0 mg), counted 43 and 68 revertants/plate, respectively.

No reports of teratogenic or reproductive effects associated with exposure to benzyl chloride have been found.

The results from experiments on bacteria and rodents indicate that benzyl chloride is a weak mutagen in microbial test systems and, following sc injection in rats, causes neoplastic changes at the injection site with

metastases. However, further animal research is required to estimate these risks following pulmonary exposure to benzyl chloride.

Data on the effects of exposure to humans and animals are presented in Tables III-2, III-3, and III-4.

TABLE III-2

SUMMARY OF EFFECTS OF EXPOSURE TO BENZYL CHLORIDE VAPOR IN HUMANS

Concentration (mg/cu m)	Duration	Effects	Reference
0.21-0.24	Brief*	Odor thresholds	30,31
6-8	5 min	Conjunctivitis	29
41	10 sec	Eye irritation threshold	30
180	One breath*	Nasal irritation threshold	30
160-17,700	Brief*	Lacrimation, conjunctivitis, respiratory tract irritation	21
Up to 2,590	Intermittent*	Conjunctivitis, upper respiratory tract irritation	27
800-23,600	Brief	Eye and respiratory irritation	22

* Authors' description

TABLE III-3

SUMMARY OF EFFECTS OF BENZYL CHLORIDE VAPOR ON ANIMALS

Species*	Concentration (mg/cu m)	Duration	Effects	Reference
Mouse	2,000	1 hr	All survived	33
"	390 (LC_{50})	2 hr	Respiratory tract inflammation, secondary infection	34
Rat	2,000	1 hr	All survived	33
"	740 (LC_{50})	2 hr	Respiratory tract inflammation, secondary infection	34
" (10)	100	"	Weight loss, eye and respiratory tract irritation	34
Rabbit (1)	480	8 hr/d for 6 d	Mild eye irritation, reddening of oral and nasal mucosa by 6th d	21
Cat (2)	480	"	Eye and respiratory tract irritation, loss of appetite	21
Dog	1,900	8 hr	Irritation of the ocular, respiratory, and oral mucosa, corneal turbidity, death within 24 hr	22

* Numbers in parentheses represent the number of animals.

TABLE III-4

SUMMARY OF EFFECTS OF BENZYL CHLORIDE ON ANIMALS

Route of Administration	Species*	Concentration (mg/kg)	Regimen	Effects	Reference
oral	Mouse	1,624	--	50% died	33
"	Rat	1,231	--	"	33
sc	"	1,000	--	"	55
"	" (8)	80	1/wk, 51 wk	Six developed local sarcomas with mean induction time of 500 d, most with lung metastases.	55
"	" (14)	40	"	Three developed local sarcomas with mean induction time of 500 d; no metastases reported.	55
ip	Mouse (20)	250	8 injections within 8 wk	No significant increase in lung adenomas by 24th wk	56
"	"	125	12 injections within 8 wk	"	56
"	"	50	12 injections	"	56
intracutaneous followed by dermal	Guinea pig	0.01 mg in saline solution followed by application of liquid to skin	2/wk for 12 wk to sensitize; dermal application 2 wk later	Skin swelling and pinkness	35

* Numbers in parentheses represent numbers of animals exposed.